UNIVERSITE ECOLOGIE BRETAGNE GEOSCIENCES LOIRE AGRONOMIE ALIMENTATION

UNIVERSITÉ DE NANTES

THESE DE DOCTORAT DE

L'UNIVERSITÉ DE NANTES

COMUE UNIVERSITE BRETAGNE LOIRE

ÉCOLE DOCTORALE N° 600

École doctorale : *Écologie, Géosciences, Agronomie et Alimentation*

Spécialité : *Microbiologie, virologie et parasitologie*

Par

Nassima ILLIKOUD

Caractérisation des mécanismes d'altération des produits carnés et de la mer par *Brochothrix thermosphacta*

Approches phénotypique, génomique, transcriptomique et analyse du volatilome

Thèse présentée et soutenue à Oniris, Nantes, le 9 juillet 2018

Unité de recherche : ***UMR 1014, INRA, Oniris, Université Bretagne-Loire***

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« La réussite est l'accumulation d'échecs, d'erreurs, de faux départs, de confusions, et la volonté de continuer malgré tout. »

Nick Gleason

Je dédie cette thèse à ma mère et ma sœur
qui nous ont quittés trop tôt…

Remerciements

Je tiens, tout d'abord, à remercier tous les membres de mon jury d'avoir accepté d'évaluer ce travail de recherche. Je remercie tout particulièrement, Christophe Chassard et Marie-Christine Champomier-Vergès d'avoir accepté d'être rapporteurs de cette thèse. Je suis également reconnaissante envers Anne-Marie Revol-Junelles, Michel Dion et Yves Le Loir d'avoir accepté de faire partie de mon jury.

Je tiens à adresser mes remerciements à Hervé Prévost, ancien directeur d'unité et Marie-France Pilet, directrice actuelle, pour m'avoir accueillie au sein du laboratoire pour effectuer mes travaux de thèse.

Je voudrais remercier particulièrement ma directrice de thèse, Monique Zagorec, dont le bureau a toujours été ouvert. Je veux lui témoigner ma profonde gratitude pour sa patience, sa lecture vigilante de mes écrits, son aide et sa disponibilité qui ont rendu possible toutes les actions menées au cours de ce projet.

Je remercie également mes co-encadrants Emmanuel Jaffrès et Marie France Pilet. Emmanuel qui a toujours été disponible tant pour les expérimentations clés de la thèse, que pour la relecture attentive ainsi que pour son soutien infaillible. Marie-France Pilet quant à elle pour ses conseils pertinents, sa bienveillance et son soutien qui ont contribué à réaliser ma thèse dans les meilleures conditions possibles. Nos discussions m'ont permis de prendre le recul nécessaire sur le sujet et sur les méthodes d'analyse.

Je tiens également à exprimer ma gratitude à Régine Talon et Hélène Falentin d'avoir accepté de faire partie de mon comité de suivi de thèse et pour leurs nombreux conseils scientifiques mais aussi concernant mon avenir professionnel. Merci également à Ségolène Calvez d'avoir accepté d'être ma tutrice dans le cadre de l'École Internationale de Recherche-Agreenium mais aussi pour m'avoir fourni la clé Bionumerics pour l'analyse de mes gels Rep-PCR et PFGE.

J'exprime également ma profonde gratitude à tous les collaborateurs avec qui j'ai travaillé au cours de ce projet de thèse : Merci à Dalal Werner et Rodérick Gohier (Aérial, Strasbourg) pour l'analyse des molécules volatiles par GC/MS. Merci également de m'avoir si bien accueillie afin que je puisse acquérir cette technique. Je remercie également Thomas Charrier et Romain Chauvet (Eurofins Laboratoires de Microbiologie Ouest) pour la collaboration pour le typage MALDI-TOF. Je remercie nos interlocuteurs de la plateforme MaGe, en particulier David Roche pour son accompagnement et sa réactivité lors des analyses des génomes et du transcriptome. Enfin, je suis reconnaissante envers le Professeur Danilo Ercolini pour ces conseils sur le plan d'expérience de l'étude du volatilome qui n'a malheureusement pas pu être réalisée en collaboration comme initialement envisagée.

Je suis reconnaissance envers Catherine Magras, pour sa précieuse aide lors de la mise en relation avec l'abattoir qui nous a permis d'obtenir les souches. Merci aussi Jean-Michel Cappelier pour le covoiturage au moment de l'échantillonnage.

Un énorme merci à Albert Rossero, le MacGyver de Secalim, qui trouvait toujours une solution, même si tout espoir semblait être perdu. Merci pour son aide lors des expérimentations de PFGE, préparation

des jus (que ferait-on sans le Thermomix !!) et bien d'autres choses. Merci également à Agnès Bouju pour les précieux dépannages.

Un grand merci à Isabelle Clouet pour son soutien et sa disponibilité, en particulier pour les commandes passées, alors qu'elle était absente de Secalim. Merci également Patricia pour son sourire constant qui égayait les journées dès le matin.

Je remercie l'ensemble des chercheurs et enseignants-chercheurs de l'UMR secalim pour les discussions enrichissantes et leurs conseils précieux. Je remercie également tous les membres de l'équipe technique de Secalim que j'ai côtoyé tout long de ma thèse. Je remercie particulièrement Valérie, Sandrine, Nicolas et Laurent pour leur bonne humeur lors de tous les moments que nous avons partagés notamment en pauses déjeuner.

Une pensée particulière à tous les doctorants et post-doctorants, anciens et actuels : Benoît, Soumaya, Manuel, Raouf, Alizée, Rached, Norman, Benjamin, Du, Nicolas, Aurélien et Georges, pour les moments passés lors des pauses rigolotes. Je remercie particulièrement Taous pour tellement de choses que je ne pourrais pas tout citer ici. Une pensée pour Ramila, pour ses délicieuses gourmandises brésiliennes. Merci à Vicky, mon ancien binôme de course à pied, pour son soutien et ses conseils en début de thèse. Je remercie Amélie de m'avoir aidée lors de mes premiers pas dans les commandes Linux, pendant sa fin de thèse chargée. Merci aussi Géraldine pour les bons moments partagés lors des différentes activités de l'ATD. Enfin un merci particulier à Juliana pour m'avoir supportée pendant ces mois de rédaction mais aussi pour son soutien et ses encouragements.

Enfin, je remercie les membres de ma famille, en particulier mon père, mes frères et ma sœur, qui m'ont toujours soutenue quels que soient mes choix et qui ont cru en moi. Un merci particulier à mon mari pour son soutien au quotidien, ses encouragements et sa patience durant cette dernière année de thèse.

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Liste des abréviations

ADN	Acide Désoxyribonucléique
ANI	Average Nucleotide Identity
ANOVA	Analysis of variance
ARN	Acide Ribonucléique
A_w	Water Activity
BAs	Biogenic Amines
BHI	Brain Heart Infusion broth
BLAST	Basic Local Alignment Search Tool
cDNA	complementary Deoxyribonucleic Acid
CDS	Coding DNA Sequence
CFU	Colony-Forming Units
COG	Clusters of Orthologous Groups of proteins
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DE	Differentially expressed
EMBL	European Molecular Biology Laboratory
ENA	European Nucleotide Archive
FAO	Food and Agriculture Organization
FC	fold change
GC/MS	Gas Chromatography coupled to Mass Spectrometry
ICFMH	International Committee of Food Microbiology and Hygiene
IFREMER	Institut Français de Recherche pour l'Exploitation de la Mer
INRA	Institut National de la Recherche Agronomique
ISO	International Organization for Standardization
KEGG	Kyoto Encyclopedia of Genes and Genomes
LAB	Lactic Acid Bacterium/Bacteria
LB	Luria Bertani broth
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time of Flight
MAP	Modified Atmosphere Packaging/Packaged
MDS	Multidimensional Scaling

MICFAM	MicroScope Family
MLST	Multi-Locus Sequence Typing
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Center for Biotechnology Information
OECD	Organization for Economic Co-operation and Development
PacBio	Pacific BioscienceS
pb	Paire de base
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
pH	Potentiel hydrogène
PkGDB	Prokaryotic Genome DataBase
PTS	Phosphotransferase System
Rep-PCR	Repetitive Element palindromic PCR
RNA-seq	RNA Sequencing
rRNA	Ribosomal RNA
SD	Standard Deviation
SSO	Specific Spoilage Organisms
STAA	Streptomycin sulphate Thallous Acetate Agar
TAE	Tris, Borate, EDTA
Taq Pol	*Thermus aquaticus* Polymerase
TBE	Tris, Borate, EDTA
Tec	Tonnes Equivalent Carcasses
TMA	Triméthylamine
tRNA	Transfer RNA
UFC	Unité Formant Colonie
UMR	Unité Mixte de Recherche
UPGMA	Unweighted Pair-Group Method using the Average approach
VOC	Volatile Organic Compound
VP	Vacuum Packaging

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Introduction générale

Malgré les progrès réalisés sur les techniques de conservation pour allonger la durée de vie des produits alimentaires (chaîne du froid, atmosphères protectrices, conservateurs chimiques), environ un tiers des denrées est perdu chaque année en raison notamment du développement de microorganismes et/ou de leur métabolisme. Tous les aliments peuvent être affectés par l'altération microbiologique. Cependant, les viandes et les produits de la mer sont considérés comme les plus sensibles en raison de leur teneur en eau, leur pH et leur richesse en nutriments. La composition de la flore d'altération varie en fonction : i) de la nature du produit (composition nutritionnelle, activité de l'eau, pH, etc.) ; ii) du traitement de stabilisation subi (séchage, fumage, fermentation, traitement thermique, etc.) ; iii) des communautés bactériennes présentes dans l'environnement ; et iv) des conditions de stockage (température, atmosphère gazeuse). La température et la disponibilité de l'oxygène sont les facteurs les plus importants car ils peuvent influencer la croissance des bactéries de manière sélective durant le stockage. Ainsi, un conditionnement des viandes sous atmosphère protectrice enrichie en CO_2 ou sous vide permet d'inhiber la croissance de bactéries aérobies à Gram négatif telles que *Pseudomonas*, mais favorise la croissance des bactéries capables de résister au CO_2 comme les bactéries lactiques, les entérobactéries, les *Clostridium* ou encore *Brochothrix thermosphacta* (Gill, 1983).

B. thermosphacta est considérée comme l'une des principales bactéries altérantes en raison de sa nature ubiquitaire, de sa capacité à croître dans un large intervalle de température (0-30 °C) et de sa présence sur de nombreux produits alimentaires où elle est systématiquement associée au processus d'altération. L'altération par *B. thermosphacta* a été étudiée dans plusieurs matrices alimentaires naturellement contaminées ou lors de challenge-tests après inoculation de matrices stériles ou faiblement contaminées (Casaburi et al., 2014; Ercolini et al., 2011; Ercolini et al., 2006; Jaffrès et al., 2011; Joffraud et al., 2001). Ces études ont montré que *B. thermosphacta* présente une forte capacité d'altération des viandes et des produits de la mer en produisant des molécules responsables d'odeurs désagréables, qui pouvaient être différentes en fonction des produits alimentaires et des conditions de stockage (Fall et al., 2010; Jaffrès et al., 2011; Laursen et al., 2006; Mejlholm et al., 2005; Stohr et al., 2001). Cependant, la diversité génotypique et les fonctions génétiques impliquées dans le

développement de l'altération par *B. thermosphacta* ont quant à elles été peu étudiées. Par exemple, on ignore si les souches issues de produits carnés ont des capacités altérantes sur d'autres matrices alimentaires, voire même si elles sont aptes à s'y développer. Des recherches sont donc à entreprendre afin de clarifier ces aspects et expliquer le rôle de cette bactérie dans l'altération des produits carnés et de la mer.

L'objectif de ce travail de thèse était donc d'élucider les mécanismes métaboliques et génétiques mis en place par *B. thermosphacta* lors de l'altération. Ce manuscrit de thèse s'articule autour de quatre chapitres :

Le chapitre 1 consiste en une étude bibliographique afin de situer le contexte socio-économique et scientifique dans lequel s'inscrit ce projet. Les connaissances actuelles sur le sujet y sont présentées à travers quatre parties. La première partie décrit la place des viandes et des produits de la mer (valeur nutritionnelle, production et pertes) dans un contexte économique, social et environnemental. La deuxième partie est dédiée à l'altération microbiologique des viandes et des produits de la mer, et la troisième partie présente les connaissances actuelles sur *B. thermosphacta* : son habitat, ses caractéristiques physiologiques, son potentiel altérant et les différentes stratégies visant à lutter contre l'altération qu'elle provoque. Cette troisième partie a fait objet d'une revue bibliographique publiée dans *Reference Module in Life Science*. Ce chapitre 1 se clôturera par une quatrième partie consacrée à la stratégie envisagée pour atteindre notre objectif.

Au vu de l'état de l'art, nous observons que le potentiel d'altération de *B. thermosphacta* est variable suivant les souches, les matrices alimentaires, et les communautés bactériennes qui l'entourent. Nous nous sommes donc posé la question si une corrélation peut être établie entre l'origine écologique des souches de *B. thermosphacta*, leur diversité et leur potentiel altérant. Pour cela, nous avons constitué une collection de souches provenant de plusieurs niches écologiques (environnement, produits de la mer, carnés et laitiers). La diversité intra-espèce a été évaluée en utilisant trois outils de typage : la rep-PCR (*repetitive element palindromic-PCR*), la PFGE (*Pulse-Field Gel Electrophoresis*) et le MALDI-TOF (*Matrix-Assisted Laser Desorption / Ionization Time-Of-Flight*). De plus, la diversité phénotypique a été évaluée par la quantification de la production d'acétoïne et de diacétyle, deux molécules associées à

l'altération par *B. thermosphacta*. Les résultats obtenus sont rapportés dans le **chapitre 2** qui a fait l'objet d'un article publié dans la revue scientifique *Food Microbiology*.

À l'issue de cette étude, quatre souches de *B. thermosphacta* représentatives de la diversité ont été sélectionnées. Leurs génomes ont été séquencés puis comparés pour mettre en évidence de potentiels gènes impliqués dans l'altération, ou conférant un avantage compétitif pour s'implanter ou s'adapter sur une matrice alimentaire donnée, ou aux environnements de production des produits alimentaires. Cette partie est présentée dans le **chapitre 3.** Elle correspond à un manuscrit soumis dans la revue scientifique *Standards In Genomic Sciences.*

Afin d'approfondir les connaissances sur le comportement de *B. thermosphacta* et les voies métaboliques liés à l'altération par cette bactérie, nous avons réalisé une étude transcriptomique couplée à l'analyse du volatilome après inoculation de deux souches sur deux matrices modèles (jus de crevettes et jus de viande). Cela a permis i) d'étudier l'effet de chaque souche sur l'altération et ii) de comparer l'effet des deux matrices alimentaires sur le potentiel d'altération de chaque souche. Cette partie est présentée dans le **chapitre 4** sous forme d'un manuscrit en préparation.

Enfin, une discussion générale permet de conclure et d'ouvrir les perspectives de ce mémoire de thèse.

Chapitre 1

Synthèse bibliographique

Nassima Illikoud
2018

Chapitre 1. Synthèse bibliographique

1.1. Les viandes et les produits de la mer

1.1.1. Valeur nutritionnelle

La viande et les produits de la mer constituent la première source de protéines animales pour de nombreuses personnes à travers le monde (Heinz and Hautzinger, 2007). En effet, ces produits fournissent des acides aminés essentiels, peu présents dans les produits végétaux. La viande constitue également une excellente source de fer facilement assimilable par notre organisme. Les produits de la mer quant à eux apportent du fer, et sont une bonne source d'iode. Ces produits apportent également des vitamines (Table 1.1).

Table 1.1 Composition nutritionnelle de quelques viandes et produits de la mer.

Les données (par 100 g) sont issues de Williams (2007) pour les viandes et de AQUIMER, www.nutraqua.com pour les produits de la mer.

	Bœuf[a]	Veau[a]	Agneau[a]	Mouton[a]	Crevette[b]	Saumon[c]	Dorade royale[c]	Cabillaud[c]
Eau (g)	73,1	74,8	72,9	73,2	71,2	65,6	72,7	79,7
Protéines (g)	23,2	24,8	21,9	21,5	26,6	20	20,8	17,9
Lipides (g)	2,8	1,5	4,7	4	0,6	12,9	4,8	0,4
Energie (KJ)	498	477	546	514	480	824	537	333
Cholestérol (mg)	50	51	66	66	237,1	55,9	63,5	41,5
Vitamine A (µg)	<5	<5	8,6	7,8	<2	8,5	4,5	<2
Vitamine B1 (mg)	0,04	0,06	0,12	0,16	<0,04	0,2	0,1	<0,04
Vitamine B2 (mg)	0,18	0,2	0,23	0,25	0,04	0,07	0,07	0,04
Niacine (mg)	5	16	5,2	8	2,91	7,12	6,88	2,68
Vitamine B5	0,35	1,5	0,74	1,33	0,13	1,08	0,26	0,15
Vitamine B6 (mg)	0,52	0,8	0,1	0,8	0,18	0,59	0,44	0,15
Vitamine B12 (µg)	2,5	1,6	0,96	2,8	2,46	3,74	2,93	1,22
Vitamine D (µg)	ND	ND	ND	ND	<0,5	5,69	0,87	0,88
Vitamine E (mg)	0,63	0,5	0,44	0,2	1,91	2,32	0,87	0,44
Sodium (mg)	51	51	69	71	138	35,5	52,7	77,9
Potassium (mg)	363	362	344	365	207	374	461	377
Calcium (mg)	4,5	6,5	7,2	6,6	81,1	4,7	7,5	1,6
Fer (mg)	1,8	1,1	2	3,3	0,84	0,24	0,46	0,5
Zinc (mg)	4,6	4,2	4,5	3,9	2	0,31	0,42	0,37
Magnésium (mg)	25	26	28	28	54,3	26,2	31,4	28,8
Phosphore (mg)	215	260	194	290	203	186	248	173
Cuivre (mg)	0,12	0,08	0,12	0,22	0,29	<0,1	<0,1	<0,1
Iode (µg)	ND	ND	ND	ND	41	14	7	143

a : viandes maigres ; **b** : chair cuite; **c** : filet frais; **ND** : non disponible

1.1.2. Production et pertes

La production mondiale de la viande et des produits de la mer ne cesse d'augmenter. Celle des viandes, par exemple, est passée de 70,91 millions de tonnes en 1961 à 315,14 millions de tonnes en 2013 (Figure 1.1) (Ritchie and Roser, 2018). Selon les perspectives agricoles de l'OCDE et de la FAO pour la période 2017-2026, celle-ci devrait augmenter de 13 % en 2026 par rapport à 2017 (OCDE/FAO, 2017). Cette tendance est également observée dans le cas des produits de la mer. En effet, leur production est passée de 40 millions de tonnes en 1961 à 160 millions de tonnes en 2013 (Ritchie and Roser, 2018)

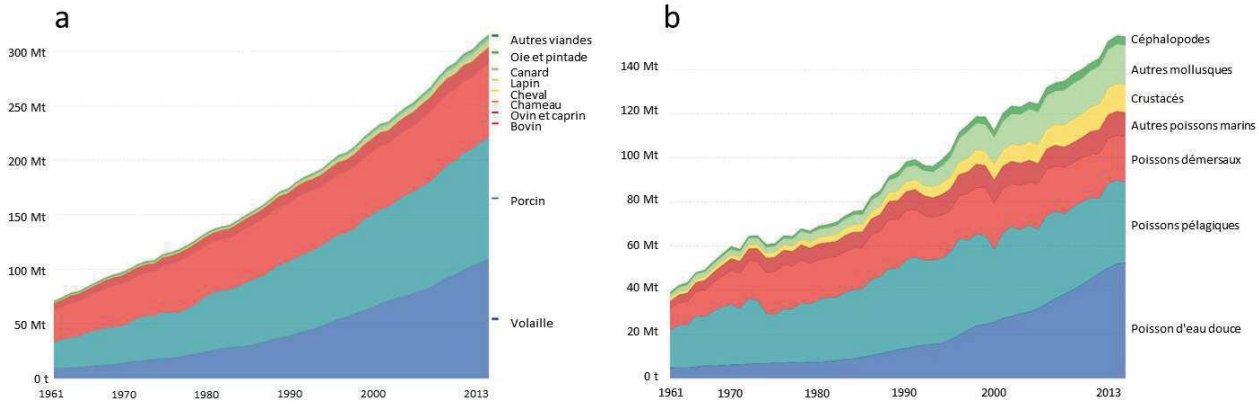

Figure 1.1 Evolution des niveaux de production de la viande et des produits de la mer (1961 – 2013).

La production des viandes **(a)** et des produits de la mer **(b)** est mesurée en millions de tonnes (Mt) par an. Données issues de OurWorldinData (Ritchie and Roser, 2018).

Cependant, parallèlement à l'augmentation de la production, des quantités importantes de produits carnés et de la mer sont perdues engendrant des pertes économiques considérables. En effet, près d'un tiers de toute la production alimentaire mondiale est perdu chaque année, représentant 1.3 milliards de tonnes de pertes, pour partie en raison de leur altération, et ce depuis la production primaire jusqu'à la consommation finale dans les ménages (Gustavsson et al., 2011). Ces pertes ont été estimées pour chacun des segments de la chaine de production de différentes filières. La Figure 1.2 résume en quelques chiffres la part attribuable à la production, la transformation, la distribution et la consommation pour les viandes de porc et de bœuf, les volailles et les produits de mer (INCOME consulting - AK2C, 2016). Outre leurs conséquences économiques non négligeables, ces pertes ont un impact sur la sécurité alimentaire et sur l'environnement. En effet, la production de ces aliments finalement perdus

compte pour 24% des ressources totales en eau douce utilisées dans la production alimentaire, 23% de la superficie totale des terres et 23% de l'utilisation totale des engrais au niveau mondial, sans oublier les émissions polluantes qui en résultent avec la production de gaz à effet de serre (Büchner, 2012; Kummu et al., 2012).

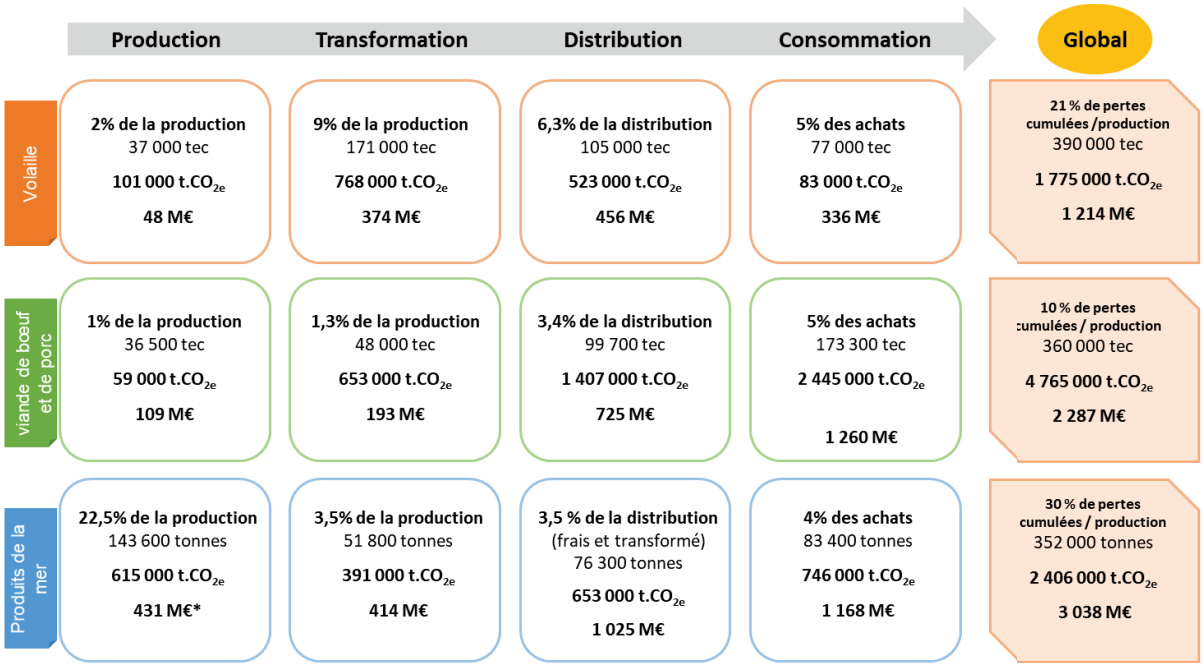

Figure 1.2 Estimation des pertes et gaspillages pour les filières volaille, porc et bœuf et pour produits de la mer au long de la chaine de production.

L'estimation de la quantité de produits écartés de l'alimentation humaine en tonnes (t) ou tonnes équivalent carcasse (tec) perdues au niveau de la production, la transformation, la distribution et la consommation est présentée. L'impact carbone en tonnes équivalent (CO_{2e}) ainsi que la valeur économique théorique sont indiqués. Modifiée de INCOME consulting - AK2C (2016).

De ce fait, de nombreuses initiatives ont été lancées au cours des dernières années au niveau national et européen pour lutter contre les pertes alimentaires notamment à travers la compréhension et la maîtrise des phénomènes d'altération (Garot, 2015). L'un des principaux objectifs est d'encourager les innovations technologiques susceptibles de contribuer à la réduction de ces pertes. L'altération des aliments peut être définie comme toute modification rendant un produit indésirable ou inacceptable pour la consommation humaine d'un point de vue organoleptique (Gram and Dalgaard, 2002). Elle peut être causée par des facteurs physico-chimiques (dommages physiques, oxydation) et l'hydrolyse enzymatique. Cependant, la croissance de microorganismes et leur métabolisme constituent

la principale cause des altérations. Ainsi, une bonne connaissance des caractéristiques des produits carnés et de la mer et de leur écologie microbienne est donc une priorité pour les exploitants du secteur alimentaire, afin de garantir leur stabilité et d'éviter des modifications indésirables pendant la période de stockage. Par conséquent, l'amélioration des connaissances sur les phénomènes d'altération, et le développement de méthodes visant à augmenter la sécurité et la qualité globale des denrées ainsi que leur durée de conservation, représentent un intérêt majeur pour la recherche dans l'industrie des viandes et des produits de la mer.

1.2. Altération microbiologique des viandes et produits de la mer réfrigérés

L'altération microbiologique, rendant un produit impropre à la consommation, peut affecter le goût, l'odeur, la couleur et l'aspect ainsi que la texture (Ellin Doyle, 2011; Gram et al., 2002). Elle résulte du développement de certains microorganismes et de leur métabolisme entraînant la formation d'amines, de sulfures, d'alcools, d'aldéhydes, de cétones ou d'acides organiques responsables de flaveurs désagréables et indésirables. Des modifications de couleur et de texture peuvent également être induites telles que la formation de limon ou poissage, la production de pigments et la formation de colonies (Casaburi et al., 2015b; Wang et al., 2017a). Le terme indésirable est spécifique à un produit alimentaire lorsqu'il est appliqué à l'altération des aliments. À titre d'exemple, l'odeur beurre/caramel associée au diacétyle fait partie des odeurs recherchées dans certains produits comme la crème ou le beurre alors qu'elle est indésirable dans les produits de la mer frais ou légèrement préservés comme le saumon fumé.

1.2.1. Les bactéries responsables d'altération

La qualité microbiologique de la viande et des produits de la mer dépend grandement de l'état de l'animal au moment de l'abattage, des conditions d'abattage et de transformation, de la température, mais également d'autres conditions de stockage et de distribution (Nychas et al., 2008a). De plus, l'industrie alimentaire crée constamment de nouveaux habitats pour les microorganismes par le développement de nouveaux produits et la reformulation de produits traditionnels. En effet, toute manipulation depuis l'abattage jusqu'à l'assiette du consommateur peut influencer la composition des communautés bactériennes d'un produit alimentaire. Ces conditions environnementales que subit l'aliment créent donc une niche

écologique spécifique qui favorise certaines espèces bactériennes initialement présentes ou introduites par contamination croisée, alors que d'autres espèces sont défavorisées (Nychas et al., 2008a). Seule une fraction des contaminants initiaux seront capables de tolérer les conditions spécifiques au produit (composition nutritionnelle, pH, activité de l'eau, température, conditionnement) et de proliférer durant le stockage. Cette fraction de bactéries constitue donc la « flore associée à l'altération » du produit. Ce terme décrit les bactéries présentes dans le produit au moment de l'altération. Cependant, ces bactéries ne sont pas toutes responsables de l'altération. Seules celles qui produisent les métabolites responsables d'odeurs et flaveurs associées à l'altération du produit sont considérées comme des bactéries altérantes, et sont donc qualifiées de bactéries spécifiques de l'altération, appelées en anglais « *Specific Spoilage Organisms* ou *SSO* » (Gram and Dalgaard, 2002; Nychas and Skandamis, 2005). Bien que les genres bactériens rapportés dans la littérature pour être dominants dans cette fraction soient similaires, les espèces varient suivant les études et les produits (Nychas et al., 2007). Parmi les principaux genres et espèces bactériens capables d'altérer la viande et/ou les produits de la mer, on retrouve *Flavobacterium, Pseudomonas*, *Acinetobacter*, *Moraxella*, *Psychrobacter, Photobacterium phosphoreum*, différents genres de la famille des *Enterobacteriaceae* comme *Hafnia, Serratia, Morganella,* des Gram positifs comme *Staphylococcus*, *Micrococcus*, *Clostridium*, des bactéries lactiques comme *Lactococccus piscium*, *Leuconostoc gasicomitatum*, *Carnobacterium maltaromaticum et Carnobacterium divergens*, mais également d'autres bactéries comme *B. thermosphacta* (Dainty and Mackey, 1992; Doulgeraki et al., 2012; García-Lopez et al., 1998; Gill and Newton, 1977; Labadie, 1999). Dans les produits de la mer frais ou légèrement préservés, les bactéries spécifiques de l'altération sont initialement présentes en faible nombre (Figure 1.3). Cependant, elles prolifèrent plus rapidement que les autres pendant le stockage sous des conditions particulières de basses températures et d'atmosphères et produisent des métabolites comme de la triméthylamine (TMA), des acides organiques et des composés volatils entrainant le rejet sensoriel du produit (Dalgaard, 2000). À ce stade, la concentration des bactéries spécifiques de l'altération est d'environ 6-7 Log (UFC/g)(Gram and Dalgaard, 2002). Le nombre de bactéries spécifiques de l'altération et la concentration de leurs métabolites peuvent être utilisés comme des indicateurs de l'altération afin de prédire la durée de vie d'un produit.

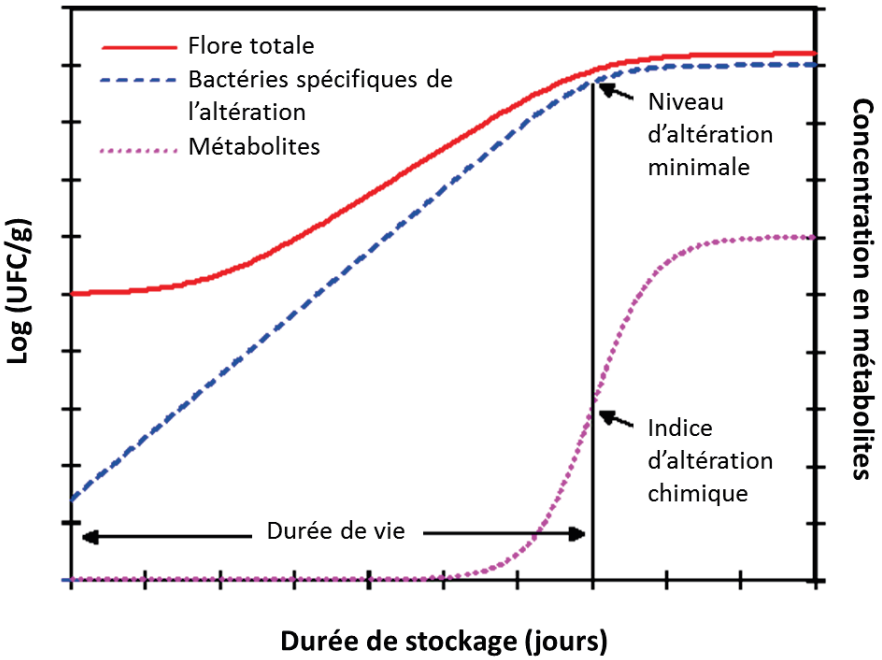

Figure 1.3 Évolution de la flore totale, des bactéries spécifiques de l'altération et des métabolites produits au cours du stockage des produits de la mer à basse température.
La durée de vie correspondant au niveau d'altération minimale et l'indice d'altération chimique est indiqué. Modifiée de Dalgaard (2000).

1.2.2. Étude du potentiel d'altération des bactéries spécifiques d'altération

La mise en évidence des microorganismes responsables de la production de molécules chimiques altérantes peut s'avérer fastidieuse, d'autant plus que des interactions entre les microorganismes peuvent influer la production de certaines molécules altérantes (Corry, 2007; Nychas et al., 1998). L'identification des bactéries impliquées dans l'altération nécessite des études sensorielles, microbiologiques et chimiques approfondies (Dalgaard, 1995). Elle s'appuie sur la comparaison des caractéristiques d'altération d'un produit naturellement contaminé avec celles provoquées par l'inoculation d'un ou plusieurs isolat(s) issu(s) de la flore d'altération naturelle, sur le même type de produit faiblement contaminé. La Figure 1.4 récapitule les différentes étapes pour l'identification des bactéries altérantes d'un produit.

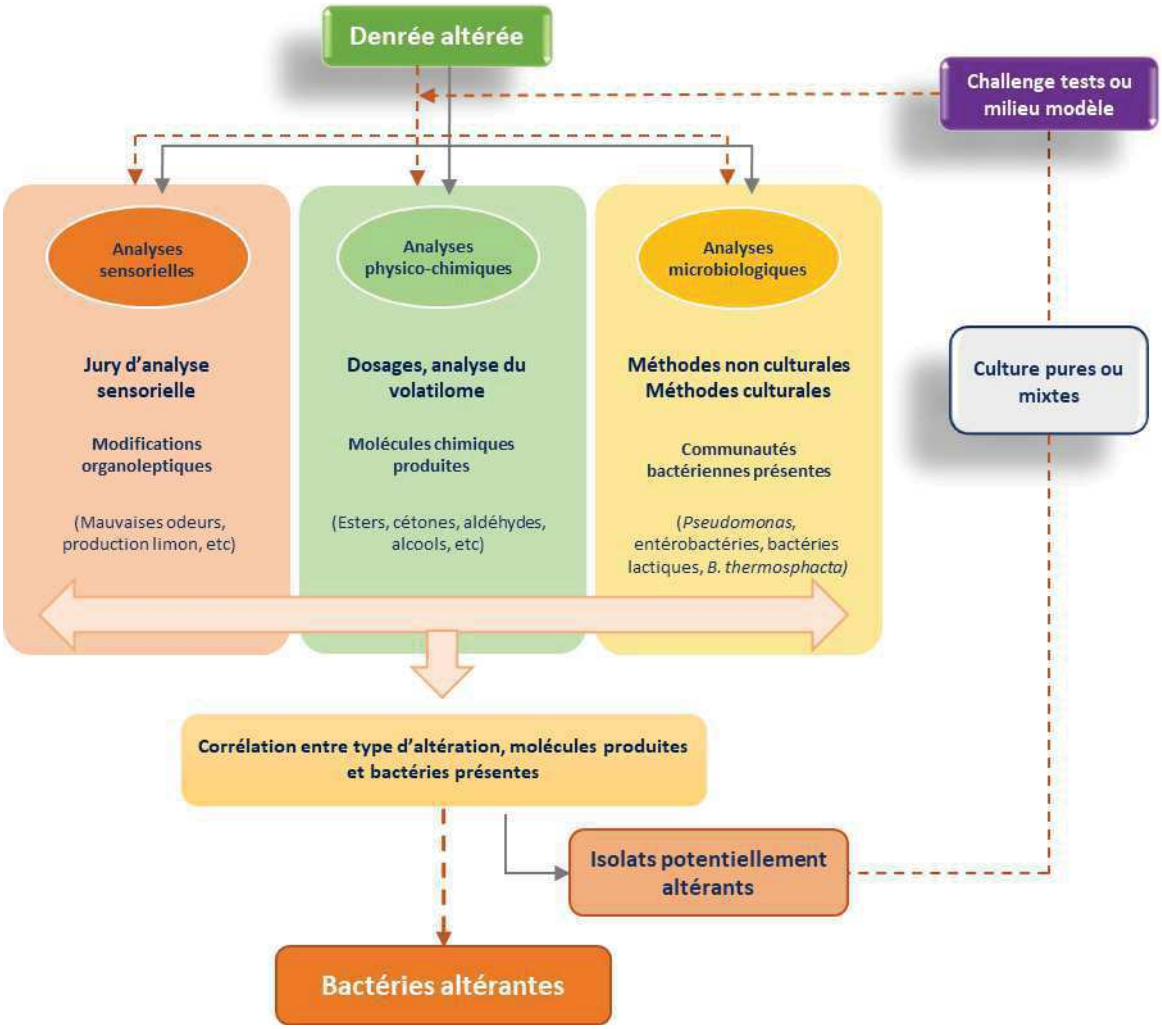

Figure 1.4 Représentation schématique des différentes étapes pour l'identification des bactéries responsables de l'altération.

Au préalable, les modifications sensorielles, microbiologiques et chimiques doivent être étudiées, caractérisées et quantifiées sur un produit altéré naturellement. Cela permet dans certains cas de déterminer le niveau d'un ou plusieurs composé(s) chimique(s) donné(s) qui est corrélé avec l'altération (indicateur(s) de l'altération chimique). Ensuite, le potentiel d'altération des souches bactériennes isolées au point de rejet sensoriel et identifiées au niveau du genre ou de l'espèce doit être évalué. Ceci peut être réalisé à l'aide de milieux modèles stériles ou sur des produits alimentaires faiblement contaminés, lors de challenge-tests après inoculation de cultures pures (un seul isolat) ou mixtes (cocktail de plusieurs isolats). Le potentiel d'altération est ainsi évalué par la capacité de cet(s) isolat(s) à reproduire des modifications sensorielles ou chimiques retrouvées à l'origine dans le produit altéré naturellement. Enfin, les souches sélectionnées doivent être testées pour déterminer leur

activité d'altération, à savoir si leur taux de croissance et leur production de facteurs altérants sont similaires aux mesures obtenues dans le produit naturellement altéré (Dalgaard, 2006; Dalgaard et al., 1993). Cette dernière étape est particulièrement importante car certaines bactéries peuvent produire les composés chimiques associés à l'altération, mais sont incapables de le faire en quantités significatives dans l'aliment. Elles ne peuvent donc pas être considérées comme des bactéries spécifiques de l'altération.

L'étude du potentiel altérant et de l'activité d'altération peut être réalisée sur deux types de substrats, à savoir une matrice alimentaire stérile ou faiblement contaminée (Ercolini et al., 2011; Ercolini et al., 2009; Jaffrès et al., 2011; Joffraud et al., 2001; Stohr et al., 2001),ou encore sur des milieux modèles stériles se rapprochant le plus possible du produit alimentaire, comme par exemple des jus de viande ou de poisson stérilisés (Fall et al., 2010; Wang et al., 2017b). L'utilisation d'une matrice alimentaire stérile offre l'avantage de se rapprocher le plus du produit mais reste difficile à mettre en œuvre (Gram and Huss, 1996).

1.2.3. Influence du conditionnement sur l'altération

Différents procédés de conservation peuvent être utilisés pour inhiber la croissance des bactéries d'altération et prolonger la durée de vie microbiologique des viandes et des produits de la mer. Celles-ci incluent le séchage, le fumage, le salage, la réfrigération et l'ajout de conservateurs (Heinz, 2007). Afin de répondre à la demande des consommateurs qui recherchent des produits frais ou peu transformés avec des quantités minimales de conservateurs, l'utilisation d'atmosphères protectrices combinées aux basses températures est devenue une pratique courante pour prolonger la durée de conservation, en inhibant la croissance des bactéries pathogènes et d'altération des produits alimentaires (Jacxsens et al., 2001; McMillin, 2008).

La température est l'un des facteurs les plus importants influençant l'altération des viandes et des produits de la mer (Lambert et al., 1991; McDonald and Sun, 1999). En effet, la conservation à froid affecte la vitesse de croissance et la survie des bactéries d'altération. Le conditionnement à basse température pourrait avoir un effet qualitatif sur les caractéristiques de l'altération en exerçant une pression de sélection empêchant la croissance des bactéries mésophiles et favorisant celle des psychrotrophes dont fait partie la majorité des espèces

bactériennes d'altération comme par exemple *B. thermosphacta* ou encore *Pseudomonas fluorescens* (Gill and Newton, 1977).

Conditionnement sous air

L'altération des viandes et des produits de la mer frais, conditionnés sous air et à basse température, est principalement causée par les bactéries psychrotrophes à Gram négatif incluant les espèces appartenant aux genres *Pseudomonas* et *Shewanella*, comme *Pseudomonas fragi*, *P. fluorescens*, *Pseudomonas putida* et *Shewanella putrefaciens* (Andreani and Fasolato, 2017; Casaburi et al., 2015b; Gram and Huss, 1996). En effet, celles-ci ont été identifiées comme dominantes dans les communautés bactériennes au moment de l'altération de divers produits carnés comme la viande de bœuf et des produits de la mer tels que la dorade royale (Labadie, 1999; Parlapani et al., 2013; Pennacchia et al., 2011; Stanbridge and Davis, 1998). L'altération par des *Pseudomonas* se manifeste par la production de limon et de mauvaises odeurs de « fruité et putréfié » (Andreani et al., 2015; Nychas et al., 2008a). Ces bactéries produisent diverses molécules volatiles comme des aldéhydes, des cétones, des esters et des composés soufrés mais ne produisent pas de sulfure d'hydrogène (H_2S) et sont incapables de réduire l'oxyde de triméthylamine (OTMA) en triméthylamine (TMA) (Dainty et al., 1984; Mikš-Krajnik et al., 2016; Miller et al., 1973). A l'inverse, *S. putrefaciens* est capable de réduire le TMAO en TMA, une molécule associée aux odeurs ammoniacales ce qui explique son importance dans l'altération du poisson stocké à basse température. Cette bactérie produit également des sulfures volatils notamment du H_2S (Gram and Huss, 1996; Herbert et al., 1971). Des bactéries identifiées comme *S. putrefaciens* ont également été associées à l'altération de la viande de bœuf et des carcasses de poulets de chair (Dainty et al., 1989; Russell et al., 1995).

D'autres bactéries comme *B. thermosphacta* et des *Enterobacteriaceae* psychrotolérantes, telles que *Hafnia alvei*, *Serratia liquefaciens* et *Enterobacter agglomerans* peuvent également se développer sur des produits réfrigérés stockés en aérobiose (Borch et al., 1996; Nychas et al., 1998). Les bactéries lactiques peuvent également être détectées dans les produits altérés stockés sous air et à basse température (Doulgeraki et al., 2010; Parlapani et al., 2013).Néanmoins, celles-ci ne sont pas considérées comme importantes pour l'altération dans ces conditions de stockage.

En conditions d'aérobiose, l'altération peut survenir suivant les produits au bout d'environ 10 jours à 0 °C ou 5 jours à 5 °C (Hood and Mead, 1993). Elle se manifeste par l'apparition d'odeurs indésirables comme les odeurs de putréfaction, de fromage, de sulfures, et des odeurs douces et fruitées associées à la production de composés soufrés, ou par un verdissement de la viande suite à la production de sulfure d'hydrogène (Borch et al., 1996; Nychas et al., 1998)

Conditionnement sous vide ou sous atmosphère protectrice

Des combinaisons de gaz avec différentes proportions de dioxygène, de dioxyde de carbone et d'azote sont utilisées pour la conservation de viandes et de produits de la mer. Le terme « atmosphère protectrice » est utilisé pour ce type de conditionnement. La durée de conservation sous ces atmosphères protectrices est déterminée par la nature du mélange gazeux, la température de stockage et le type de produit.

L'emballage sous vide et sous atmosphère protectrice des viandes et des produits de la mer modifie leur microbiote et par conséquent leur évolution dans le temps et le type d'altération. En effet, de fortes concentrations en dioxyde de carbone, en général plus de 20 %, permettent de limiter la croissance des bactéries aérobies à Gram négatif, en particulier les *Pseudomonas* (Gram and Huss, 1996; Mastromatteo et al., 2010). Cependant, certaines bactéries comme *Photobacterium phosphoreum*, bactérie résistante au CO_2, ont été retrouvées comme faisant partie de la flore d'altération dans des produits de la mer conditionnés sous vide et sous-atmosphère protectrice, notamment le saumon fumé, le cabillaud, et d'autres poissons comme le flétan (Dalgaard et al., 1993; Hovda et al., 2007; Leroi et al., 1998). Sur ce type de produits *P. phosphoreum* produit des odeurs aigres et aminées liées à la production de TMA (Dalgaard et al., 1997).

Des bactéries à Gram positif, en particulier les bactéries lactiques appartenant aux genres *Lactobacillus*, *Leuconostoc*, *Lactococcus* et *Carnobacterium*, mais également d'autres bactéries comme *B. thermosphacta* se développent particulièrement bien sur les produits conditionnés sous atmosphères enrichies en dioxyde de carbone. Elles sont les principales causes d'altération dans ces conditions (Borch et al., 1996; Nieminen et al., 2011; Pothakos et al., 2015). C'est le cas par exemple de *Lactobacillus curvatus* qui est considérée comme l'une des bactéries spécifiques de l'altération du saumon fumé (Jørgensen et al., 2000), de

L. gasicomitatum responsable de la production de gaz, de limon, d'odeurs de beurre et de verdissement de la viande (Johansson et al., 2011), ou encore de *L. piscium* qui est impliquée dans l'altération de la hachée de bœuf, de porc et du saumon frais conditionné sous atmosphère protectrice. Cette dernière était à l'origine d'odeurs aigres et de beurre (Macé et al., 2012; Macé et al., 2013; Rahkila et al., 2012). L'altération des produits stockés sous atmosphère protectrice est retardée par rapport à un stockage sous air. Elle est associée aux activités microbiennes qui modifient les propriétés organoleptiques et la texture du produit alimentaire. Ces activités comprennent la production de gaz, de limon et d'amines biogènes, la décoloration, la formation de mauvaises odeurs et l'acidification (Casaburi et al., 2015b; Pothakos et al., 2015; Remenant et al., 2015).

Parmi toutes ces bactéries responsables de l'altération des aliments, l'espèce bactérienne *B. thermosphacta* est particulièrement intéressante. En effet, cette espèce, anaérobie facultative, psychrotrophe et ubiquitaire, a été mise en évidence dans de très nombreuses matrices alimentaires (produits carnés, produits de la mer, etc.), et elle est systématiquement associée au processus d'altération. Elle a été décrite comme l'espèce emblématique systématiquement présente dans les produits carnés et les produits de la mer frais et altérés (Chaillou et al., 2015). Une revue bibliographique a été réalisée pour établir un état de l'art des connaissances actuelles sur cette espèce. Ce travail a donné lieu à la rédaction d'un chapitre publié dans la revue *Reference Module in Life Sciences – Module Microbiology* et présenté ci-après.

1.3. *Brochothrix thermosphacta:* revue bibliographique

Brochothrix thermosphacta

Nassima Illikoud, Emmanuel Jaffrès, and Monique Zagorec, SECALIM, INRA, Oniris, University Brittany Loire, Nantes, France

12106. Microbiology/ *Brochothrix thermosphacta*

Nassima ILLIKOUD, Emmanuel JAFFRÈS, Monique ZAGOREC

Corresponding Author: Monique Zagore, UMR1014 SECALIM, INRA, Oniris, Route de Gachet 44307, Nantes, France

Abstract

Brochothrix thermosphacta is a Gram positive non-spore forming bacterium, closely related to *Listeria*. This nonpathogenic species is often isolated from meat and seafood products where it can cause spoilage by the production of off-odors. Nevertheless, the metabolic pathways leading to spoilage are not well understood, although the genome of several strains has been sequenced. The knowledge available about this species is presented.

Keywords

Bacteria; Bacteriocin; Biopreservation; Genomics; Meat; Seafood; Spoilage; Taxonomy; Volatile organic compounds;

Abbreviations

BA: Biogenic Amines

CDS: Coding Sequence

CFU: Colony Forming Unit

MAP: Modified Atmosphere Packaging

STAA: Streptomycin-Thallous Acetate-Actidione

VOC: Volatile Organic Compound

VP: Vacuum Packaging

Glossary

Bacteriocin: a peptide produced by bacteria, with an antimicrobial activity toward bacterial species closely-related or non-related to the producer bacterium.

Biopreservation: the most commonly used definition has been proposed by Michael E. Stiles in 1996 as “Biopreservation refers to extended storage life and enhanced safety of foods using the natural microflora and (or) their antibacterial products”(Stiles, 1996).

Coding sequence: portion of a gene that is transcribed into a messenger RNA and translated into a protein.

Contig: a set of overlapping DNA sequences (reads) that together represent a consensus region of DNA.

Genome: the collection of an organism's entire genetic information, organized in genes. In bacteria the genome is most of the time constituted by a single chromosome and sometimes plasmids, the number of which varies depending on strains.

Genomics: science that studies all genes in an organism as well as the function of genes, their regulation mode, and their interactions (functional genomics or post-genomics).

Microbial spoilage: defect of the organoleptic properties of a food product including aspect, texture, color, odor, and flavor resulting from microbial growth and metabolism with the production of molecules (volatile compounds, pigments, gas, exopolysaccharides…).

Microbiota: all microorganisms that typically inhabit a particular environment (here the food environment) considered as a group.

Operon: a functional unit of DNA containing a cluster of genes controlled by a single promoter. The genes are transcribed together into a messenger RNA.

Peptidoglycan: also called murein, is an essential and specific component of the bacterial cell wall preserving cell integrity and located on the outside of the cytoplasmic membrane of bacteria.

Psychrotrophic: ability of microorganisms to grow at 0 °C. Their maximum growth temperature is above 20 °C. They are widespread in natural environments and in foods.

Scaffold: a set of non-contiguous series of oriented genomic sequences or contigs separated by gaps of known length but of unknown sequence.

Taxonomy: classification and description of living organisms. It includes naming and defining of species, and collecting data about their biology (phenotypic, genotypic characteristics), biogeography, and ecology).

1.3.1. Introduction

The genus *Brochothrix* belongs to the *Listeriaceae* family which encompasses only two genera: *Listeria* and *Brochothrix*. To date, in the *Brochothrix* genus only two closely related species have been described (Stackebrandt and Jones, 2006). *Brochothrix thermosphacta* has been

first isolated from pork sausages (McLean and Sulzbacher, 1953) and *Brochothrix campestris* from grass and soil (Talon et al., 1988). Although, these two species are non-pathogenic, *B. thermosphacta* gained a scientific interest because of its implication in the spoilage of meat and seafood products. Indeed, *B. thermosphacta* is a psychrotrophic and ubiquitous bacterium that has been reported as present in various meat and seafood products stored at cold temperatures, and as responsible for the production of undesired off-odors such as cheesy or buttery odors (Casaburi et al., 2015b; Ercolini et al., 2006; Rattanasomboon et al., 1999; Russo et al., 2006). In the case of meat, *B. thermosphacta* was reported as the dominant spoiler of products stored under vacuum packaging (VP) or modified atmosphere packaging (MAP) (Gill, 1983; Gill and Newton, 1977).

Food spoilage consists in any change of a food product leading to its rejection based on sensory characteristics (Gram et al., 2002). Food spoilage can result from physical damages or chemical and enzymatic reactions, but microbial growth and the resulting microbial metabolic activities are the major causes of food spoilage. Although spoiled foods may be not dangerous for human consumption, they are rejected by the consumer or earlier in the food production chain because they do not fit with quality standards. Therefore, spoilage has important economic and ecological consequences. Indeed, one third of food produced for the human consumption is lost or wasted every year (Gustavsson et al., 2011) and part of losses are the consequence of microbial spoilage. All types of food can be concerned by microbial spoilage, but meat and seafood products are recognized as the most perishable ones due to their near neutral pH, water content, and abundance of nutrients essential for the growth of various microorganisms (Gram et al., 2002). Several studies showed that bacteria are the dominant spoilage microorganisms of meat and seafood (Gram et al., 2002). Microbial spoilage may appear as a visible bacterial growth, as changes in the texture, aspect, or as off-odor and off-flavor development (Gram et al., 2002). It is a complex process to which many bacteria can contribute. The food microbiota and its dynamics during the shelf life is affected by the nature of the food matrix (pH, water activity, and chemical composition), the stabilization process (cooking, smoking, drying, salting…), the hygienic conditions during the processing, and the packaging and storage conditions from the distribution of the food product until its consumption (Borch et al., 1996; Samelis et al., 1998; Samelis et al., 2000a). Although many preservation methods have been developed to enhance the storage stability and maintain

quality of foods for extended periods, excessive amounts of foods are lost every year due to microbial spoilage (Gustavsson et al., 2011).

Thus, understanding the behavior of *B. thermosphacta* during food production and storage and the mechanisms responsible for its spoilage activity should contribute to find solutions for avoiding its presence or development in perishable meat and seafood products. This review focuses on the characteristics of this species, its role in food spoilage, and some strategies for fighting it in the food industry.

1.3.2. Taxonomy history

B. thermosphacta first isolated from pork sausage (Sulzbacher and McLean, 1951) was initially named *Microbacterium thermosphactum* (McLean and Sulzbacher, 1953). However, the same authors already mentioned notable discrepancies in the cell morphology between *M. thermosphactum* and the type species of the genus *Microbacterium lacumin*. Later studies confirmed the differences in the morphology and noted other dissimilarities especially in enzymology and protein profiles, in the DNA base composition and in peptidoglycan structure (Stackebrandt and Jones, 2006). Moreover, *M. thermosphactum* isolates formed a distinct cluster sufficiently distant from other species of the genus *Microbacterium* and from the closest Gram-positive genera (Stackebrandt and Jones, 2006). Consequently, this species was reclassified to a new genus *Brochothrix* and renamed as *B. thermosphacta* and first placed in the *Lactobacillaceae* family (Sneath and Jones, 1976). Thereafter, bacteria of the genus *Brochothrix* were found to be phenotypically closer to those of the genus *Listeria* than to those of *Lactobacillus* and were therefore moved to the *Listeriaceae* family (Sneath and Jones, 1986). The phylogenetic relationship of *B. thermosphacta* with *Listeria monocytogenes* was later reinforced by the results of a study analyzing the 16S rRNA sequence of the two species (Ludwig et al., 1984). In 1988, the isolation and description of a new species belonging to *Brochothrix*, *B. campestris* (Talon et al., 1988) confirmed the genus and its classification in the *Listeriaceae* family. To date it encompasses two species: *B*. *thermosphacta* (type strain ATCC 11509/DSM 20171) and *B. campestris* (type strain ATCC 43754/DSM 4712), which are highly related as their 16S rDNA sequences share 99.3% identity (Stackebrandt and Jones, 2006). Recently, the *rpoB* gene sequence, encoding the beta subunit of the RNA polymerase, appeared as a powerful tool for bacterial identification and phylogenetic analyses, especially

for studying closely related isolates. Alignments of *rpoB* gene sequences have been reported to enable resolution at the species and subspecies levels (Adékambi et al., 2009). Additionally, it has been reported that *rpoB* gene sequencing provided higher discriminating power than 16S rRNA gene sequencing (Adékambi et al., 2009). We have drawn a phylogenetic tree based on the alignment of *rpoB* gene sequences from 12 *B. thermosphacta* genomes and from the unique *B. campestris* genome described to date. *Listeria* species were used as an outgroup (Figure 1.5). The resulting tree confirms that *B. campestris* is located on a separate branch.

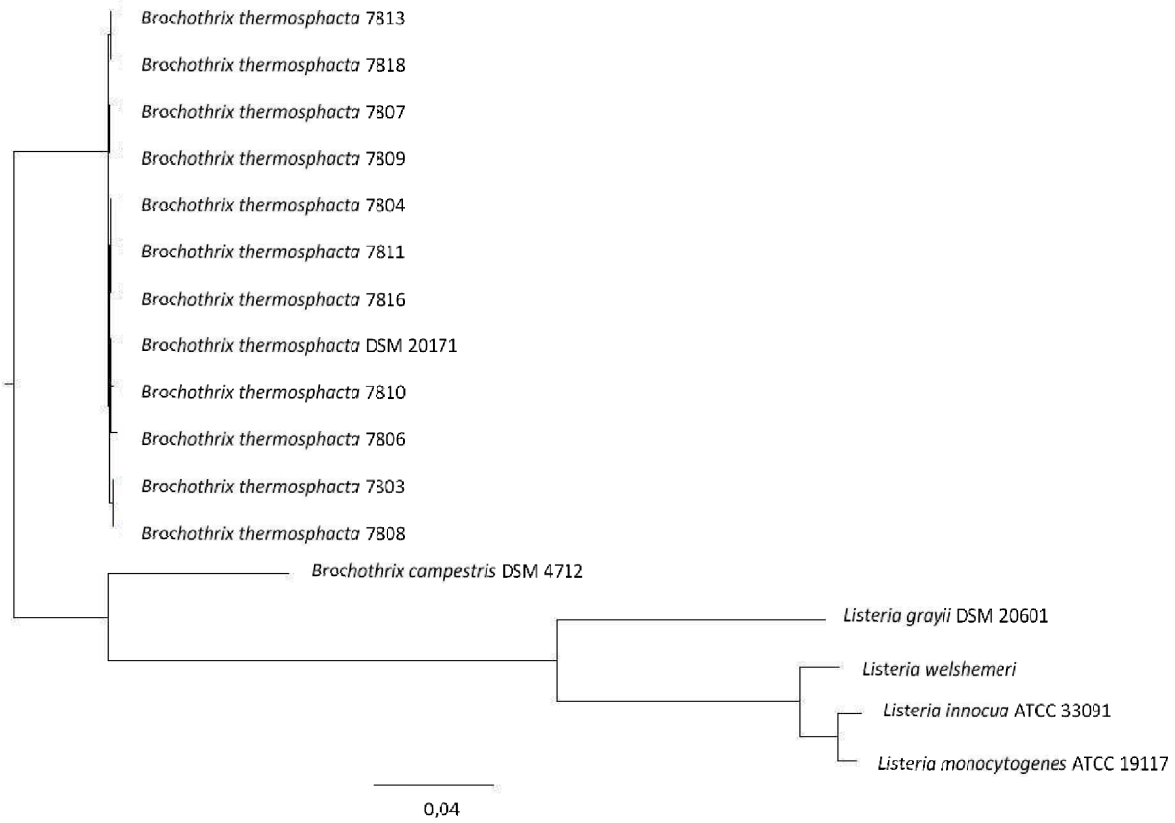

Figure 1.5 Phylogenetic tree of the *rpoB* gene from *B. thermosphacta* and *B. campestris*.

The *rpoB* gene sequences were retrieved from GenBank (http:// www.ncbi.nlm.nih.gov); MAFFT alignment was performed using Geneious software R9; The phylogenetic tree was drawn with FastTree 2.1.5 then visualized with FigTree (v1.4.3); *rpoB* gene sequences from different species of the genus *Listeria* were used as outgroup species.

1.3.3. Characteristics and ecology of *B. thermosphacta*

1.3.3.1. Habitat

The natural habitat of *B. thermosphacta* is not fully known. Indeed, initially isolated from pork sausage and considered as a psychrotrophic bacterium responsible for the spoilage of meat products, it did not attract the attention of microbiologists from other fields. In addition, its

successive reclassifications mentioned above may have contributed to misidentification of some isolates. Nevertheless other possible sources of *B. thermosphacta* have been reported such as soil and feces (Gardner, 1966). More recently, it has been also isolated from a wide variety of food of animal origin *i.e.* meat from mammals and birds, seafood, milk and dairy products (Stackebrandt and Jones, 2006). Moreover, *B. thermosphacta* was described omnipresent along the production chain of beef meat, from the animal to the final product. It was isolated from beef carcasses, animal skin and rumen, walls, equipment of slaughterhouses, processing plants, hands of manipulators, and ambient air of chilling rooms (Nychas et al., 2008b). It is therefore possible that this species lives in a wide range of habitats but has been found only in some environments where it was systematically searched, or in which it can be selected as growth conditions are optimal, such as cold temperatures and nutritious meat matrixes.

B. thermosphacta has been isolated from food, even in the absence of detectable spoilage (Liang et al., 2012; Nychas et al., 2007). Because of its ubiquitous and psychrotolerant nature, *B. thermosphacta* is frequently found as a dominant spoiler bacterium of a wide range of foodstuffs stored at low temperature. It has been identified as the emblematic bacterial species among the microbiota of 8 spoiled meat and seafood products stored at low temperature (Chaillou et al., 2015). Indeed, it has been associated to beef, veal, pork, and poultry spoiled meat (Bohaychuk and Greer, 2003; Delhalle et al., 2016; Dias et al., 2013; Ercolini et al., 2011; Liang et al., 2012; Nieminen et al., 2012; Piotrowska-Cyplik et al., 2017; Rattanasomboon et al., 1999; Sakala et al., 2002; Samelis et al., 2000b; Vasilopoulos et al., 2008) and seafood products (Drosinos et al., 1997; Drosinos and Nychas, 1996; Jaffrès et al., 2011; Mejlholm et al., 2005; Noseda et al., 2012; Parlapani et al., 2014). In most of these reported cases, *B. thermosphacta* numbers in the spoiled food had reached levels above 7 log CFU g^{-1}.

B. thermosphacta commonly belongs to aerobic microbiota of chilled meats and is a specific spoiler of meat stored under MAP and VP (Dainty and Mackey, 1992; Ercolini et al., 2011; Nychas et al., 2007; Nychas et al., 2008b; Piotrowska-Cyplik et al., 2017; Remenant et al., 2015; Vasilopoulos et al., 2008).

1.3.3.2. General phenotypic description of the species

Strains belonging to the genus *Brochothrix* are Gram positive, non-spore forming, non-motile regular unbranched rods (0.6 - 0.8 µm diameter and 1-2 µm long) that occur singly or in pairs. Unlike *B. campestris*, *B. thermosphacta* can arise in short chains or in long filamentous chains. In old cultures, the cell morphology of this bacterium changes from rod to coccoid but cells resume the rod form when subcultured in a suitable culture medium (Stackebrandt and Jones, 2006). *Brochothrix* sp. are aerobe and facultative anaerobe, catalase positive, and oxidase negative. These bacteria can grow at temperatures ranging from 0 to 30°C with an optimum growth between 20 and 25°C (Stackebrandt and Jones, 2006). The optimum pH for growth is pH 7.0, but *B. thermosphacta* is able to grow within the pH range from 5 to 9.

Glucose is the preferred carbon source used by *B. thermosphacta* in meat (Gill and Newton, 1977). In the IMViC tests (indole, methyl-red, Voges-Proskauer, and citrate) used for differentiating bacterial isolates, the species is considered as methyl-red and Voges-Proskauer positive meaning ability to mixed acid fermentation and production of 3-hydroxy-2-butanone (acetoin) and 2,3-butanediol. Its metabolism is influenced by dioxygen and carbon dioxide contents in the atmosphere. Under aerobic conditions, 3-hydroxy-2-butanone (acetoin) and 2,3-butanedione (diacetyl) are the major metabolites produced from the consumption of glucose, and also free fatty acids as isobutyric and isovaleric acids which result from valine and leucine consumption at limiting concentrations of glucose (Dainty and Hibbard, 1980). In the absence of O_2, *B. thermosphacta* produces mainly L-(+)-lactic acid and ethanol (Dainty et al., 1985; Pin et al., 2002). When glucose is depleted, glycerol appears to be an alternative carbon source for growth (Macaskie et al., 1984). Nitrate is not reduced to nitrite by *B. thermosphacta* and this species does not appear to have a nitrite reductase system (Collins-Thompson and Rodriguez-Lopez, 1980; Holley, 2014). Indole and H_2S are not produced by *B. thermosphacta* (Stackebrandt and Jones, 2006).

Information about lipolytic and proteolytic activities of *B. thermosphacta* is scarce and remains ambiguous. Several studies reported that the optimal temperature for production of lipases by this species is 20°C (Braun and Sutherland, 2004; Nowak and Piotrowska, 2012; Papon and Talon, 1988). Nevertheless Nowak et al. (2012b) found that most *B. thermosphacta* strains showed a lipolytic activity at 25°C and only some at 4°C, while Casaburi et al. (2014)

did not observe any lipolytic activity from strains neither *in vitro* nor in meat. These contradictory results may be explained by the different *B. thermosphacta* strains used by these authors and suggest important intra-species diversity.

1.3.4. Factors affecting growth of *B. thermosphacta*

Various parameters are used to prolong the shelf life of food by diminishing microbial growth. These include the use of various MAP conditions (varying the O_2, CO_2 and N_2 ratios), storage at low temperature, addition of ingredients such as salts (especially in fermented and cured meat or in salted fish products), but also preservatives such as nitrite and sodium lactate. The growth of *B. thermosphacta* on meat and seafood products is highly affected by such abiotic factors (Blickstad, 1983; Hitchener et al., 1979; Labadie, 1999; Nychas et al., 2008b; Pin et al., 2002). Table 1.2 summarizes the main effects of various factors on the growth of *B. thermosphacta* recorded from the literature.

1.3.5. Isolation and identification tools

Methods developed for isolating *B. thermosphacta* have been mainly devoted to its recovery from meat products. A selective medium has been designed and is still routinely used (Gardner, 1966, 1985). This so-called STAA medium contains streptomycin, thallium acetate, and actidione. *B. thermosphacta* is resistant to high concentrations of streptomycin-sulfate, which inhibits other Gram-positive and most Gram-negative bacteria. Thallium acetate inhibits most yeast species as well as many facultative aerobic and anaerobic microorganisms. The incorporation of actidione (cycloheximide) inhibits yeast and filamentous fungi (Gardner, 1966). Standard ISO norm 13722:1996 reports a colony-count technique for enumerating *B. thermosphacta* from meat products by plating on the selective medium STAA followed by incubation of the plates at temperatures ranging from 22°C to 25°C during 48 hours. Further tests may be necessary to check the colonies selected on STAA plates, such as an oxidase test for differentiating *B. thermosphacta* from some Pseudomonads that can also grow on this medium. Indeed, although the medium preferentially selects *B. thermosphacta*, a number of other bacterial species can also grow on it (Gardner, 1966).

Table 1.2 Abiotic factors encountered in meat products and their influence on growth of *B. thermosphacta*.

Factor	Effect on *B. thermosphacta* growth	Reference
Gas composition of the atmosphere	Presence of O_2 improves growth	(Samelis et al., 2000a) (Argyri et al., 2011)
	High level of CO_2 is not inhibitory as long as O_2 is present	(Samelis et al., 2000a) (Koutsoumanis et al., 2006) (Asensio et al., 1988) (Lopez-Galvez et al., 1995)
Temperature	Growth is favored at low temperature (0 and 5°C)	(Argyri et al., 2011) (Koutsoumanis et al., 2006)
pH	Growth is inhibited at low pH	(Blickstad, 1983)
Glucose	Increasing concentrations improve growth	(Macaskie et al., 1984) (Nychas et al., 2008b)
NaCl	Growth still possible with 8-10%	(Collins-Thompson and Rodriguez-Lopez, 1980)
Nitrate	Growth inhibition by nitrate, more important inhibition in combination with low pH and low temperature	(Talon et al., 1988)
Nitrite	Combination of low pH + low temperature + nitrite, increase the growth inhibition	(Collins-Thompson and Rodriguez-Lopez, 1980)
Sodium lactate	Levels reduced by sodium lactate in acidified chicken meat model (pH = 5.0) stored at 22°C	(Lemay et al., 2002)

Both phenotypic and genotypic methods can contribute to a further fast screening of colonies isolated on STAA medium. Some key tests for the phenotypic identification of new isolates of *Brochothrix* spp. have been traditionally used such as Gram staining, detection of catalase activity, and assessment of growth at different temperatures. Table 1.3 summarizes the main characteristics that differentiate *Brochothrix* from other non-sporulating Gram-positive bacilli that can contaminate the same food matrices (Stackebrandt and Jones, 2006).

Table 1.3 Main characteristics that differentiate *Brochothrix spp*. from phylogenetically close genera

Characteristics	*Brochothrix*	*Listeria*	*Carnobacterium*	*Lactobacillus*
Growth at 35°C	-	+	+	+
Growth on STAA agar	+	-	-	-
Motility	-	+	-	-
Catalase activity	+	+	-	-
GC content (%)	36-38	36-42	33-37	32-53

However, the above-mentioned tests do not enable the discrimination of *B. thermosphacta* from *B. campestris*. Those can be differentiated by their ability to grow in media containing 8-10% NaCl or 0.05% of potassium tellurite, as *B. thermosphacta* can grow on these media whereas *B. campestris* cannot. Moreover, unlike *B. thermosphacta, B. campestris* is able to hydrolyze hippurate and produces acids from rhamnose (Stackebrandt and Jones, 2006).

Recently, molecular techniques have been widely applied for detecting and typing foodborne pathogens and spoilers (Böhme et al., 2014; Ceuppens et al., 2014; Scheu et al., 1998; Van Der Vossen and Hofstra, 1996). For example, 16S rRNA gene sequencing is the most commonly used method for studying bacterial phylogeny and taxonomy. However, although it is a powerful tool for many genera and species, it has poor discriminatory power in some cases (Bosshard et al., 2006; Mignard and Flandrois, 2006). As mentioned above, the two *B. thermosphacta* and *B. campestris* type strains share >99.3% sequence identity with regard to their 16S rRNA genes (Stackebrandt and Jones, 2006). Real-time PCR assays have been developed for detecting and quantifying *B. thermosphacta* from beef, seafood, and pork sausage (Fougy et al., 2016; Mamlouk et al., 2012; Papadopoulou et al., 2012a). Some were based on primers designed from the 16S rRNA gene sequence (Mamlouk et al., 2012; Papadopoulou et al., 2012a) and may not differentiate the two species. On the contrary, in a most recent report, the primers designed from the *rpoC* gene were specific for *B. thermosphacta* only (Fougy et al., 2016). Two PCR-based tests for specific detection and differentiation of *B. thermosphacta* and *B. campestris* were developed (Gribble and Brightwell (2013). The first is a real-time TaqMan PCR based on a single nucleotide polymorphism (T/A) and targeting 16S rRNA gene sequences. This test allows the specific differentiation of *Brochothrix* species from the closely related *Listeria* and *Lactobacillus* genera. The second is a standard PCR assay for the specific identification of *B. campestris* based on the amplification of the brochocin-C encoding operon. This bacteriocin is produced by the *brcABIDT* operon (McCormick et al., 1998) which is absent from *B. thermosphacta* (Gribble and Brightwell, 2013; Siragusa and Cutter-Nettles, 1993). However, this reportedly species-specific PCR based test may not be reliable as the studies on brochocin-C have all been performed with the type strain, *B. campestris* ATCC 43754. Thus, there is no information about the presence of the genes for brochocin-C production in other *B. campestris* strains that could validate this PCR test as accurate on more strains.

Other DNA based methods such as PFGE (Pulsed Field Gel Electrophoresis), REP-PCR (Repetitive Extragenic Palindromic PCR) were also applied on *B. thermosphacta* strains with the aim to characterize the intra-species diversity (Papadopoulou et al., 2012a; Xu et al., 2010). The results obtained by these authors confirmed a genetic diversity between the strains. MALDI-TOF MS (Matrix assisted laser desorption/ionization time of flight Mass spectrometry) based on the analysis of protein profiles enabled the identification of *B. thermosphacta* colonies isolated from poultry cuts but no indication about the possible identification of *B. campestris* with this method was mentioned (Höll et al., 2016).

The use of culture-independent methods, performed with no need of prior bacterial cultivation, has greatly improved the knowledge about the diversity and the structure of microbial communities of food. By such methods the presence of *B. thermosphacta* among the dominant species has been highlighted in many food matrixes including meat and seafood products (Chaillou et al., 2015; Delhalle et al., 2016; Drosinos and Nychas, 1996; Ercolini et al., 2011; Jaffrès et al., 2009; Nieminen et al., 2012; Pennacchia et al., 2009; Pennacchia et al., 2011; Piotrowska-Cyplik et al., 2017).

1.3.6. Spoilage potential of *B. thermosphacta*

1.3.6.1. Production of malodorous molecules

The spoilage of food can be evaluated by sensory analyses which reveal color, aspect, odor, taste with attributes (such as buttery odor or wet dog as examples) and sometimes notation. Chemical analyses can also be performed to identify and sometimes quantify the molecules that are produced and considered as responsible for spoilage. In the case of off-odor production, methods aiming at detecting volatile molecules have been largely used. As *B. thermosphacta* has been reported to be one of the most common species associated with spoilage, many studies have analyzed volatile organic compounds (VOCs) in food contaminated by this species, to establish a link with its presence or activity in spoiled products. Table 3 summarizes the different molecules that have been identified as putatively linked to the presence of *B. thermosphacta* and its involvement in spoilage of various food matrices.

The spoilage effect of *B. thermosphacta* has been studied in different food matrices either from naturally contaminated products (Dainty et al., 1985; Dainty et al., 1989; Dainty and Mackey, 1992; Ercolini et al., 2011; Noseda et al., 2012) or in challenge test experiments performed after inoculating sterile or low contaminated food matrices (Casaburi et al., 2014; Franke and Beauchamp, 2017a; Jaffrès et al., 2011; Joffraud et al., 2001; Mikš-Krajnik et al., 2016; Stanley et al., 1981). As shown in Table 1.4, these studies reported that *B. thermosphacta* brings about spoilage by producing various undesirable VOCs comprising aldehydes, ketones, esters, alcohols, and small amounts of short chain fatty acids causing off-odors and off-flavors. (Casaburi et al., 2014; Casaburi et al., 2015b; Ordonez et al., 1991)This species also produces organic acids (lactic and acetic acids) and ethanol (Hitchener et al., 1979; Pin et al., 2002).

Table 1.4 Volatile Organic Compounds (VOCs) produced by *B. thermosphacta*.

VOCs produced by *B. thermosphacta* during storage of meat or seafood under air, vacuum packaging (VP) and modified atmosphere packaging (MAP) were presented.

Volatile Organic Compounds (VOC)	Matrix	Storage conditions	Odor description	Other spoilage bacteria	References
Hydrocarbons					
Dimethylbenzene	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 30% N_2, 20% O_2), 5°C, 10 days	Strong butter, buttermilk, sour, nauseous sweet	/	(Laursen et al., 2006)
2-Octene	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 30% N_2, 20% O_2), 5°C, 10 days	Strong butter, buttermilk, sour, nauseous sweet	/	
Alcohols					
Ethanol	Inoculated beef meat	VP, 5°C, 15 days		/	(Stanley et al., 1981)
	Inoculated chicken breast fillets	MAP (30% CO_2, 70% O_2), 4°C, 7 days		/	(Franke and Beauchamp, 2017a)
1-Propanol	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
2-Methyl-1-propanol	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	
2-Methyl-1-butanol	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50% N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	
3-Methyl-1-butanol	Spoiled chicken carcasses	Air		*Shewanella, Serratia liquefaciens*	(Nychas et al., 1998)
	Inoculated beef meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014)
	Inoculated chicken breast fillets	MAP (30% CO_2, 70% O_2), 4°C, 7 days		/	(Franke and Beauchamp, 2017a)
	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 30% N_2, 20% O_2), 5°C, 10 days	Wet dog	*Carnobacterium maltaromaticum, Carnobacterium divergens Carnobacterium mobile*	(Laursen et al., 2006)
	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50% N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
2-Methylpropanol	Spoiled chicken carcasses	Air		*Pseudomonas* spp. *S. liquefaciens*	(Nychas et al., 1998)
	Inoculated beef meat	VP, 5°C, 15 days		/	(Stanley et al., 1981)
2-Methylbutanol	Inoculated beef meat	VP, 5°C, 15 days		/	

This table continues in the following pages

1-Octanol	Inoculated beef meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014)
1-Octen-3-ol	Inoculated beef meat	Air, 4 °C, 7 days		/	
2-Ethyl-1-hexanol	Inoculated beef meat	Air, 4 °C, 7 days		/	
	Beef meat chops	Air, 4°C		/	(Ercolini et al., 2011)
2-Ethyl-1-decanol	Inoculated beef meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014)
2,3-Butanediol	Inoculated beef meat	Air, 4 °C, 7 days		/	
	Beef meat chops	Air, 4°C		*Pseudomonas* spp., *Enterobacteriaceae*	(Ercolini et al., 2011)
	Inoculated beef meat	VP, 5°C, 15 days		/	(Stanley et al., 1981)
Butanediol	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 30% N_2, 20% O_2), 5°C, 10 days	Strong butter, buttermilk, sour, nauseous sweet	/	(Laursen et al., 2006)
2-Butoxy-ethanol	Inoculated beef meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014)
Hepanol	Inoculated beef meat	Air, 4 °C, 7 days		/	
Phenylethyl alcohol	Beef meat chops	Air, 4°C		*Pseudomonas* spp., *Enterobacteriaceae*	(Ercolini et al., 2011)
2-Hexanol	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
Aldehydes					
Acetaldehyde	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
2-Methyl-1-propanal	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	
2-Methylpropanal	Spoiled chicken carcasses	Air		*Pseudomonas* spp., *S. liquefaciens,*	(Nychas et al., 1998)
2-Methyl-1-butanal	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
2-Methylbutanal	Spoiled chicken carcasses	Air		*Pseudomonas* spp. *S. liquefaciens*	(Nychas et al., 1998)

This table continues in the following pages

Volatile Organic Compounds (VOC)	Matrix	Storage conditions	Odor description	Other spoilage bacteria	References
3-Methyl-1-butanal	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 30% N_2, 20% O_2), 5°C, 10 days	Strong butter, buttermilk, sour, nauseous sweet	/	(Laursen et al., 2006)
	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 30% N_2, 20% O_2), 5°C, 10 days	Wet dog	*C. maltaromaticum, C. divergens, C. mobile*	
	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
3-Methylbutanal	Inoculated beef meat	Air, 5°C, 6-8 days		*Hafnia. alvei, Enterobacter agglomerans, S. liquefaciens, Alteromonas putrefaciens, Aeomonas hydrophila, Pseudomonas fragi*	(Dainty et al., 1989)
	Inoculated beef meat	VP, 5°C, 15 days		/	(Stanley et al., 1981)
Pentanal	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
Hexanal	Inoculated beef meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014)
Heptanal	Inoculated beef meat	Air, 4 °C, 7 days		/	
Nonanal	Inoculated beef meat	Air, 4 °C, 7 days		/	
	Inoculated beef meat	VP, 5°C, 15 days		/	(Stanley et al., 1981)
Decanal	Inoculated beef meat	VP, 5°C, 15 days		/	
Ketones					
3-Hydroxybutanone (acetoin)	Inoculated beef meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014)
	Inoculated beef meat	VP, 5°C, 15 days		/	(Stanley et al., 1981)
	Inoculated beef meat	Air, 5°C, 6-8 days		*H. alvei, E. agglomerans, S. liquefaciens, A. putrefaciens, A. hydrophila, P. fragi*	(Dainty et al., 1989)
	Beef meat chops	Air/MAP (60% O_2, 40% CO_2), 4°C		*Pseudomonas* spp., *Enterobacteriaceae, C. maltaromaticum*	(Ercolini et al., 2011)
	Spontaneously spoiled meat	Air		*Enterobacteriaceae*	(Dainty and Mackey, 1992)
	Inoculated chicken breast fillets	MAP (30% CO_2, 70% O_2), 4°C, 7 days		/	(Franke and Beauchamp, 2017a)
	Raw atlantic salmon	Air; 4°C, 4 days		*Pseudomonas* spp., LAB, H_2S producing bacteria	(Mikš-Krajnik et al., 2016)

2,3-Butanedione (diacetyl)	Inoculated beef meat	Air, 5°C, 6-8 days		*H. alvei, E. agglomerans, S. liquefaciens, A. putrefaciens, A. hydrophila, P. fragi*	(Dainty et al., 1989)
	Spontaneously spoiled meat	Air,		*Enterobacteriaceae*	(Dainty and Mackey, 1992)
	Inoculated chicken breast fillets	MAP (30% CO_2, 70% O_2), 4°C, 7 days		/	(Franke and Beauchamp, 2017a)
	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 30% N_2, 20% O_2), 5°C, 10 days	Strong butter, buttermilk, sour, nauseous sweet	/	(Laursen et al., 2006)
	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 30% N_2, 20% O_2), 5°C, 10 days	Wet dog	*C. maltaromaticum, C. divergens, C. mobile*	
2-Propanone	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
	Inoculated chicken breast fillets	MAP (30% CO_2, 70% O_2), 4°C, 7 days		/	(Franke and Beauchamp, 2017a)
2-Hexanone	Inoculated cold-smoked salmon	VP, 6°C, 40 days (Spoiled)	Blue cheese odor	/	(Joffraud et al., 2001)
2-Heptanone	Inoculated cold-smoked salmon	VP, 6°C, 40 days (Spoiled)	Blue cheese odor	/	
2,3-Heptanedione	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50%N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
3-Octanone	Inoculated beef meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014)
Esters					
Ethyl acetate	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 50% N_2), 8°C, several weeks (spoiled)	Cheese/feet and sour/fermented	/	(Jaffrès et al., 2011)
Ethyl butanoate	Inoculated sterile and none sterile meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014)
Ethyl 3-methylbutanoate	Inoculated beef meat	Air, 4 °C, 7 days		/	
Ehylhexanoate	Beef meat chops	Air, 4°C		/	
	Inoculated beef meat	Air, 4 °C, 7 days		/	

This table continues in the following pages

Volatile Organic Compounds (VOC)	Matrix	Storage conditions	Odor description	Other spoilage bacteria	References
Ethyloctanoate	Inoculated beef meat	Air/MAP (60% O_2,40% CO_2), 4°C		/	(Ercolini et al., 2011)
	Inoculated beef meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014)
Ethyldecanoate	Inoculated beef meat	Air, 4 °C, 7 days		/	
Acids					
Acetic acid	Inoculated meat	Air		/	(Dainty and Hibbard, 1983)
	Inoculated chicken breast fillets	MAP (30% CO_2, 70% O_2), 4°C, 7 days		/	(Franke and Beauchamp, 2017a)
	Inoculated peeled and cooked shrimp	MAP (50% CO_2, 30% N_2, 20% O_2), 5°C, 10 days	Wet dog	*C. maltaromaticum, C. divergens, C. mobile*	(Laursen et al., 2006)
	Sea bream filet	Air/ MAP (60%CO_2, 10% O_2, 30% N_2), 5°C		*Pseudomonas* spp., *Enterobacteriaceae,* LAB, H_2S producin bacteria	(Parlapani et al., 2014)
Formic acid	Model system and spoiled meat	Air,		*Lactobacillus* spp. *Leuconostoc* spp., *Carnobacterium* ssp.	(Nychas et al., 2007) (Nychas et al., 2008b)
	Inoculated chicken breast fillets	MAP (30% CO_2, 70% O_2), 4°C, 7 days		/	(Franke and Beauchamp, 2017a)
Hexanoic acid	Beef meat chops	Air, 4°C		*Pseudomonas* spp., *Enterobacteriaceae*	(Ercolini et al., 2011)
2-Methyl butanoic acid	Inoculated beef meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014) (Dainty and Hibbard, 1983)
	Model system and spoiled meat	Air		/	(Nychas et al., 2007) (Nychas et al., 2008b)
	Spoiled meat	Air, 5°C, 3 days		/	(Dainty et al., 1985)
	Beef meat chops	Air, 4°C		*Pseudomonas* spp., *Enterobacteriaceae*	(Ercolini et al., 2011)
3-Methyl butanoic acid	Inoculated beef meat	Air, 4 °C, 7 days		/	(Casaburi et al., 2014) (Dainty and Hibbard, 1983)
	Model system and spoiled meat	Air		/	((Nychas et al., 2007) (Nychas et al., 2008b)
	Spoiled meat	Air, 5°C		/	(Dainty et al., 1985)

Iso-butyric acid	Model system and spoiled meat	Air	/	(Nychas et al., 2007) **(Nychas et al., 2008b)**
	Spoiled meat	Air, 5°C, 3 days	/	(Dainty et al., 1985)
	Inoculated chicken breast fillets	MAP (30% CO_2, 70% O_2), 4°C, 7 days	/	(Franke and Beauchamp, 2017a)
	Inoculated meat	Air	/	(Dainty and Hibbard, 1983)
Sulphur compounds				
Dimethyl sulphide	Spoiled meat	VP, 4°C	*Enterobacteriaceae, Clostridium algidicarnis, Clostridium putrefaciens, H. alvei, Lactobacillus curvatus, Lactobacillus sakei, S. liquefaciens, Shewanella Baltica*	(Ercolini et al., 2011)
	Inoculated chicken breast fillets	MAP (30% CO_2, 70% O_2), 4°C, 7 days	/	(Franke and Beauchamp, 2017a)

Some of the spoilage-related molecules produced by *B. thermosphacta* may have a very intense off-odor or off-flavor, and therefore affect the sensory quality of the product even at low levels (Refsgaard et al., 1999). For example, acetic acid has an undesirable pungent, acidic, and vinegar flavor even at low concentrations (Vermeiren et al., 2005). The amounts of the spoilage related molecules may vary depending on strains and on the nature and concentration of the substrates available (glucose, glycerol, ribose, branched-chain amino acid, and fatty acids among others) (Borch and Molin, 1989; Macaskie et al., 1984; Vermeiren et al., 2005).

The apparition of creamy/dairy and buttery odors are associated with the production of high amounts of acetoin and diacetyl, respectively (Casaburi et al., 2014; Dainty and Hibbard, 1983; Dainty and Mackey, 1992; Nychas et al., 2008b; Stanley et al., 1981). Both compounds are major end products of the aerobic catabolism of glucose by *B. thermosphacta* (Blickstad, 1983; Pin et al., 2002).

In relation to seafood products, *B. thermosphacta* has been reported to have an important role in the spoilage of cooked and peeled shrimps stored under MAP (Jaffrès et al., 2011; Mejlholm et al., 2005), and of fresh and VP smoked salmon filets (Mikš-Krajnik et al., 2016); (Joffraud et al., 2001; Stohr et al., 2001). For example, in cold-smoked salmon *B. thermosphacta* produced mainly 2-hexanone and 2-heptanone responsible for the formation "blue-cheese" off-odor (Joffraud et al., 2001; Laursen et al., 2006; Mejlholm et al., 2005). In cooked and peeled shrimp, *B. thermosphacta* was related to the strong butter, buttermilk-like, nauseous, sour/fermented and cheese/feet off-odors associated with the production of 2,3-butanedione (diacetyl), 3-methyl-1-butanal, and 3-methyl-1-butanol (Jaffrès et al., 2011; Laursen et al., 2006; Mejlholm et al., 2005). It has been shown that factors affecting the biochemical composition of food products may determine the nature of the VOC and therefore affect the type and the intensity of spoilage (Koutsoumanis and Nychas, 1999; Nychas et al., 2008b; Skandamis and Nychas, 2002). Other factors can influence spoilage by *B. thermosphacta*, as notably the presence of diverse bacterial genus and species in the food microbiota, and the possible interactions between them. Indeed, it has been reported that when co-inoculated on cooked and peeled shrimps, *B. thermosphacta* and *C. maltaromaticum* were able to form a typical wet dog off-flavor, whereas neither produced this off-flavor when

inoculated separately (Laursen et al., 2006). No new metabolite was detected in *B. thermosphacta–C. maltaromaticum* co-cultures by comparison with those produced by both species inoculated alone. It can therefore be hypothesized that the wet dog off-flavor is most probably resulting from an interaction between metabolites formed by both species.

Obviously, a more in-depth investigation is necessary to clearly understand the metabolic pathways which lead to the production of VOCs by *B. thermosphacta*.

1.3.6.2. Production of Biogenic amines (BAs)

B. thermosphacta has been also associated with the production of biogenic amines (BAs) in some studies (Casaburi et al., 2014; Paleologos et al., 2004). These compounds, such as histamine, tyramine, tryptamine, putrescine, and cadaverine, often result from decarboxylation of free amino acids. Some BAs are responsible for strong putrid off-odors and some, as histamine, for foodborne poisoning. Histidine decarboxylase and tyrosine decarboxylase, responsible for histamine and tyramine synthesis, respectively, have been reported for several bacterial species. Although histidine decarboxylase activity has been reported in *B. thermosphacta* (Casaburi et al., 2014), no histidine decarboxylase gene was found in the genome (Stanborough et al. (2017). This may suggest that BAs production is strain dependent in *B. thermosphacta*, as already described in lactic acid bacteria (Coton and Coton, 2009). Furthermore, during co-cultures, *B. thermosphacta* has been reported to enhance the cadaverine production of *Escherichia coli* and can also promote the production of histamine by *Lactobacillus sakei* (Nowak and Czyzowska, 2011). This shows the difficulty to clearly demonstrate the spoilage activity of bacteria in food products.

1.3.7. Strategies for fighting *B. thermosphacta* food spoilage

1.3.7.1. Biopreservation

Biopreservation has been described in 1996 by M.E. Stiles as “extended storage life and enhanced safety of foods using the natural microflora and (or) their antibacterial products” (Stiles, 1996). The addition of bacteria as protective cultures, of their metabolites like bacteriocins or other antagonistic compounds, or the use of bacteriophages having antagonistic effects reflect examples of biopreservation. The inhibition of *B. thermosphacta* using bacteriocins has primarily focused on nisin. This bacteriocin, produced by *Lactococcus lactis,* is applied as a food preservative (E234) in the food industry, particularly in some dairy

products but not in meat or fish where *B. thermosphacta* commonly occurs. However, several studies focused on the addition of nisin in beef carcasses and meat products to inhibit the growth of *B. thermosphacta* (Cutter-Nettles and Siragusa, 1996a, b, 1997; Tu and Mustapha, 2002). Cutter-Nettles and Siragusa (1994) demonstrated that nisin spray treatment of beef carcasses can reduce the level of *B. thermosphacta* by up to 3.6 log·CFU cm^{-2} after aerobic incubation during 24 h at 4°C. The same authors showed later on that nisin spray treatment combined by VP was more effective as resulting in reducing *B. thermosphacta* by up 4.5 log CFU cm^{-2} (Cutter-Nettles and Siragusa, 1996b). Additionally, nisin immobilized in a calcium alginate gel limited the growth *B*. *thermosphacta* on ground beef and beef carcasses to undetectable levels (< 1.30 log CFU g^{-1}) (Cutter-Nettles and Siragusa, 1996a, 1997). Other authors demonstrated that nisin and nisin combined with EDTA treatments followed by VP could increase the shelf life of fresh beef stored at 4°C up to 25 days by inhibiting *B. thermosphacta* (Tu and Mustapha, 2002).

Brochocin-C, the two peptide bacteriocin produced by *B. campestris* ATCC 43754, inhibits *B. thermosphacta* and other closely related Gram-positive species associated with food and meat such as *Listeria* ssp., *Lactobacillus* spp. and *Carnobacterium* spp. (Greer and Dilts, 2006; Siragusa and Cutter-Nettles, 1993). Brochocin-C has been described as a potential food preservative since this bacteriocin was shown to be heat stable at 100°C and its activity preserved at pH ranging from 2 to 9 (McCormick et al., 1998). However *B. campestris* can also grow and cause spoilage on vacuum packed lamb making the use of this bacteriocin producing bacterium not suitable for meat biopreservation (Gribble and Brightwell, 2013).

Protective cultures active against *B. thermosphacta* in laboratory medium and also in meat and seafood products have been reported (Fall et al., 2010; Fall et al., 2012; Hwanhlem et al., 2017; Leroi et al., 2015; Leroi et al., 2012; Olaoye et al., 2015). Inoculation of cooked and peeled shrimps with *Lactococcus piscium* CNCM I-4031 inhibited the growth of *B. thermosphacta* by 3–4 log CFU g^{-1} (Fall et al., 2012) and totally stopped its growth in cooked-smoked salmon (Leroi et al., 2015). Additionally, *L. lactis* subsp. *lactis* I23, a nisin producer, and the multi-bacteriocin producer strain *Lactobacillus curvatus* CRL705 and other lactic acid bacteria prevented the growth of *B. thermosphacta* in meat (Castellano and Vignolo, 2006; Olaoye et al., 2015; Russo et al., 2006).

Bacteriophages have also been proposed for biopreservation as an alternative for extending the storage quality of refrigerated meats (García et al., 2008). Sensory analysis of pork tissue showed a better acceptability of samples inoculated with *B. thermosphacta* and a phage solution than those inoculated only with *B. thermosphacta*, which produced undesirable odors within 4 days making the product rejected by the panel (Greer and Dilts (2002).

Organic acids, often naturally produced by microorganisms, are used in food industry as food preservatives (Theron and Lues, 2007). The effect of lactic acid on *B. thermosphacta* has been evaluated (Grau, 1980; Greer and Dilts, 1995; Niemand et al., 1983). According to Grau (1980), lactic acid is more effective for inhibiting the growth of *B. thermosphacta* under anaerobic condition. Moreover, the product characteristics influence the inhibitory effect of lactic acid on *B. thermosphacta*. For example, lactic acid causes a stronger inhibition in fat tissue than in lean tissue (Greer and Dilts, 1995).

1.3.7.2. Plant-derived and other antimicrobials

Many plants and their derived essential oils are known to delay or inhibit the growth of bacteria, yeast, and molds. These compounds have been therefore investigated for their potential and applicability to preserve food (Tiwari et al., 2009). Some studies have been published on the inhibitory effects of plant-derived antimicrobials on *B. thermosphacta* (Casaburi et al., 2015a; D'Amato et al., 2016; Nowak et al., 2012a). For example, in beef stored under oxygen-enriched MAP, thyme and rosemary essential oils have been shown to prevent the growth of *B. thermosphacta.* However, the level of their bactericidal effect varied depending on the strain they were applied to (Nowak et al., 2012a). Thyme essential oil was also found to strongly inhibit the growth of *B. thermosphacta* in pork meat (D'Amato et al., 2016). Thus, these antimicrobial molecules could be good alternative methods for extending the shelf life of meat. However to be suitable for commercial application, the amount of essential oils to be added is determinant for the acceptance, since their strong aromas might be transmitted to food products (Chouliara et al., 2007).

1.3.7.3. Physical treatments

The effect of physical treatments used in food processing, as heating, gamma irradiation of high hydrostatic pressure, has been evaluated. *B. thermosphacta* is heat sensitive bacterium that is destroyed by a heating at 63°C during 5 min (Stackebrandt and Jones, 2006).

B. thermosphacta has been reported more resistant to irradiation than other meat spoilage organisms such as *Pseudomonas* (Giroux et al., 2001). Although it has been frequently isolated from irradiated meat and poultry, this species is affected by irradiation doses of 0.5 and 2.0 kGy (Stackebrandt and Jones, 2006). Various studies have shown that high hydrostatic pressure is of great interest for food industry to extend the shelf-life of solid food products as it can inactivate most micro-organisms. Inactivation of *B. thermosphacta* has been evaluated in high pressure treated ground beef (Ouattara et al., 2002), ready-to-eat meats (Hayman et al., 2004), cooked ham (Han et al., 2010), and Indian white prawn (Ginson et al., 2015). In the case ready-to-eat meat stored at 4°C, high pressure was shown to reduce *B. thermosphacta* counts to undetectable levels until 95 days after the treatment (Hayman et al., 2004). In vacuum-packed cooked ham similar results were observed with *B. thermosphacta* counts maintained at 2 log CFU g^{-1} 90 days after the treatment (Han et al., 2010).

1.3.8. Genomic characteristics of *B. thermosphacta*

To date, 12 *B. thermosphacta* and one *B. campestris* draft genome sequences are publicly available. Genome sequences of the type strains *B. thermosphacta* ATCC 11509 and *B. campestris* ATCC 43754 were sequenced by DOE Joint Genome Institute (JGI) in 2014 in the framework of the project: Genomic Encyclopedia of Bacteria and Archaea (GEBA). More recently, eleven draft genome sequences of *B. thermosphacta* strains were determined (Stanborough et al., 2017). These strains were isolated from various meat products stored aerobically or under MAP.

Table 1.5 summarizes the characteristics of these genomes. All are draft genome sequences. Their size is quite similar, ranging from 2.43 to 2.64 Mb with a G+C content from 36.2 to 36.4%. Genome-based analysis performed by Stanborough et al. (2017) showed a high degree of genomic sequence similarity between the *B. thermosphacta* strains. The genome analysis highlighted diverse repertoire of substrate-specific genes from the phosphotransferase system (PTS). This includes the genes for glucose, maltose, sucrose, fructose, mannose, trehalose, cellobiose, β-glucoside, mannitol, and N-acetylglucosamine transport and phosphorylation systems. Ribose, glycerol-3-phosphate, maltose/maltodextrin and inositol transporter genes were also identified. All genes required for glycolysis were present. All genes required for the pentose phosphate pathway were also found, while coding sequences

(CDS) for only four of the eight enzymes of the citrate cycle (TCA cycle) were detected. This analysis revealed also the presence of genes involved in the production of malodorous compounds as acetoin and 2,3-butanediol, with the presence of genes coding for α-acetolactate synthase, acetolactate decarboxylase and an (S,S)-butanediol dehydrogenase. As it has been suggested that free fatty acid production in *B. thermosphacta* results from amino acid catabolism rather than from lipolysis (Holley, 2014). Stanborough et al. (2017) have also searched for genes involved in the degradation of the branched-chain amino acids. They found all enzymes of these pathways leading to the production by *B. thermosphacta* of the branched-chain fatty acids isovaleric, isobutyric, and 2-methylbutyric acids from the degradation of leucine, valine, and isoleucine, respectively. These fatty acids are also responsible for off-odors production on food (Casaburi et al., 2014; Casaburi et al., 2015b). As mentioned above no gene for histidine decarboxylase and histamine production was detected. Stress response regulatory genes were also identified, as for example the alternative sigma factor σ^B, which most probably plays important roles in the survival and growth under high-salt conditions and adaptation to refrigeration temperatures, and in the spoilage process. This suggests that despite the high degree of genomic sequence similarity between the *B. thermosphacta* strains, the spoilage potential for each strain is most probably related to the regulation of spoilage-specific genes expression.

Table 1.5 Genome overview of *B. thermosphacta* strains and *B. campestris*.

Strain name	Source	Genbank accession Number	Genome size (Mb)	GC content (%)	CDS	Contigs / scaffolds
B. thermosphacta						
DSM 20171 [a]	Pork sausage	MDLK00000000	2.49 Mb	36.4 %	2,290	33/33
7803	Beef[b]	MDLL00000000	2.57 Mb	36.3 %	2,348	61/61
7804	Beef[b]	MDLU00000000	2.61 Mb	36.3 %	2,415	56/56
7806	Beef[c]	MDLM00000000	2.54 Mb	36.3 %	2,328	41/41
7807	Lamb[c]	MDLN00000000	2.48 Mb	36.4 %	2,277	31/31
7808	Lamb[c]	MDLO00000000	2.56 Mb	36.3 %	2,328	68/68
7809	Beef[c]	MDLV00000000	2.49 Mb	36.3 %	2,273	22/22
7810	Beef[c]	MDLP00000000	2.47 Mb	36.4 %	2,244	22/22
7811	Beef[c]	MDLT00000000	2.55 Mb	36.3 %	2,292	186/186
7813	Minced veal[b]	MDLQ00000000	2.64 Mb	36.2 %	2,417	166/166
7816	Lamb[c]	MDLR00000000	2.58 Mb	36.3 %	2,342	154/154
7818	Beef[c]	MDLS00000000	2.59 Mb	36.3 %	2,364	130/130
B. campestris						
DSM 4712[a]	Soil	NZ_AODH01000000	2.37	40.2 %	2,424	128/128

a: Type strain
b: stored under MAP
c: stored under aerobic conditions

Despite the temporal and the geographic diversities of the strains used for genome comparison, and also the difference in term of the meat source and their packaging conditions for strain isolation, the strain panel used does not represent an exhaustive diversity of *B. thermosphacta*. Indeed, this species has been isolated from various other ecological niches including seafood products and the environment (Stackebrandt and Jones, 2006). Therefore, sequencing other strains isolated from wider origin may help to better estimate and evaluate the diversity of *B. thermosphacta*.

1.3.9. Conclusion

Figure 1.6 summarizes the information available on the spoilage potential of this bacterium depending on the food matrix and the storage conditions. *B. thermosphacta* is gaining the interest of researchers because it is increasingly isolated from various meats and seafood. It has been even recognized as a specific spoilage microorganism of meat products. Several studies aimed at understanding its metabolism as a function of the environmental parameters. Recently, analyzing the genome content of several *B. thermosphacta* isolates identified several genes involved in the metabolic pathways related to the development of spoilage reactions already described in *B. thermosphacta.* However, most of the genes responsible for the high number of molecules involved in the spoilage caused by

B. thermosphacta remain yet unknown (Figure 1.6). The limited genetic diversity among *B. thermosphacta* genomes may result from the narrow ecological niche (red meats) for selecting strains to be sequenced. Therefore, sequencing the genome of other strains isolated from wider sources may help to better estimate and evaluate the diversity of *B. thermosphacta.*

In order to better understand *B. thermosphacta* spoilage, there is still a need for revealing the role of specific genes that contribute significantly to food spoilage. Discovering the influence of abiotic factors like food composition and storage conditions and of biotic factor (composition of the microbiota contaminating food) should help identifying genes responsible for spoilage activity and proposing strategies for fighting *B. thermosphacta*. A link between spoilage molecules and gene expression should be established for assessing the metabolic pathways involved in food spoilage. This should enable to deduce pertinent spoilage biomarkers (genes or molecules) for developing technologies that can prevent meat and seafood spoilage by this bacterium.

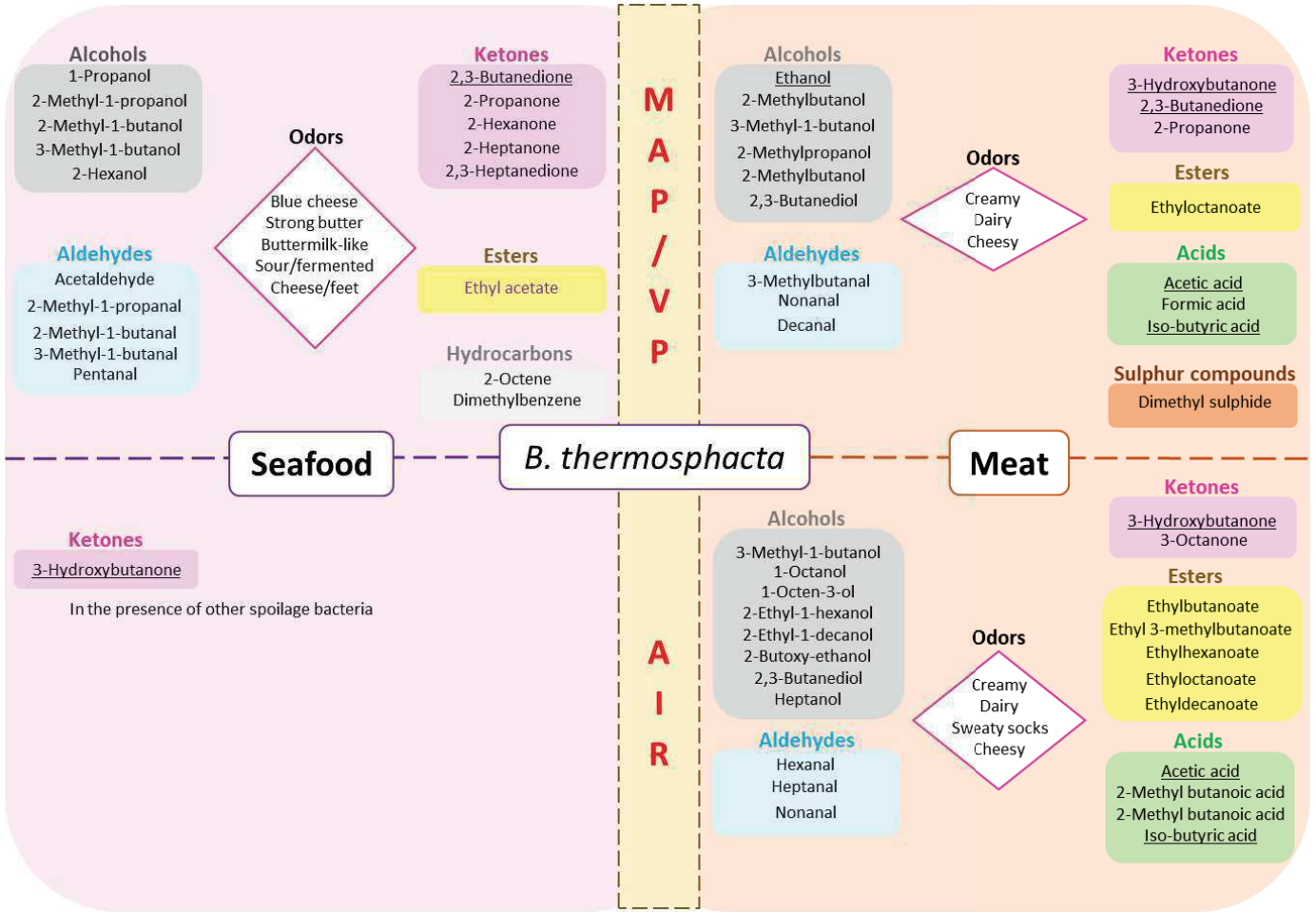

Figure 1.6 Spoilage potential of *B. thermosphacta*.

VOCs and off-odors produced in meat and seafood stored under air or MAP/VP are indicated. VOCs, which biosynthetic pathways are known, are indicated in underline

1.4. Stratégie pour l'étude des mécanismes d'altération chez *B. thermosphacta*

L'altération de différentes matrices alimentaires par diverses bactéries a été décrite et documentée (Remenant et al., 2015). Cependant, les mécanismes spécifiques conduisant à l'altération restent peu étudiés et peu compris. Récemment, différentes études se sont intéressées à l'investigation des mécanismes impliqués dans l'altération chez plusieurs espèces bactériennes incluant *Leuconostoc gelidum* subsp *gasicomitatum*, *L. piscium* et *P. fluorescens*, etc (Andreani et al., 2015; Andreevskaya et al., 2016; Andreevskaya et al., 2015; Johansson et al., 2011).

Dans le cadre de notre étude, nous avons établi une stratégie pour élucider les mécanismes spécifiques de l'altération par *B. thermosphacta*. Elle est destinée à élargir les connaissances sur sa physiologie, sa diversité, les caractéristiques de son génome et les fonctions potentiellement impliquées dans l'altération. Les grandes lignes de cette stratégie sont présentées dans la Figure 1.7. Elles comportent l'analyse de la diversité génétique et phénotypique de l'espèce *B. thermosphacta*. En effet, cette bactérie ubiquitaire est isolée de diverses matrices alimentaires altérées ou non. De plus, elle a été décrite comme responsable de la production d'odeurs indésirables variées suivant les matrices sur lesquelles elle se développe. Nous nous sommes donc posé la question de l'étendue et du rôle de la diversité génétique et phénotypique (potentiel d'altération) de cette espèce suivant l'origine écologique des souches, à savoir le type de matrice d'où elles proviennent ou le fait que celle-ci soit altérée ou non. Ensuite, nous avons envisagé une analyse plus fine de génomique comparative, afin d'identifier des gènes ainsi que des fonctions caractérisant potentiellement la niche écologique et le potentiel altérant d'une souche. Puis, nous avons choisi de réaliser une analyse fonctionnelle afin d'étudier l'effet souche et l'effet matrice sur les capacités altérantes de *B. thermosphacta*. Ces trois grandes lignes sont développées ci-dessous.

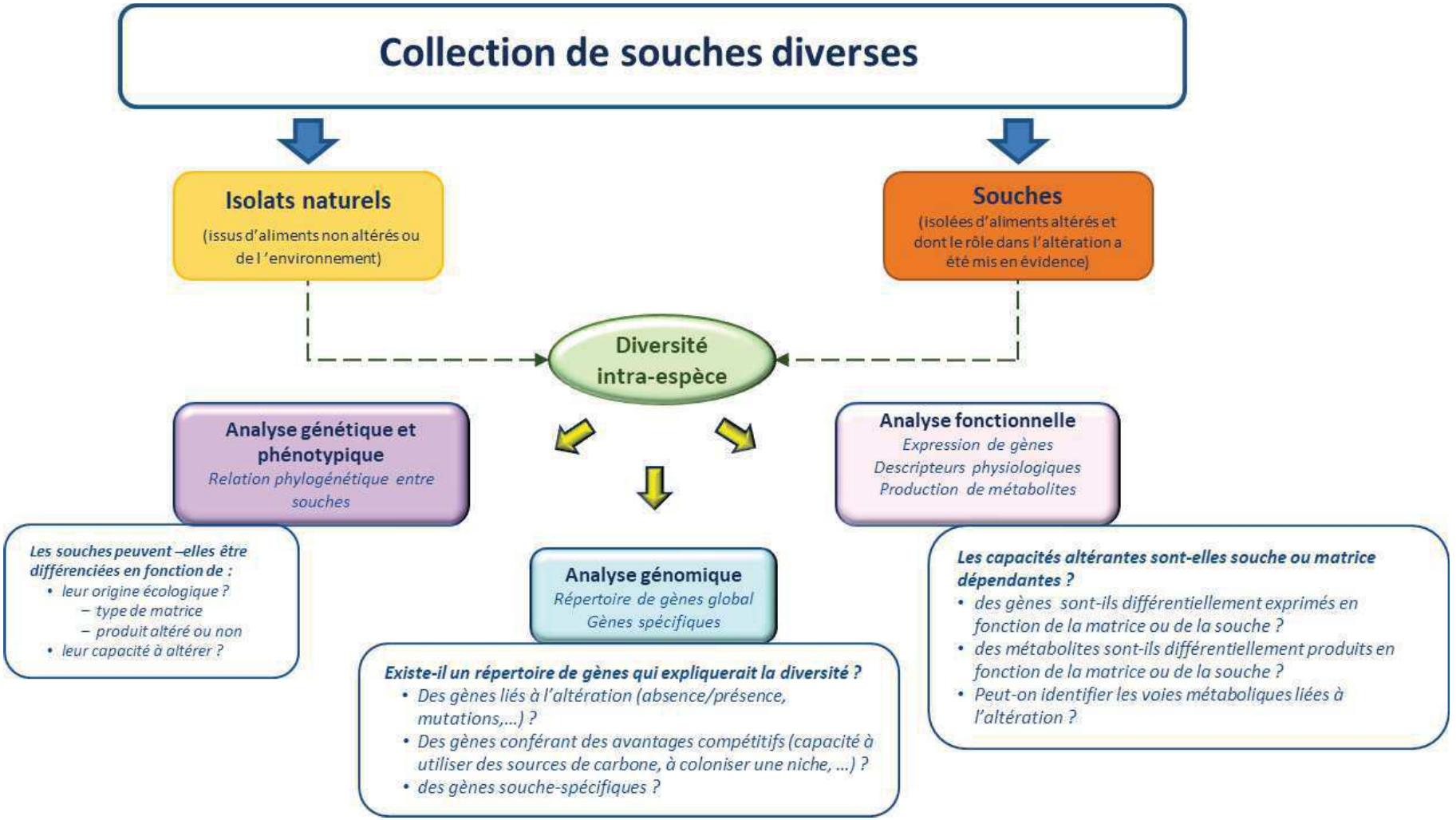

Figure 1.7 Démarche expérimentale pour explorer les mécanismes d'altération de *B. thermosphacta.*

1.4.1. Analyse génétique et phénotypique

Les microorganismes doivent s'adapter aux différents processus technologiques que subissent les produits alimentaires. Les souches appartenant à une même espèce partagent un ensemble commun de gènes et possèdent des gènes qui leur sont spécifiques et peuvent traduire une adaptation particulière à un environnement ou une condition ou leur confèrent une capacité particulière. Il est donc nécessaire de caractériser les variations génétiques intra-spécifiques des bactéries d'origine alimentaire. La diversité intra-spécifique des bactéries résulte principalement de trois phénomènes génétiques : l'acquisition de gènes par transfert horizontal, la perte de gènes, et l'accumulation de mutations. La fréquence de ces trois événements rend l'étude de la diversité intra-spécifique assez compliquée (Fraser-Liggett, 2005).

Il existe deux systèmes de typage distincts permettant d'évaluer la diversité intra-spécifique des bactéries : le phénotypage et le génotypage. Au cours des deux dernières décennies, le génotypage a progressivement remplacé les tests phénotypiques, en raison de sa forte résolution. En effet, il a été largement utilisé pour le typage des souches bactériennes comme l'utilisation de l'électrophorèse en champ pulsé (PFGE) pour le typage de *L. gasicomitatum* (Vihavainen and Björkroth, 2009), ou encore l'analyse *Multilocus Sequence Typing* (MLST) par exemple pour le typage de *L. sakei* (Chaillou et al., 2013) ou *P. fluorescens* (Andreani et al., 2014).

Les techniques de génotypage se sont considérablement développées et diversifiées avec l'accessibilité des outils de la biologie moléculaire pour de nombreux laboratoires. Leur diversité a notamment résulté de la nécessité d'adapter les techniques existantes aux particularités génétiques des micro-organismes étudiés. Elles peuvent être classées en trois catégories principales : méthodes basées sur l'analyse des profils de bandes d'ADN (PFGE par exemple), sur le séquençage de l'ADN et sur l'hybridation ADN-ADN (Li et *al*., 2009). Nous avons choisi de coupler des méthodes phénotypiques et génétiques pour l'étude de la diversité de *B. thermosphacta* dans notre stratégie présentée dans la figure 1.7.

1.4.2. Analyse génomique

La disponibilité de nombreux génomes bactériens dans les bases de données publiques permet de réaliser des comparaisons inter- et intra-espèce. Cette approche puissante

détermine les similarités (la parenté) et les différences (la diversité génomique) entre les génomes de différentes espèces et souches. La comparaison de séquences génomiques entières permet de détecter les régions de synténie (régions dans lesquelles l'ordre des gènes est conservé entre deux espèces apparentées), les réarrangements génomiques (insertions, délétions, translocations, et duplications), les transferts horizontaux de gènes et la présence de prophages (Binnewies et al., 2006; Blakely, 2015). La comparaison de génomes entiers peut être effectuée en utilisant des outils d'alignement, ou en comparant le répertoire de gènes (Daubin and Ochman, 2004; Edwards and Holt, 2013). Plus précisément, une espèce microbienne peut être décrite par son pan-génome (Medini et al., 2005; Tettelin et al., 2008), qui est composé de trois parties: (i) un génome de base qui est partagé par toutes les souches, appelé « core génome », (ii) un ensemble de gènes accessoires qui sont partagés par certains mais pas par tous les isolats, appelé « génome variable », et (iii) un ensemble de gènes spécifiques à la souche qui sont uniques à chaque isolat, appelé « génome spécifique ». L'analyse de ces groupes de gènes permet d'identifier des gènes spécifiques de certaines souches isolées de niches environnementales particulières ou présentant un phénotype particulier (virulence, capacité d'altération, résistance aux antibiotiques, etc.), assurant ainsi un lien entre leur phénotype et leur génotype (Medini et al., 2005; Tettelin et al., 2008). Habituellement, la taille du pan-génome d'une espèce bactérienne est corrélée avec le niveau de diversité des niches colonisées par cette espèce (Medini et al., 2005; Tettelin et al., 2008). Un profil de type présence/absence de gènes dans les génomes comparés peut refléter non seulement les relations phylogénétiques, le transfert horizontal de gènes mais aussi peut aider à la compréhension des différences phénotypiques (Snipen and Ussery, 2010). Nous avons utilisé cette approche pour caractériser les particularités de *B. thermosphacta.*

1.4.3. Analyse fonctionnelle

De nombreuses méthodes analytiques bien établies sont utilisées pour évaluer la qualité des aliments, dont l'analyse sensorielle (Hyldig and Green-Petersen, 2005), l'analyse des communautés microbiennes (Gram and Huss, 1996) et les méthodes biochimiques (Dainty, 1996). Des études ont mis en évidence le rôle de différentes molécules volatiles (ester, alcools, aldéhydes, etc.) dans l'altération des produits carnés et de la mer (Casaburi et al., 2015b; Odeyemi et al., 2018). De plus, le type de molécules volatiles produites permettrait de

déterminer le type d'altération du produit mais aussi l'étendue de son altération (Aru et al., 2016). L'analyse du volatilome peut être définie comme l'étude de l'ensemble des composés organiques volatils de faible poids moléculaire produits par un organisme, et qui constituent les produits finaux des processus cellulaires (Dunn and Ellis, 2005). La chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC / MS) a été largement appliquée pour l'analyse des molécules volatiles liées à l'altération de divers produits carnés et de la mer (Casaburi et al., 2015b; Ercolini et al., 2011; Jaffrès et al., 2011; Joffraud et al., 2001; La Storia et al., 2012). Cette méthode génère un spectre de masse unique à chaque composé, grâce à l'utilisation de l'ionisation électronique, permettant donc une identification relativement plus facile par rapport à d'autres techniques analytiques. En outre, un grand nombre de composés peuvent être facilement identifiés en utilisant la base de données des spectres de masse NIST (National Institute of Standards and Technology) (Tsugawa et al., 2011).

Les molécules volatiles liées à l'altération ont été identifiées pour divers produits carnés et de la mer, et pour de nombreuses bactéries comme *Shewanella*, *Pseudomonas*, *Photobacterium*, *B. thermosphacta* et *Carnobacterium* (Casaburi et al., 2015b; Odeyemi et al., 2018). Cependant, les mécanismes génétiques conduisant à la production de ces molécules restent à l'heure actuelle peu étudiés et peu compris. La compréhension du rôle spécifique d'un micro-organisme dans le processus d'altération d'un aliment nécessite de coupler ces analyses biochimiques avec l'information génomique et l'expression génique. (Tsugawa et al., 2011).

Le RNA-seq (*RNA sequencing* en anglais, ou séquençage de l'ARN) est une technologie qui utilise le séquençage à haut débit. Elle permet d'identifier, qualitativement et quantitativement, le transcriptome (l'ensemble des transcrits) d'un organisme à un stade de développement spécifique ou dans une condition physiologique donnée (Chu and Corey, 2012; Wang et al., 2009). L'étude du transcriptome est essentielle pour interpréter les éléments fonctionnels du génome et révéler les constituants moléculaires des cellules (Wang et al., 2009). La technologie RNA-seq a de nombreuses applications telles que : i) la catégorisation de différents types d'ARN (ARNm, petits ARNs, ARNs antisens, etc.) ; ii) la détermination de la structure transcriptionnelle (localisation de promoteurs alternatifs) ; et iii) la détection des différences dans les niveaux d'expression des gènes au cours de la

croissance d'un organisme ou en réponse à certaines conditions (Chu and Corey, 2012; Han et al., 2015; Sharma and Vogel, 2014).

En comparaison avec les autres techniques, la technologie RNA-Seq offre plusieurs avantages tels que l'absence de nécessité d'avoir une séquence génomique connue, et donc la possibilité de détecter de nouveaux transcrits. De plus, elle est très fiable en termes de reproductibilité technique. Cependant, elle requiert une expérience dans l'utilisation des méthodes bio-informatiques et statistiques (Chu and Corey, 2012; Yendrek et al., 2012).

Dans le cadre de notre stratégie expérimentale permettant d'étudier les mécanismes génétiques mis en jeu par *B. thermosphacta*, qui conduisent à la production de molécules volatiles altérantes (Figure 1.7), nous avons donc choisi de coupler une analyse du volatilome et la recherche de gènes différentiellement exprimés par RNA-seq pour explorer la capacité de *B. thermosphacta* à adapter son métabolisme à des conditions de croissance différentes.

Chapitre 2

Diversité génotypique et phénotypique de *Brochothrix thermosphacta*

Nassima Illikoud
2018

Chapitre 2. Diversité génotypique et phénotypique de *Brochothrix thermosphacta*

2.1. Préambule

La revue de la littérature, nous a permis de constater que le potentiel d'altération de *B. thermosphacta* est variable en fonction des matrices alimentaires et des conditions de leur stockage. En effet, cette bactérie produit des odeurs indésirables différentes selon qu'elle se retrouve sur de la viande, sur de la crevette ou encore sur du saumon. De plus, les capacités altérantes peuvent être variables en fonction des souches, de la niche écologique d'où celles-ci ont été isolées. Cependant, peu d'informations sont disponibles quant à la diversité intra-espèce de cette bactérie. Les quelques articles disponibles traitent uniquement des souches issues de viandes et qui ne sont donc pas représentatives des différentes niches écologiques que *B. thermosphacta* est capable de coloniser. Le but de ce chapitre était d'étudier la diversité intra-espèce de *B. thermosphacta*, afin de savoir si une corrélation pouvait être établie entre l'origine écologique des souches, leur diversité et leur potentiel d'altération. Pour cela, nous avons constitué une collection de souches provenant de plusieurs matrices alimentaires altérées ou non (produits de la mer, carnés et laitiers) et de l'environnement d'un abattoir de bovins. La diversité intra-espèce a été évaluée par trois méthodes de typage, la rep-PCR (*repetitive element palindromic*-PCR), la PFGE (*Pulsed-Field Gel Electrophoresis*) et le MALDI-TOF (*Matrix-Assisted Laser Desorption / Ionization Time-Of-Flight*). De plus, la diversité du potentiel d'altération a été évaluée par la quantification de la production d'acétoïne et de diacétyle, deux molécules associées à l'altération par *B. thermosphacta* après inoculation dans deux milieux modèles (jus de viande et jus crevette).

Ces travaux ont fait l'objet d'un article sous presse dans *Food Microbiology* présenté ci-après.

2.2. Genotypic and phenotypic characterization of the food spoilage bacterium *Brochothrix thermosphacta*

Special issue of Food Microbiology on Microbial Spoilers of Food 2017 - In press

Genotypic and phenotypic characterization of the food spoilage bacterium *Brochothrix thermosphacta*

Nassima Illikoud [a], Albert Rossero [a], Romain Chauvet [b], Philippe Courcoux [c], Marie-France Pilet [a], Thomas Charrier [b], Emmanuel Jaffrès [a], Monique Zagorec [a, *]

[a] SECALIM, INRA, Oniris, Université Bretagne Loire, 44307, Nantes, France
[b] EUROFINS, Laboratoire Microbiologie Ouest, 44300 Nantes, France
[c] Oniris, StatSC Sensometrics and Chemometrics Laboratory, Nantes F-44322, France

Nassima Illikoud[a], Albert Rossero[a], Romain Chauvet[b], Philippe Courcoux[c], Marie-France Pilet[a], Thomas Charrier[b], Emmanuel Jaffrès[a] and Monique Zagorec[a#]

[a] SECALIM, INRA, Oniris, Université Bretagne Loire, 44307, Nantes, France.

[b] EUROFINS, Laboratoire Microbiologie Ouest, 44300 Nantes, France.

[c] Oniris, StatSC Sensometrics and Chemometrics Laboratory, Nantes F-44322, France.

[#] Corresponding author: Monique Zagorec, monique.zagorec@oniris-nantes.fr, Oniris - INRA, UMR 1014 SECALIM - Route de Gachet-CS40706 -44307 Nantes cedex 3, France

Abstract

Microbial food spoilage is responsible for significant economic losses. *Brochothrix thermosphacta* is one of the major bacteria involved in the spoilage of meat and seafood. Its growth and metabolic activities during food storage result in the production of metabolites associated with off-odors. In this study, we evaluated the genotypic and phenotypic diversity of this species. A collection of 161 *B. thermosphacta* strains isolated from different foods, spoiled or not, and from a slaughterhouse environment was constituted from various laboratory collections and completed with new isolates. A PCR test based on the *rpoB* gene was developed for a fast screening of *B. thermosphacta* isolates. Strains were typed by MALDI-

TOF MS, rep-PCR, and PFGE. Each typing method separated strains into distinct groups, revealing significant intra-species diversity. These classifications did not correlate with the ecological origin of strains. The ability to produce acetoin and diacetyl, two molecules associated with *B. thermosphacta* spoilage, was evaluated in meat and shrimp juices. The production level was variable between strains and the spoilage ability on meat or shrimp juice did not correlate with the substrate origin of strains. Although the *B. thermosphacta* species encompasses ubiquitous strains, spoiling ability is both strain- and environment-dependent.

Keywords

Meat; spoilage; seafood; off-odors; diversity; acetoin; diacetyl; *rpoB* species-specific PCR

Highlights

There is significant diversity between *B. thermosphacta* strains

Diversity is not related to the ecological origin of the isolates

The ability to produce acetoin and diacetyl depends on strains and food matrices

2.2.1. Introduction

Brochothrix thermosphacta is recognized as the dominant food spoiler of meat and seafood products stored under modified atmosphere packaging (Remenant et al., 2015). This ubiquitous microorganism has been isolated from foods of animal origin such as meat, seafood and dairy products (Stackebrandt and Jones, 2006). Moreover, it has been described as widely disseminated along the food chain, from the raw material to the final product, as well as in the food processing environment (Nychas et al., 2008a; Stackebrandt and Jones, 2006). *B. thermosphacta* can cause serious economic losses in the food industry due to its ability to produce metabolites associated with off-odors. For example in beef meat, it has been shown to produce cheesy and creamy dairy off-odors associated with the production of 3-hydroxy-2-butanone (acetoin), 2,3-butanedione (diacetyl), and 3-methyl-1-butanol (Casaburi et al., 2014; Dainty and Mackey, 1992). In cold-smoked salmon, *B. thermosphacta* produces 2-hexanone and 2-heptanone, two compounds responsible for the formation of the blue-cheese off-odor (Joffraud et al., 2001; Laursen et al., 2006; Mejlholm et al., 2005). Strong butter, buttermilk-like, sour, and nauseous off-odors caused by *B. thermosphacta* in cooked and

peeled shrimp have been associated with the production of 2,3-butanedione (diacetyl), 3-methyl-1-butanal, and 3-methyl-1-butanol (Jaffrès et al., 2011; Laursen et al., 2006; Mejlholm et al., 2005). Therefore, the molecules produced by *B. thermosphacta* seem to depend on the food matrix. However, the above-mentioned studies used different strains and thus it is possible that the spoilage potential is also strain-dependent.

B. thermosphacta is a facultative anaerobe that can grow on chilled meats and fish stored under low O_2 and under vacuum packaging (Borch et al., 1996; Drosinos and Nychas, 1997; Ercolini et al., 2006). Although glucose is not present at high concentration in meat, Gill and Newton (1977) reported that it is the preferred substrate of *B. thermosphacta* when grown in meat juice. In addition, glucose metabolism is greatly affected by the composition of the gas used for storage. Under aerobic conditions, 3-hydroxy-2-butanone (acetoin) and 2,3-butanedione (diacetyl) are the major metabolites produced by the consumption of glucose while under anaerobic conditions, *B. thermosphacta* produces lactic acid and ethanol (Dainty et al., 1985; Pin et al., 2002). Thus, spoilage activity may also vary depending on food storage conditions.

Brochothrix and *Listeria* genera constitute the *Listeriaceae* family, and the *Brochothrix* genus encompasses two non-pathogenic species: *B. thermosphacta* and *Brochothrix campestris*. However, little information is available for *B. campestris*, most of the available information refers mainly to a single strain (ATCC 43754, the type strain) (Talon et al., 1988).

Various molecular techniques have been widely applied to genotype foodborne pathogenic or spoilage bacterial species. Pulsed Field Gel Electrophoresis (PFGE) has been described as highly discriminatory, robust and reproducible (Gerner-Smidt et al., 2006; Graves and Swaminathan, 2001; Lukinmaa et al., 2004). It has been used successfully for genotyping *B. thermosphacta* meat isolates (Papadopoulou et al., 2012b). Repetitive-element Palindromic PCR (rep-PCR) has been widely applied for molecular typing and has proven to be a powerful tool in environmental and food microbiology (Ishii and Sadowsky, 2009). It can differentiate a wide range of bacterial species at the subspecies or even the strain level (Wolska and Szweda, 2012) and has been applied to the differentiation of *B. thermosphacta* (Papadopoulou et al., 2012b; Xu et al., 2010). Recently, various studies have also shown the applicability of Matrix Assisted Laser Desorption/Ionization Time Of Flight Mass Spectrometry (MALDI-TOF MS) for

bacterial identification, taxonomy and strain typing (Singhal et al., 2015). Widely used in clinical microbiology for identification purposes (Carbonnelle et al., 2011), this technique is an effective tool for the intra-specific typing of bacteria from the genera *Listeria* (Barbuddhe et al., 2008) and *Salmonella* (Dieckmann et al., 2008) and is increasingly applied for identifying and typing microorganisms associated with food (Böhme et al., 2011; Kern et al., 2014).

Several studies have led to different conclusions about the intra-species diversity of *B. thermosphacta* (Papadopoulou et al., 2012b; Stanborough et al., 2017). This may be due to the small number of strains, the low diversity of the ecological origin of the studied collections, or the use of different methods. In addition, the genetic functions involved in spoilage remain little studied. The analysis of draft genome sequences of 13 *B. thermosphacta* strains pointed out some genes potentially involved in spoilage activity, but did not reveal any strong diversity (Stanborough et al., 2017). Therefore, genetic diversity and its potential link with the ecological niches or spoilage ability of *B. thermosphacta* remain unsolved.

In order to investigate whether a correlation can be established between the ecological origins of *B. thermosphacta* strains, their diversity and their spoilage potential, we constituted a collection of strains from a wide range of ecological environments. MALDI-TOF MS, PFGE, and rep-PCR were used to assess diversity in the collection while phenotypic diversity was evaluated through the quantification of acetoin and diacetyl production, two molecules associated with spoilage by *B. thermosphacta*.

2.2.2. Materials and methods

2.2.2.1. Bacterial strains and growth conditions

The 161 *B. thermosphacta* strains isolated during this study (N = 80) or sourced from various collections (N = 81) are listed in Table 2.1. In addition, *B. campestris* ATCC 43754 (= DSM 4712), *Listeria innocua* ATCC 33090, *L. innocua* CLIP 11262, *L. monocytogenes* ATCC 35152, *L. monocytogenes* 08-5578 (Gilmour et al., 2010), *Carnobacterium maltaromaticum* ATCC 27865, *Carnobacterium divergens* V41 (Pilet et al., 1995), *Staphylococcus epidermidis* ATCC 12228, *S. epidermidis* RP62A (Gill et al., 2005), *Serratia liquefaciens* ATCC 27592, and *Escherichia coli* K12 were used as controls for various purposes. Bacteria were routinely grown in BHI broth (VWR Chemicals, France) at 25 °C for *Brochothrix* sp., 30 °C for *Listeria* sp., *Carnobacterium* sp., *S. epidermidis*, and *S. liquefaciens*, and at 37 °C for *E. coli*.

Minced beef meat and peeled shrimp juices were used as broth to quantify acetoin and diacetyl production by *B. thermosphacta* strains. Meat juice was prepared by stomaching ground beef, collected frozen from a local supermarket, as previously described by Rantsiou et al. (2012), and filtered through a 0.45 µm membrane filter before sterilization with a 0.2 µm membrane filter. Shrimp juice was prepared by crushing frozen raw peeled shrimp from Ecuador (91/100 without sulfite, purchased from industry, Nantes) in sterile distilled water. The shrimp based mixture was heated (100 °C; 2 min), filtered and autoclaved (100 °C; 30 min) as previously described by Fall et al. (2010). Five milliliter aliquots were then stored frozen at -20 °C in 15 ml tubes until use.

Bacterial enumeration was performed after 48 h of incubation on Plate Count Agar (PCA) (Biomerieux, France) at 30 °C and *B. thermosphacta* selective STAA agar base containing STAA selective supplement (Oxoid, France) at 25 °C, to determine the total aerobic and *B. thermosphacta* counts, respectively.

Table 2.1 *B. thermosphacta* isolates used in this study

Ecological origin (a)	**Strains**	**Laboratory collection**	**Reference**
Beef slaughterhouse environment (7)	BSAS1 1, BSAS1 3, BSAS2 4, BSBS1 3, BSBS1 6, BSAS2 3, BSK1 3	INRA-SECALIM	This study
Chicken legs (48)	TAP 54, TAP 56, TAP 57, TAP 58, TAP 61, TAP 62, TAP 63, TAP 64, TAP 68, TAP 69, TAP 73, TAP 74, TAP 76, TAP 78, TAP 81, TAP 104, TAP 108, TAP 109, TAP 110, TAP 111, TAP 123, TAP 125, TAP 129, TAP 105, TAP 107, TAP 126, TAP 142, TAP 143, TAP 144, TAP 146, TAP 147, TAP 148, TAP 164, TAP 166, TAP 168, TAP 169, TAP 170, TAP 171, TAP 172, TAP 175, TAP 176, TAP 180, TAP 199, TAP 201, TAP 202, TAP 203, TAP 204, TAP 206, TAP 207	INRA-SECALIM	This study
Beef meat (25)	VHB2, VHB3, VHU1, VHU2, VHU3, VHF DLC1 1, VHF DLC1 2, VHF DLC1 3, VHF DLC2 1, VHF DLC2 2, VHF DLC2 3	INRA-SECALIM	This study
	V2, G8, G6, G7, MFPA17A17-02, MFPA19A15-05, MFPA22A14-04, MFPA22A14-05, MFPA42A14-07, MFPA43A14-06, MFPB17A13-02, MFPB42A12-05, MFPB43D06-02, MFPB43A12-01	INRA-MICALIS/FME	(Lucquin et al., 2012)
Lamb meat (1)	8727		
Horse meat	160X7, 160X8		
Beef and lamb sausages (4)	M1, M2, M4, M6		
Pork meat (10)	DSM 20171T = ATCC 11509, DSM 20599	DSM/ATCC	(McLean and Sulzbacher, 1953; Sneath and Jones, 1976)
	FMCC B-427, FMCC B-428, FMCC B-429, FMCC B-430, FMCC B-431, FMCC B-432, FMCC B-433, FMCC B-434	LFMB	
Shrimps (20)	CD 251, CD 252, CD 266, CD 274, CD 280, CD 290, CD 321, CD 322, CD 326, CD 331, CD 337, CD 340, CD 350, CD 352, CD 355, CD 357, CD 358, CD 372, CRE 2330, CRE 2333	INRA-SECALIM / IFREMER-EM3B	(Jaffrès et al., 2009)
Cod fillet (4)	EBP 3017, EBP 3018, EBP 3032, EBP 3033	IFREMER-EM3B	(Chaillou et al., 2015)
Salmon (30)	EBP 3069, EBP 3070, EBP 3083, EBP 3084, SF 677, SF 678, SF 711, SF 712, SF 713, SF 746, SF 748, SF 750, SF 779, SF 781, SF 782, SF 1173, SF 1186, SF 1216, SF 1234, SF 1820, SF 1838, SF 1849, SF 1926, SF 1930, SF 1939, MIP 2440, MIP 2490, MIP 2576, MIP 2599, MIP 2622	INRA-SECALIM / IFREMER-EM3B	
Sea bream (8)	FMCC B-112, FMCC B-113, FMCC B-114, FMCC B-115, FMCC B-116, FMCC B-117, FMCC B-118, FMCC B-119	LFMB	
Cheese rind (1)	cH8.14	INRA-URF	(Almeida et al., 2014)
Unknown (1)	5X10003	INRA-MICALIS/FME	

a: Number of strains; IFREMER-EM^{3}B: French Research Institute for Exploitation of the Sea, Nantes, France; INRA-MICALIS/FME: UMR INRA/AgroParisTech (Microbiologie de l'alimentation au service de la santé), Jouy en Josas, Paris, France; INRA-SECALIM: UMR INRA/Oniris (Sécurité des Aliments et Microbiologie), Nantes, France; INRA-URF: Unité de Recherches Fromagères, INRA Aurillac, France ; LFMB: Laboratory of Food Microbiology and Biotechnology, Agricultural University of Athens, Greece

2.2.2.2. Sampling new B. thermosphacta isolates

Sampling was carried out in a beef slaughterhouse at five points: (i) the chilling room (walls and floors), (ii) the nacelle receiving the viscera, (iii) the knives used for skinning, (iv) animal skin and (v) cattle barns (floor and walls). About 10 cm^2 of knife surfaces were sampled by rubbing cotton swabs five times in both vertical and horizontal directions for 30 seconds. Other surfaces (walls, floors, nacelles, skin) were sampled using sterile wipes impregnated with peptone water. Samples were transported from the collection site to the laboratory in a cooler (4 °C) and analyzed immediately. Swab and wipe samples were homogenized by shaking manually with 10 ml and 25 ml of peptone water (Biokar Diagnostics, France), respectively. New isolates from ground beef meat and chicken cuts were also collected in the present study. Chicken cuts were rinsed in peptone water as previously described (Rouger et al., 2017) whereas beef meat was stomached in 0.9% NaCl solution for 3 min. Then, appropriate decimal dilutions were plated on PCA and STAA plates for bacterial enumeration. Three to four colonies were selected from STAA plates, then purified on Brain Heart Infusion (BHI) agar (VWR Chemicals, France) and stored at -80 °C in BHI broth supplemented with 20% (v/v) glycerol (VWR Chemicals, France).

2.2.2.3. DNA extraction

DNA was extracted from 2 ml of overnight cultures with the DNeasy blood and tissue kit (Qiagen, France) according to the manufacturer's instructions. DNA concentration and purity were estimated after electrophoresis on 1% agarose. DNA extracts were stored at -20 °C.

2.2.2.4. rpoB species-specific primer design and PCR conditions

A PCR primer set was designed to amplify a DNA fragment specific to *B. thermosphacta,* excluding *B. campestris*, other closely related species (such as *Listeria*) and species present in the same environments or reported as growing on STAA medium (such as *Carnobacterium,* or *Staphylococcus* sp.). The *in-silico* primer design was based on the multiple alignment of *rpoB* gene sequences available from the GenBank database. The *rpoB* sequences of *B. thermosphacta* ATCC 11509, *B. campestris* ATCC 43754, and the most closely related bacterial species were aligned using the BioEdit-ClutalW Sequence Alignment program (Hall, 1999).

Specific primers were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/). The *in-silico* specificity of primers was assessed by nucleotide BLAST [National Center for Biotechnology Information (NCBI)] analysis and Primer3. Oligonucleotides with the highest specificity for the *B. thermosphacta* sequence, without hairpin structures or dimers, were selected. This resulted in the design of the forward primer (*rpoB*-Fw1_154-175: 5'-GCGTGCATTAGGTTTCAGTACA-3') and the reverse primer (*rpoB*-Rev1_525-547: 5'-TCCAAGACCAGACTCTAATTGCT-3') for the specific amplification of 394 bp of the *B. thermosphacta rpoB* gene. Primer specificity was then assessed by PCR amplification on the DNA extracted from *B. campestris*, *Listeria* sp., *Carnobacterium* sp., and *Staphylococcus* sp.

Amplifications were performed in a 50 µl reaction volume containing: 1 µl (50-100 ng) of DNA, 5 µl of 10X *Taq* Buffer (New England Biolabs, France), 0.2 µM of dNTP (New England Biolabs, France), 0.4 µM of each primer and 1.5 U of *Taq*-polymerase (New England Biolabs, France). PCR reactions were carried out with a PTC-100 Thermocycler (Bio-Rad Laboratories, France) using the following amplification conditions: initial denaturation step at 95 °C for 5 min, followed by 25 cycles of [denaturation (95 °C for 30 s), primer annealing (66 °C for 30 s), primer extension (72 °C for 30 sec)], and a final extension step at 72 °C for 5 min. Amplicons were separated in a 1.5% (w/v) agarose gel containing 0.05X of Sybr Safe (Invitrogen Life Technologies, France) in TAE buffer. The gel was visualized under UV transillumination (Bio-Rad Laboratories, France).

2.2.2.5. 16S rDNA sequencing

The 16S rDNA (about 1500 bp) was amplified by PCR according to Jaffrès et al. (2009). Fragments were partially sequenced (about 800 bp) using the Eurofins Genomics service (Les Ulis, France). The resulting sequences were cleaned then assembled into a unique contig sequence with BioEdit software (Hall, 1999). A BLAST search of partial 16S rRNA gene sequences was performed in the NCBI database (NCBI, Bethesda, USA).

2.2.2.6. MALDI-TOF

For MALDI-TOF MS analysis, fresh cultures of Brochothrix sp. incubated at 25 °C were centrifuged (10 min; 3,000 g; 4 °C) and rinsed in 1 ml of molecular biology grade water. One microliter of the bacterial suspension was spotted in a square-form onto the sample target plate in 8 replicates (Bruker Daltonics, Germany) and allowed to dry in a biosafety cabinet at

ambient temperature. Each droplet was overlaid with 1 µl of HCCA matrix solution, a saturated solution of alpha-cyano-4-hydroxy cinnamic acid in 50% acetonitrile with 2.5% trifluoroacetic acid (Bruker Daltonics, Germany), and then dried as above. As a positive control for each run and for calibration purposes, one spot was also covered by the Bacterial Test Standard (BTS) mixture (Bruker Daltonics, Germany). Measurements were made using the manufacturer's recommended settings (linear positive mode, Nitrogen Laser with 60 Hz repetition rate, 20-kV acceleration voltage, 18.5-kV IS2 voltage, 250 ns extraction delay, and 2,000 to 20,000 m/z range). For each sample, mass spectra were examined visually using FlexAnalysis (Bruker Daltonics V3.4) to identify large spot-to-spot inconsistent variations. Spectra were then imported into a Matlab (MathWorks) script, which performs smoothing, normalization, baseline subtraction and peak selection automatically. From a selected peak list associated to intra-species variations, a dendrogram was generated using the Euclidean distance measure and an average linkage.

2.2.2.7. Rep-PCR

DNA was subjected to rep-PCR analysis according to Ouoba et al. (2008) using (GTG)5 primer (5'-GTGGTGGTGGTGGTG-3'). Amplicons were separated in a 2% (w/v) agarose gel in 1 x TAE at 3 V/cm for 3 h. After the run, gels were stained with 0.5 µl/ml Syber Safe (Invitrogen Life Technologies, France) for 1 h and then visualized with UV transillumination (Bio-Rad Laboratories, France). DNA profiles were analyzed with Bionumerics software, version 6.5 (Applied-Maths, Belgium). Isolates were compared using the band-based Dice coefficient (optimization: 0.5%; tolerance: 1%) and UPGMA (unweighted pair-group method using the average approach) cluster analysis.

2.2.2.8. PFGE

Genomic DNA from 2 ml of overnight cultures was prepared in low-melting-point agarose plugs as described by Doulgeraki et al. (2010), and digested with the endonuclease *Apa*I (New England Biolabs, France) according to the manufacturer's instructions. Electrophoresis was performed on the CHEF-DRIII PFGE system (Bio-Rad Laboratories, France) in 1% (w/v) agarose gels with 0.5 x TBE as the running buffer, at 14 °C. A lambda ladder (Bio-Rad Laboratories, France) was used as the molecular weight marker. Restriction fragments were resolved at a constant voltage of 6 V/cm with switch times of 4–40 s for 18 h and 4–12 s for 4 h. Gels were

stained with 0.5 mg/ml ethidium bromide, and DNA bands were visualized with UV transillumination (Bio-Rad Laboratories, France). PFGE profiles were analyzed using the BioNumerics Software, version 6.5 (Applied-Maths, Belgium), and then compared using the Pearson coefficient (optimization: 0.5%; curve smoothing: 0%) and UPGMA cluster analysis.

2.2.2.9. Acetoin/diacetyl production

Voges-Proskauer reaction

Tests were performed in 48-well plates (Falcon, France) on 0.5 ml samples pipetted from 10 ml cultures collected after 48 h of growth at 25 °C and gentle resuspension of cells. A volume of 75 µl of alpha-naphthol (5% (w/v) in 95% ethanol) and 50 µl of KOH [40% (w/v) in water] was added. Plates were incubated at room temperature for 1 h. The level of acetoin production was assessed visually using a six-point scale based on the color intensity and noted from (0): not produced or light yellow to (5): very high production or strong red color.

Acetoin and diacetyl quantification

Five milliliter aliquots of meat or shrimp juice were gently defrosted at 4 °C and then inoculated (1:100) with overnight cultures grown at 25 °C in BHI broth. After incubation for 48 h at 25 °C, 2 ml aliquots were centrifuged for 10 min at 10,000 *g* and the supernatant was recovered for acetoin and diacetyl quantification as described by (Nicholson, 2008; Westerfeld, 1945). The reaction was carried out on 200 µl of culture supernatant by the addition of 140 µl of creatine [0.5% (w/v) in water], 200 µl of alpha-naphthol [5% (w/v) in 95% ethanol], and 200 µl of KOH [40% (w/v) in water]. A blank was prepared simultaneously with non-inoculated meat and shrimp juices. Absorbance at 560 nm of the samples was measured in a spectrophotometer (Spectronic Genesys 5) after incubation at room temperature for 10 min (for diacetyl) and 1 h (for acetoin). Standard curves were constructed with controls containing increasing acetoin and diacetyl concentrations and used to calculate acetoin and diacetyl production. pH was measured at the end of the experiments using a Crison pH-meter (Crison micro pH 2000, Spain). Analyses were performed in triplicate.

2.2.2.10. Statistical analysis

Hierarchical clustering and multidimensional scaling

Similarities between isolates were first converted into dissimilarities by complement to 1 of the Dice coefficient (for rep-PCR) and the Pearson coefficient (for PFGE). These dissimilarities were analyzed using two different statistical techniques: the hierarchical clustering of isolates and the factorial representation of individuals by Multidimensional Scaling (MDS).

Dendrograms of isolates were obtained by hierarchical clustering of the two matrices of dissimilarities. The agglomerative procedure was the UPGMA, also known as the group average linkage (Everitt et al., 2001).

Multidimensional scaling aims to derive a factorial representation of individuals from a measure of the dissimilarity between them (Borg and Groenen, 2005). The two matrices of dissimilarities between isolates were summed and submitted to MDS in order to produce a spatial configuration representing the distances between isolates. The quality of fit was measured by the stress index, which is the sum of the squared differences between the initial dissimilarities and distances in the configuration. The obtained stress value was 0.147 with 6 dimensions, indicating an acceptable goodness of fit of the MDS configuration (Krzanowski, 1990). The final configuration was rotated in order to interpret the results more easily.

Data were statistically analyzed with the R packages *cluster* (for hierarchical clustering) and *smacof* (for multidimensional scaling).

Analysis of variance

Analysis of variance (ANOVA) with R version 3.3.2 (C) 2016 (The R Foundation for Statistical Computing) was performed to determine statistically significant differences between strains grown in meat and shrimp juices. P-values < 0.05 were considered statistically significant.

2.2.3. Results

2.2.3.1. Constitution of a B. thermosphacta strain collection

As the purpose of the study was to investigate the genotypic and phenotypic diversity of the species and to determine whether a correlation exists between genotype and ecological origin or spoilage potential, we wanted to constitute as diverse a strain collection as possible. First, 79 isolates from spoiled or non-spoiled meat, seafood and milk products were provided by different laboratories. We included DSM 20599 and the type strain ATCC 11509 (= DSM 20171), both isolated from pork meat. To complete the collection, chicken cuts and a beef slaughterhouse environment were sampled as no such isolate was represented in the available collection. New isolates from non-spoiled ground beef meat were also added for comparison with those provided by other laboratories. More than 200 new isolates were collected and 80 were kept for further analysis after removal of putative redundant strains, using a preliminary rep-PCR analysis. In total, a collection of 161 *B. thermosphacta* strains was selected for analysis (Table 2.1).

2.2.3.2. rpoB species-specific PCR test

As *B. campestris* isolates are scarce (only 5 strains have been reported in the literature ((Illikoud et al., 2018) and references therein), we developed an accurate and reproducible PCR assay for a fast identification of *B. thermosphacta* isolates since both species cannot be discriminated through their 16S rDNA sequence. The PCR assay was designed to target a 394 bp region of the *B. thermosphacta rpoB* gene. The specificity of the primer set (*rpoB*-Fw1_154-175/*rpoB*-Rev1_525-547) was tested against a range of DNA from closely related bacterial species, such as *B. campestris*, and some *Listeria* or lactic acid bacteria species (Figure 2.1).

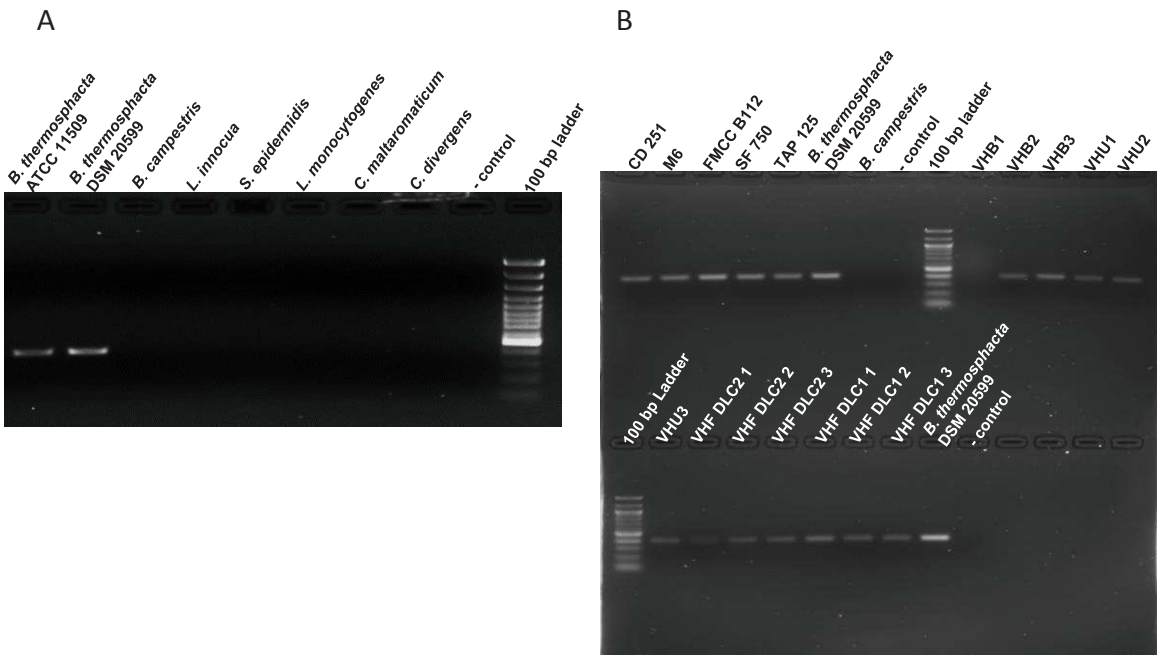

Figure 2.1 Specificity of the specific PCR assay.

A: Performance of the *B. thermosphacta*-specific PCR assay with DNA obtained from representative *Brochothrix* species and closely related bacteria. **B:** New isolates from chicken cuts (TAP 125) and beef meat (VHB2, VHB3, VHU1, VHU2, VHU3, VHF DLC2 1, VHF DLC2 2, VHF DLC2 3, VHF DLC1 1, VHF DLC1 2, and VHF DLC1 3) were identified as *B. thermosphacta*. Strains from external laboratories: *B. thermosphacta* ATCC 11509, *B. thermosphacta* DSM 20599, *B. campestris* ATCC 43754, CD 251, M6, FMCC B-112, and SF 750 were included. VHB1 isolate was negative and thus considered as not belonging to the *B. thermosphacta* species. A 100 bp ladder (New England Biolabs, France) and a negative (-) control were included.

Amplification was only observed from *B. thermosphacta* DNA, showing the specificity of the assay. The 80 new isolates were then tested by this species-specific *rpoB*-PCR assay and all were identified as *B. thermosphacta*.

2.2.3.3. Characterization of genotypic the intra-species diversity

The diversity among the 161 *B. thermosphacta* strains was assessed by MALDI-TOF MS, rep-PCR, and PFGE typing methods. The *B. campestris* type strain ATCC 43754 was included as a control.

MALDI-TOF MS spectra were obtained for all strains. Cluster analysis was performed on all the spectra to visualize similarities between those of different strains. This generated a dendrogram composed of 14 groups, named from A to N (Supplementary Figure 2.5). Two

groups (M and N) encompassed about 87% of the collection (N =113 and 26 strains, respectively) with other groups containing only 1 to 4 strains. Strains from different ecological origins were distributed in all groups. Most groups included strains isolated from different ecological origins while groups G, H, J, and L encompassed only 2-4 chicken cut isolates. *B. thermosphacta* TAP 107 and TAP 104 from group G came from the same sample, thus we cannot exclude that these two isolates are redundant. Conversely, *B. thermosphacta* TAP 204 and TAP 206 from group H were isolated from different batches. *B. campestris* ATCC 43754 was the only member of group B. By exploiting only the intra-species spectral variations, typing with MALDI-TOF did not report accuracy the phylogenetic distances between the two species of *Brochothrix* sp. For this reason, MALDI-TOF MS did not clearly differentiate *B. thermosphacta* from *B. campestris* since another *B. thermosphacta* isolate (MFPA43A14-06) stayed as an outgroup (Supplementary Figure 2.5).

The electrophoresis profiles of rep-PCR products yielded 3 to 11 bands, depending on strains. The amplification products were mainly in the range of 0.5 to 3 kb and a quite large diversity between profiles was observed. The UPGMA clustering analysis of the rep-PCR profiles obtained for all strains produced the dendrogram shown in Supplementary Figure 2.6. *B. campestris* ATCC 43754 was clearly located in a separate external cluster whereas all *B. thermosphacta* strains formed a single cluster. By applying a 60% similarity coefficient, the dendrogram analysis generated 12 groups (named A to L) in the *B. thermosphacta* cluster. Each group was composed of at least two isolates from different ecological origins and different laboratory collections, except for group L, which included two isolates (TAP 105 and TAP 199) from different chicken cut batches. Some isolates, all from sea bream and the same collection (such as FMCC B-116, FMCC B-118, and FMCC B-119 from group I) had very similar profiles and may be redundant. This was again observed for TAP 107 and TAP 104 in group C. However, these two chicken meat isolates also harbored a profile very close to that of CRE 2333, EBP 3069, EBP 3070, SF 677, and SF 711, all from different seafood products and collected from different laboratories. Using a similarity coefficient of 80% was not more informative as it generated 50 groups, 20 of which were composed of a single strain.

By PFGE, using *Apa*I as the restriction enzyme, *B. thermosphacta* CD 355, MIP 2622, TAP 57, TAP 63 and TAP 78 showed only one band or could not be lysed. These isolates were therefore

excluded from the analysis. The UPGMA cluster analysis of the PFGE profiles obtained for the remaining strains resulted in the dendrogram shown in Supplementary Figure 2.7. The dendrogram analysis, applying a 35% similarity coefficient, generated 18 groups (A to R) with only 2 single-strain groups (*i.e.* D and R). Except for these two groups and group M composed of two strains isolated from cooked and peeled shrimp, each group comprised at least two strains from different ecological origins. In addition, these groups comprised strains from at least two different laboratory collections. With a 60% similarity coefficient, 75 groups were differentiated including 34 groups consisting of single strains. The dendrogram analysis with an 80% similarity coefficient generated 130 groups including 108 single-strain groups. PFGE did not differentiate *B. campestris* ATCC 43754 from *B. thermosphacta* strains. In fact, this strain appeared in group I with *B. thermosphacta* FMCC B-116, FMCC B-118, M1 and VHB2.

Multidimensional scaling (MDS), obtained by summing the two matrices of dissimilarities from the rep-PCR and PFGE analyses, produced a spatial configuration representing the distances between isolates (Figure 2.2). It revealed significant intra-species diversity within the strain collection. MDS also clearly illustrated the absence of ecotype in *B. thermosphacta*: the intra-species diversity was not related to the ecological origin of the strains as isolates from the various origins were widely distributed and no strain clustering associated with a particular environment was observed.

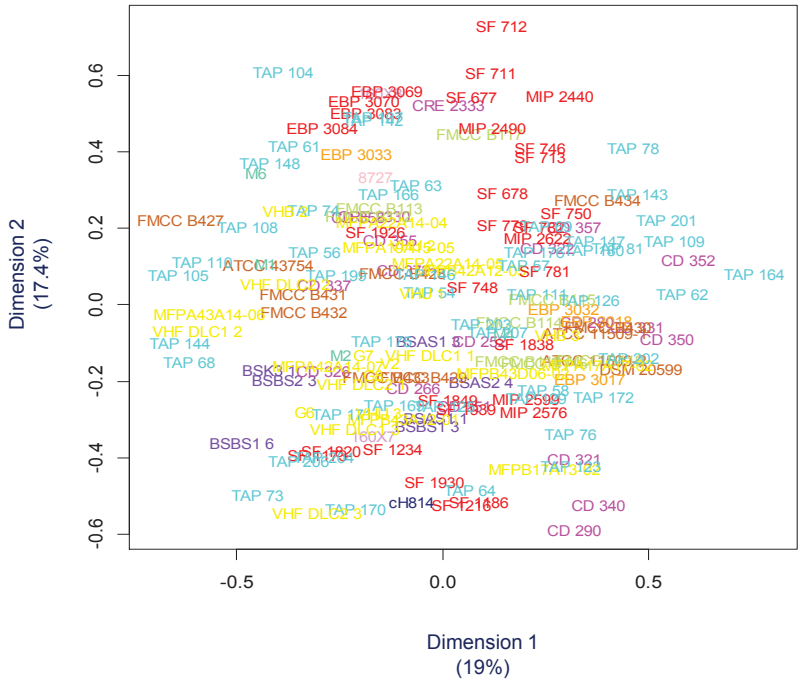

Figure 2.2 Multidimensional scaling of summed rep-PCR and PFGE distance matrices.

Each strain is presented with a color code illustrating its ecological origin: (■) beef meat, (■) pork meat, (■) lamb meat, (■) horse meat, (■) beef + lamb meat, (■) chicken meat, (■) shrimp, (■) salmon, (■) sea bream, (■) cod fillet, (■) cheese, and (■) slaughterhouse environment.

2.2.3.4. Acetoin and diacetyl production

All strains were first screened to estimate their ability to produce acetoin in a laboratory medium using the Voges-Proskauer reaction. Levels of acetoin and diacetyl were estimated using a six-point scale, based on the color intensity, noted from (0): no production to (5): very high production. Negative (*E. coli* K12, which does not produce acetoin) and positive (acetoin-producing *S. liquefaciens* ATCC 27592) controls were included (Figure 2.3A). The production level varied between strains. For example, 12% of the strains similar to the negative control and noted (0) did not produce acetoin, while only 2% produced very high levels (noted (5), far above the positive control) (Figure 2.3B).

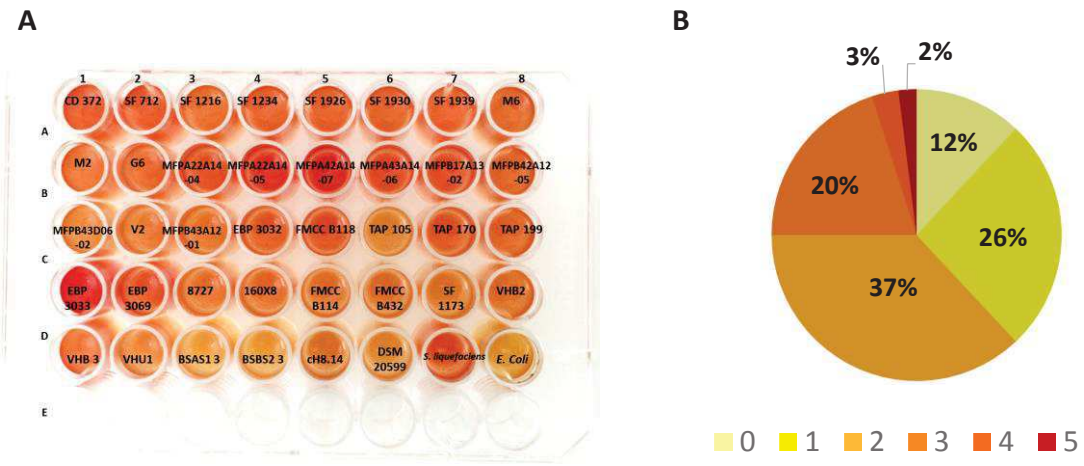

Figure 2.3 Acetoin production using the Voges-Proskauer test.

The level of acetoin production was visually estimated using a six-point scale based on the color intensity and noted from: (0): not produced (light yellow color) to (5): very high production (strong red color). **A:** Example of the results of the acetoin production assay for *B. thermosphacta* strains, *S. liquefaciens* (positive control), and *E. coli* (negative control). **B:** Pie-chart representing the overall results for 161 *B. thermosphacta* strains

Thirteen strains representing the 6 classes mentioned above (low to high producers) were chosen for further analysis to measure acetoin/diacetyl production after growth in meat and shrimp juices. Diverse strains were selected, taking into account the ecological and geographical origins and the diversity assessed by MALDI-TOF MS, rep-PCR and PFGE. The *B. thermosphacta* and *B. campestris* type strains and *B. thermosphacta* DSM 20599 were also included. All tested strains produced both molecules in meat and shrimp juices, and the production level was both strain- and food matrix-dependent (Figure 2.4). For all strains, acetoin and diacetyl were produced at higher concentrations in meat juice than in shrimp juice. In meat juice, the production level ranged from 26.87 ± 8.56 µg/ml to 129.06 ± 41.32 µg/ml for diacetyl, and from 51.65 ± 6.32 µg/ml to 111.48 ± 2.84 µg/ml for acetoin. *B. thermosphacta* EBP 3070, a strain isolated from a spoiled fish product, was the highest producer in shrimp juice, with 22.86 ± 3.19 µg/ml acetoin and 30.24 ± 2.78 µg/ml diacetyl. In shrimp juice, the lowest production levels were observed with the *B. thermosphacta* type strain (5.30 ± 1.01 µg/ml acetoin and 5.41 ± 1.67 µg/ml diacetyl) and *B. thermosphacta* BSAS1 3 (7.05 ± 1.20 µg/ml acetoin and 7.94 ± 1.71 µg/ml diacetyl), which was isolated from the environment. *B. thermosphacta* EBP 3033, EBP 3070, and TAP 175 were among the higher producers of acetoin and diacetyl in shrimp juice and lower producers in meat juice.

Conversely, CD 337 and BSAS1 3 were high producers of acetoin and diacetyl in meat juice and lower producers in shrimp juice. Finally, *B. campestris* ATCC 43754 was among the lowest acetoin and diacetyl producer, whatever the juice used. The quantitative data obtained on shrimp juice correlated with those of the screening test performed in BHI medium. Indeed, the highest producers EBP3033, EBP 3070, and TAP 175 (Figure 2.4) were noted (5), (5), and (4), respectively using the six-point scale-based color intensity of the preliminary test. On the opposite, the lowest producers ATCC 11509 and BSAS1 3 were noted (2) and (1), respectively.

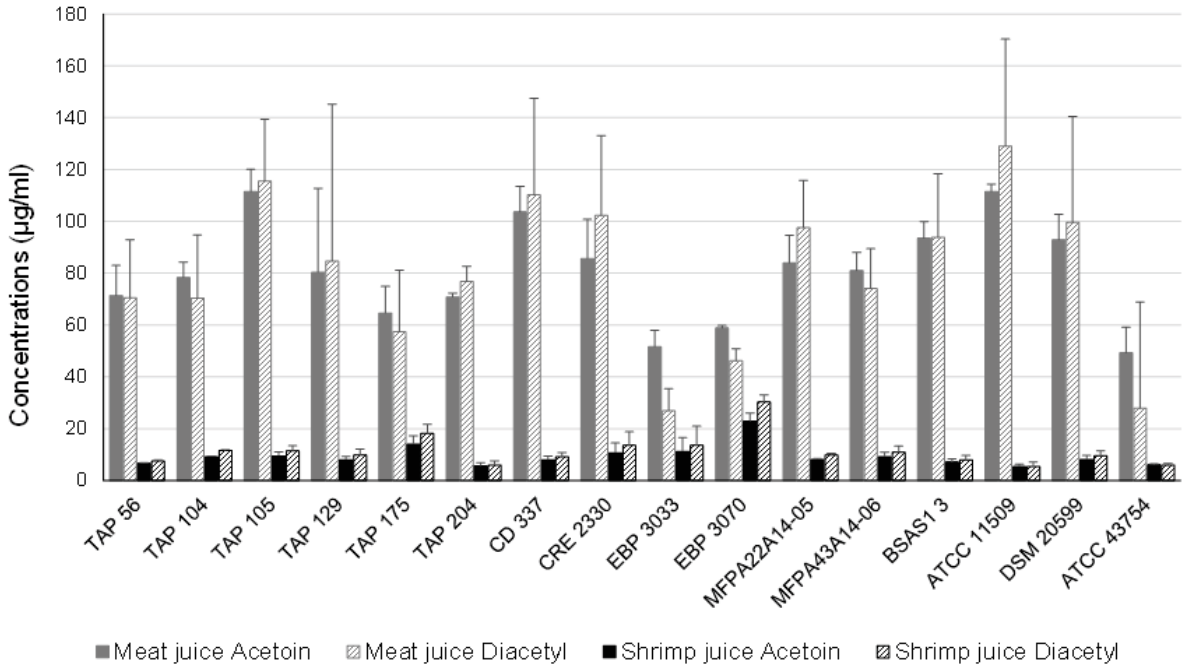

Figure 2.4 Acetoin and diacetyl production by *Brochothrix* sp. strains in meat and shrimp juices.

Fifteen *B. thermosphacta* strains and *B. campestris* ATCC 43754 were tested for their ability to produce acetoin and diacetyl after 48 h of culture in meat and shrimp juices. Data are expressed as the mean ± SD of three biological replicates.

Bacterial enumeration showed that the bacterial population reached at the end of the experiment was higher in shrimp juice than in meat juice. Counts varied according to strains from 6.06 ± 0.05 log CFU/ml to 6.72 ± 0.20 log CFU/ml in meat juice and from 7.75 ± 0.11 log CFU/ml to 8.31 ± 0.12 log CFU/ml in shrimp juice. After 48 h of incubation, the pH of non-inoculated meat and shrimp juices was 5.54 ± 0.19 and 6.40 ± 0.16, respectively. For all strains, the final pH reached 5.03 ± 0.062 and 5.66 ± 0.104 in meat and shrimp juices, respectively.

2.2.4. Discussion

The diversity of the microbial populations involved in the spoilage of meat and seafood products has been widely studied and documented (Dainty and Mackey, 1992; Jaffrès et al., 2011; Koutsoumanis and Nychas, 1999; Nychas et al., 2008a; Remenant et al., 2015). These studies have shown that *B. thermosphacta* plays an important role in the spoilage of these products. Although the diversity of its spoilage potential depending on the strains has been described, it remains poorly understood (Casaburi et al., 2014). The aims of our study were to evaluate the intra-species diversity in a large and diverse collection of *B. thermosphacta* isolates and to investigate whether this diversity could be correlated with their ecological origin and/or their ability to produce spoilage compounds.

Half of the collection was constituted of new isolates collected for this study. Since the two closely related species *B. campestris* and *B. thermosphacta* cannot be distinguished through their 16S rDNA sequence, we developed a PCR assay based on the *rpoB* gene to identify *B. thermosphacta* accurately among the new isolates. The *rpoB* housekeeping gene, encoding the RNA polymerase beta-subunit, has been described as a useful and relevant target for bacterial identification and phylogenetic studies (Adékambi et al., 2009; Case et al., 2007; Mollet et al., 1997). The specificity of this PCR assay was confirmed against a range of DNA from closely related bacterial species, such as lactic acid bacteria and *Listeria* species. This enabled the differentiation of both *Brochothrix* species and the identification of new isolates as *B. thermosphacta.* In fact, of 207 chicken cut isolates, 206 were positive to the *rpoB* PCR test, and the other one was identified as *Enterococcus faecalis* by partial 16S rDNA sequencing. Similarly, 11 out of 12 ground beef new isolates were identified as *B. thermosphacta* by this PCR assay (Figure 2.1B).

The collection included 161 *B. thermosphacta* isolates from the environment and various food matrices of animal origin (cheese; chicken, pork, beef, horse, and lamb meats; cod, salmon, sea bream, and shrimp). Some were isolated from spoiled food and others were collected from unspoiled products and before the use-by-date. MALDI-TOF MS, rep-PCR, and PFGE typing methods were selected to investigate the diversity in the collection because they are based on different principles. Rep-PCR provides fingerprints related to the presence of small repeats within the genome (Ishii and Sadowsky, 2009) while electrophoretic PFGE profiles are

generated by the separation of DNA fragments after genomic DNA digestion with rare-cutting restriction enzymes (Li et al., 2009b). MALDI-TOF generates peptide mass fingerprinting of proteins, mainly ribosomal ones as they are the most abundant and are constitutively synthesized (Rahi et al., 2016).

PFGE and rep-PCR revealed a significant diversity between *B. thermosphacta* isolates. Analysis of rep-PCR profiles by applying 60% or 80% similarity coefficients generated 12 and 50 groups, respectively. With PFGE dendrograms, these similarity coefficients distinguished 75 and 130 groups. Our results thus revealed a greater diversity than reported in previous studies on *B. thermosphacta*. For example, a PFGE analysis performed on 302 *B. thermosphacta* pork isolates distinguished only 8 groups (Papadopoulou et al., 2012b), while rep-PCR used on 27 *B. thermosphacta* isolates from meat, poultry and seafood reported only minor differences between isolates (Xu et al., 2010). The unique pork ecological origin in the first study and the small number of isolates in the second one most probably explain the low diversity reported by these authors. Compared to the observations made with the two DNA-based methods, MALDI-TOF MS typing showed a lower diversity as a major cluster encompassed more than 70% of the strains we tested. MALDI-TOF MS has been shown to discriminate *L. monocytogenes* (Barbuddhe et al., 2008; Ojima-Kato et al., 2016), *Lactobacillus brevis* (Kern et al., 2014), and *E. coli* (Siegrist et al., 2007) strains successfully. However, this method was not suitable for differentiating the two subspecies *Lactococcus lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* (Tanigawa et al., 2010). The same failure was reported for species belonging to the genera *Bacillus* and *Pseudomonas* (Ghyselinck et al., 2011), suggesting that the resolution of MALDI-TOF MS is taxon-dependent (Ghyselinck et al., 2011). To our knowledge, MALDI-TOF MS has been successfully used for identifying *B. thermosphacta* isolates from food (Höll et al., 2016), but never for investigating diversity within this species. Moreover, the available databases contain a limited number of spectra for some bacterial species.

Whatever the method used, we observed that the different ecological origins (meat, milk, seafood, or slaughterhouse environment) were widely distributed in all groups. In addition, most groups encompassing a reasonable number of strains (more than 2) included isolates from different ecological and geographical origins. Furthermore, most of the groups contained

isolates from spoiled and non-spoiled food and from both processed and unprocessed products. This suggests that the strains belonging to the different groups may have a common contamination pattern in the various meat and seafood products. These strains have adapted to grow in chilled meat and seafood products. We also noted that the groups comprised isolates recovered from products of different meat animal species as well as from the processing environment. This probably reflects the physiological capability of *B. thermosphacta* strains to grow in various food matrix ecosystems independently of the product type (raw or processed), the animal species from which it is derived (beef, pork, salmon, shrimp, etc.), and the packaging conditions (under air, vacuum packaged, modified atmosphere). In other words, no ecotype (strains sharing the same ecological niche) was observed. Of the 80 new isolates, for which information about batch origin was available, a few clustered together systematically, whatever the typing method used. Five pairs of such isolates (TAP 57/TAP 63, TAP 104/TAP 107, TAP 143/TAP 147, TAP 176/TAP 180, and TAP 204/TAP 206) were noticed and were collected from the same batches. Nevertheless, TAP 104, TAP 107, TAP 176, TAP 180, TAP 204, and TAP 206 showed differing acetoin production ability in the Voges-Proskauer test and were therefore not considered redundant.

Whether the spoiling potential of *B. thermosphacta* is strain-dependent is unknown. To evaluate this, we focused on acetoin and diacetyl production since both molecules have already been reported as associated with the spoilage of beef and chicken meat and seafood products (Casaburi et al., 2014; Franke and Beauchamp, 2017b; Jaffrès et al., 2011). We first screened the ability to produce acetoin using the Voges-Proskauer reaction from glucose fermentation in BHI laboratory medium. Our results showed that the production of this molecule was highly variable between strains and did not correlate with their origin (spoiled or non-spoiled products, nature of the food) or their genotypic clustering.

The combination of rep-PCR, MALDI-TOF and PFGE clustering and Voges-Proskauer reaction data was used to select a sub-set of thirteen distant strains for acetoin and diacetyl quantification. For this, sterile juices from two food matrices, beef meat and shrimp, were used in this study in order to avoid interference with the endogenous microbiota. Both acetoin and diacetyl were produced by *B. thermosphacta* in both matrices. These results differ from previous studies that reported no acetoin but only diacetyl production in cooked and peeled

shrimp packed under modified atmosphere (Jaffrès et al., 2011; Laursen et al., 2006). Conversely, Casaburi et al. (2014) reported that *B. thermosphacta* produced acetoin but not diacetyl in beef meat. These apparent contradictions may result from different methods and experimental conditions (shrimp packed under modified atmosphere *vs.* shrimp juice; pieces of beef meat stored aerobically *vs.* beef juice). Acetoin and diacetyl production was higher in meat juice than in shrimp juice although the bacterial population reached after 48 h of incubation was higher in shrimp juice than in meat juice. These observations could suggest that the production of these molecules is not related to the growth level of *B. thermosphacta*, but more probably to the biochemical composition of the food matrix. Moreover, acetoin and diacetyl production levels varied between strains. We noticed that *B. thermosphacta* EBP 3070 (isolated from spoiled salmon) and TAP 175 (isolated from non-spoiled chicken cuts) were among the highest producers of acetoin and diacetyl in shrimp juice but among the lowest ones in meat juice. Conversely, *B. thermosphacta* CD 337 (isolated from spoiled shrimp) and BSAS1 3 (isolated from the slaughterhouse environment) belonged to the highest acetoin and diacetyl producers in meat juice and to the lowest in shrimp juice. This shows that the spoilage ability of *B. thermosphacta* was both strain- and matrix-dependent but was not correlated to the food from which environment strains were isolated. This might result from the regulation of genes involved in the metabolic pathways that produce these molecules, which may vary depending on strains. This was recently suggested by the comparison of 13 *B. thermosphacta* draft genomes, which highlighted a large number of transcriptional regulators in these genomes but a small difference between strains (Stanborough et al., 2017). However, the genomes were all sequenced from strains of meat origin and may not represent the diversity of the *B. thermosphacta* species.

2.2.5. Conclusion

The present study revealed a significant diversity within the strain collection using rep-PCR, PFGE and MALDI-TOF typing methods. All these methods showed that there was no ecotype in this *B. thermosphacta* strain collection. The ability to produce acetoin and diacetyl in meat and shrimp juices varied between strains and did not correlate with the isolation from a spoiled or non-spoiled food product, suggesting that the spoiling ability of *B. thermosphacta* was most probably linked to strain properties rather than to the food environment from which

they were isolated. Based on these results four strains were selected for genome sequence comparison, and transcriptomics coupled to volatilome analysis will be performed for better understanding of the *B. thermosphacta* spoilage mechanisms.

Acknowledgements

This work was financed by the “Région Pays de la Loire” (grant to MZ). NI was the recipient of a PhD fellowship from the French Ministry of Higher Education and Research. We thank Stéphane Chaillou, Marie Champomier-Vergès, Marie-Christine Montel, Françoise Leroi, and George J. Nychas for kindly providing strains from INRA-MICALIS/FME, INRA-URF, IFREMER-EM^{3}B and LMBF, University of Athens, Greece collections, respectively. We are grateful to Amélie Rouger for providing isolates from poultry meat. We thank Carol Robins (Scientific English, Clisson, France) for reviewing English.

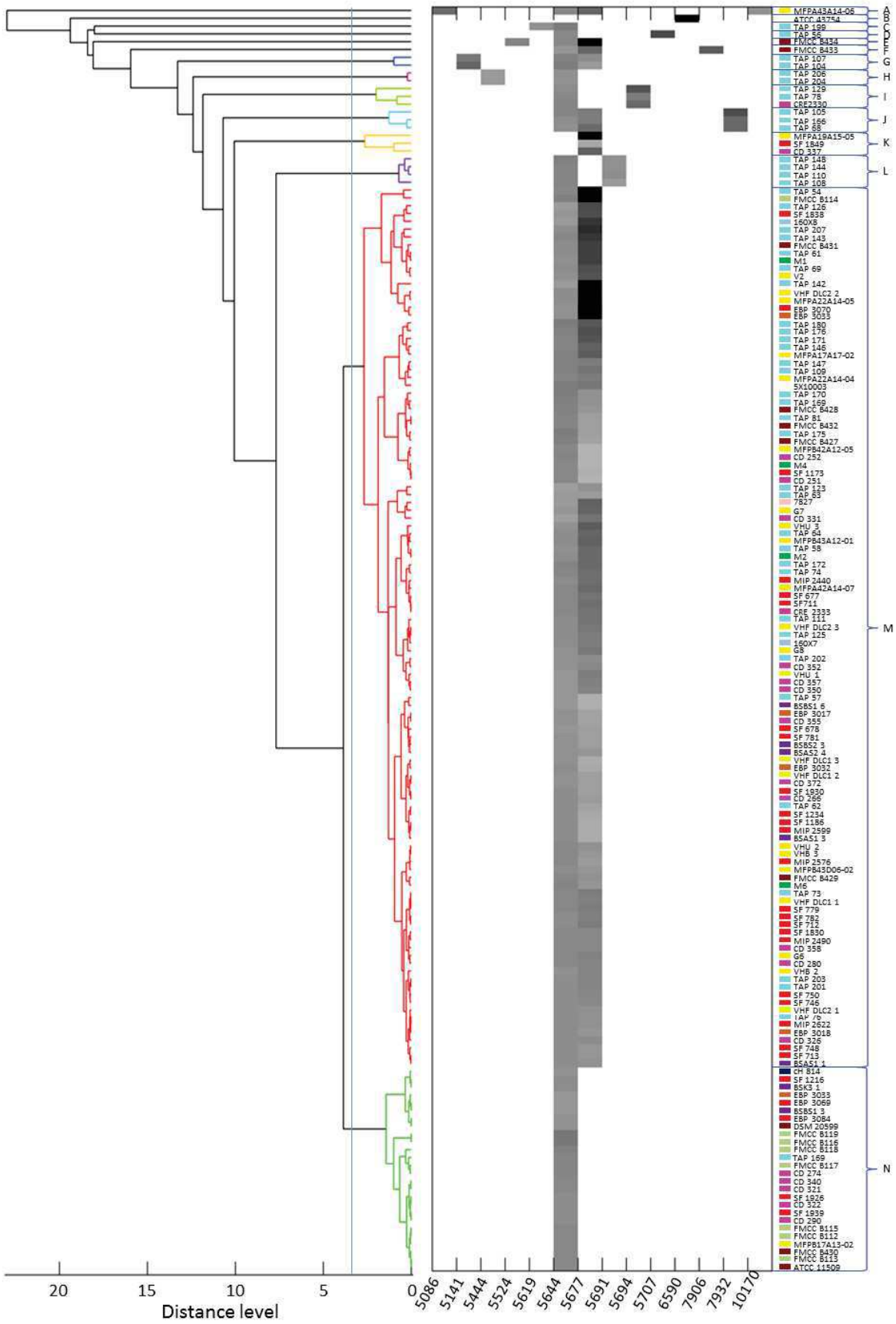

Figure 2.5 Cluster analysis of the MALDI-TOF MS of 161 *B. thermosphacta* isolates listed in Table 2.1.

The type strain *B. campestris* ATCC 43754 was included as a control. The color code refers to the ecological origin of the strains: (■) beef meat, (■) pork meat, (■) lamb meat, (■) horse meat, (■) beef + lamb meat, (■) chicken meat, (■) shrimp, (■) salmon, (■) sea bream, (■) cod fillet, (■) cheese, and (■) slaughterhouse environment. The distance level indicates the similarity of spectra ranging from 0 (identical spectra) to 1 (maximum variability).

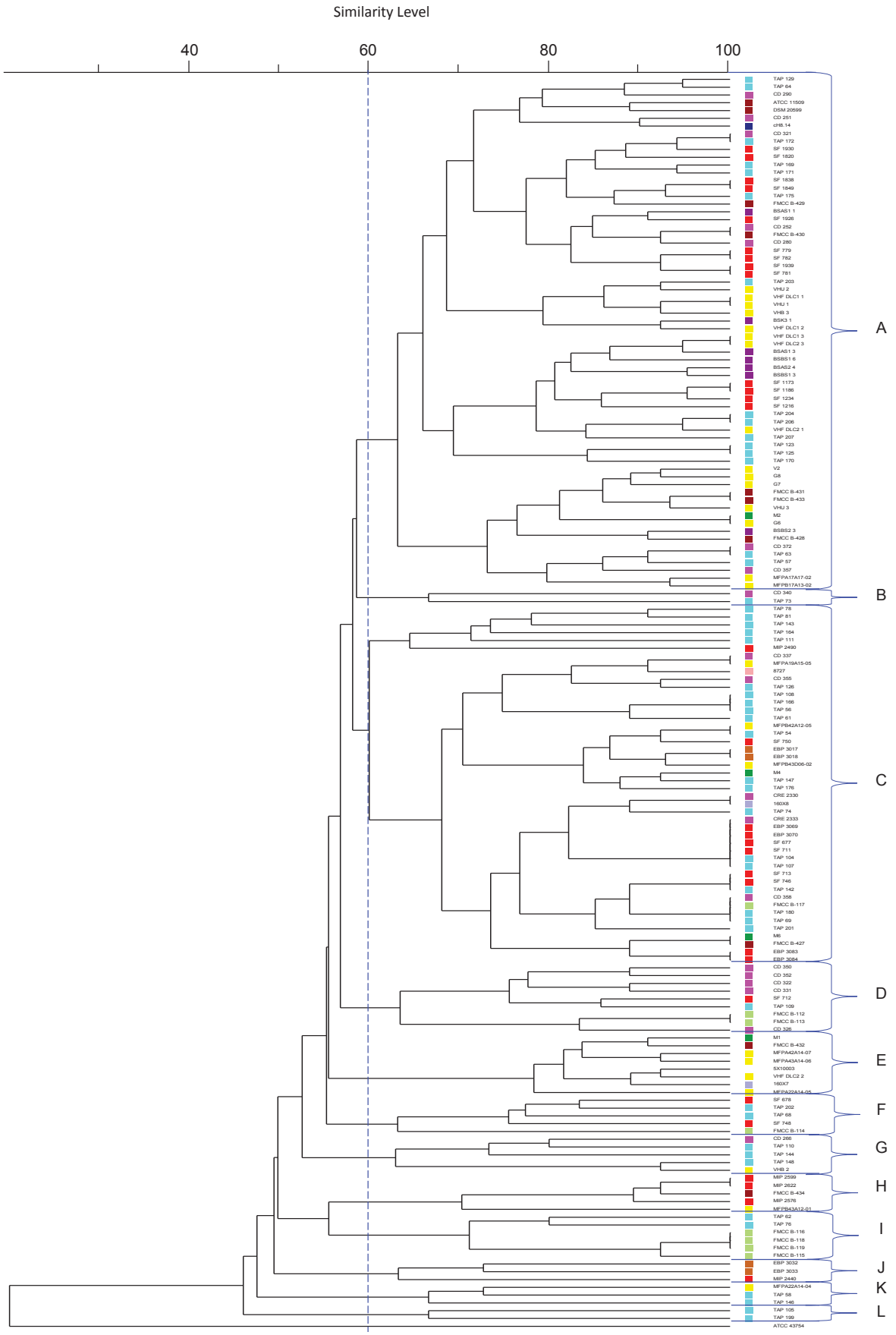

Figure 2.6 Dendrogram obtained by the cluster analysis of rep-PCR fingerprints of the 161 *B. thermosphacta* isolates listed in Table 2.1.

The type strain *B. campestris* ATCC 43754 was included as a control. Similarities were calculated using the Dice coefficient and the data clustered using the UPGMA. The color code refers to the ecological origin of the strains: (■) beef meat, (■) pork meat, (■) lamb meat, (■) horse meat, (■) beef + lamb meat, (■) chicken meat, (■) shrimp, (■) salmon, (■) sea bream, (■) cod fillet, (■) cheese, and (■) slaughterhouse environment. The vertical blue dashed line shows the delineation level of 60%. Groups from A to L were formed at the similarity level of 60%.

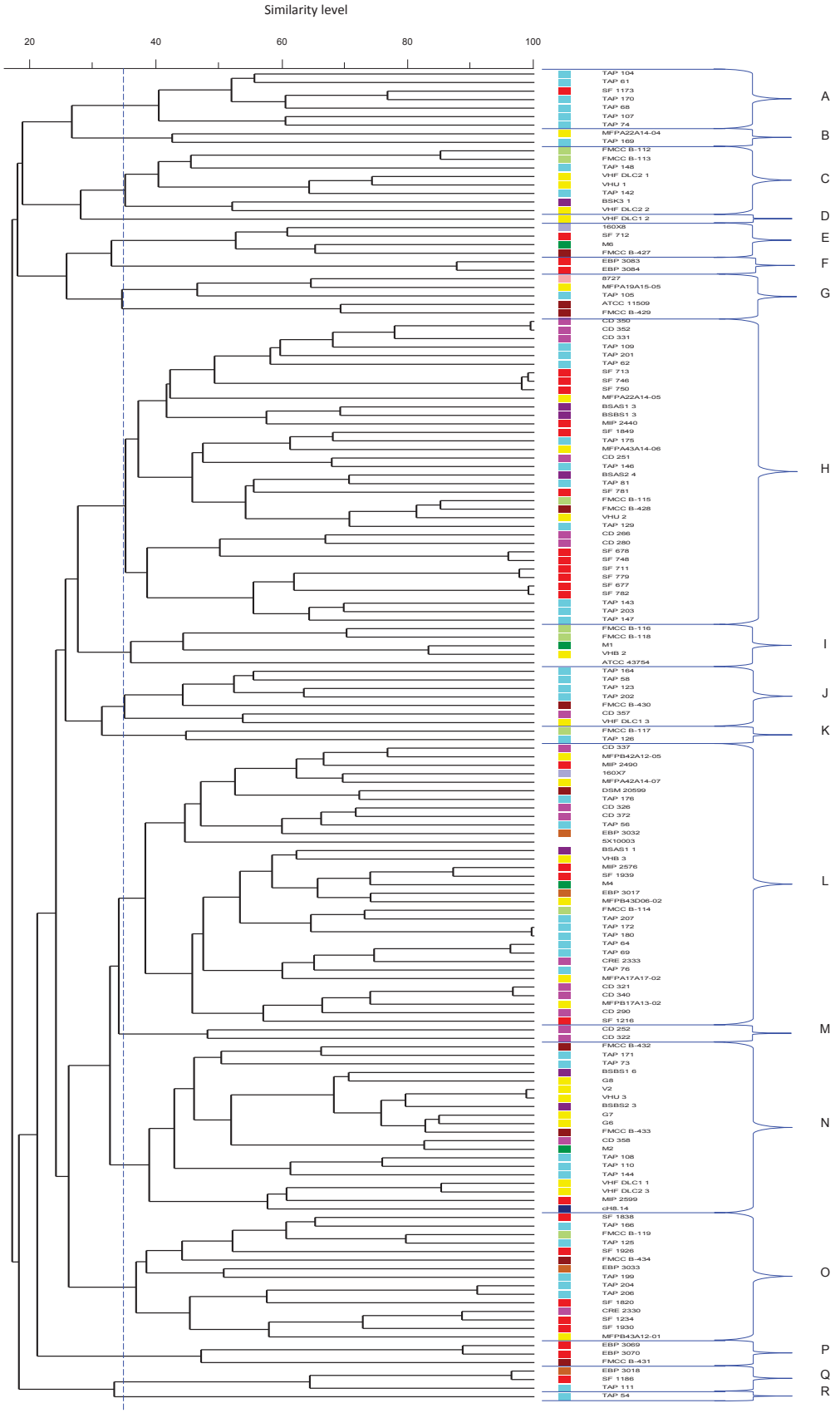

Figure 2.7 Dendrogram obtained by the cluster analysis of PFGE fingerprints of the 161 *B. thermosphacta* isolates listed in Table 2.1.

The type strain *B. campestris* ATCC 43754 was included as a control. Similarities were calculated using the Pearson coefficient and the data clustered using the UPGMA. The color code refers to the ecological origin of the strains: (■) beef meat, (■) pork meat, (■) lamb meat, (■) horse meat, (■) beef + lamb meat, (■) chicken meat, (■) shrimp, (■) salmon, (■) sea bream, (■) cod fillet, (■) cheese, and (■) slaughterhouse environment. The vertical blue dashed line shows the delineation level of 35%. Groups from A to R were formed at the similarity level of 35%.

2.3. Ce qu'il faut retenir de ce chapitre

Dans le but d'étudier la diversité intra-espèce de *B. thermosphacta* et de son potentiel d'altération, nous avons tout d'abord constitué une collection de 159 souches isolées de l'environnement de l'abattoir de bovins et de diverses matrices alimentaires (fromage, produits de la mer et viandes de porc, de bœuf, d'agneau, de cheval et de poulet). Nous avons développé un test rapide, basé sur l'amplification PCR d'un fragment du gène *rpoB* pour l'identification spécifique des souches au niveau de l'espèce. Cette collection a ensuite été caractérisée par trois méthodes de typage, la rep-PCR, la PFGE et le MALDI-TOF.

La comparaison des profils obtenus avec chacune des techniques a permis de discriminer les souches selon des groupes distincts révélant une diversité génétique importante au sein de la collection. Cependant, aucune corrélation claire entre les résultats obtenus avec les trois méthodes n'a été observée. De plus, la diversité intra-spécifique observée n'était pas liée à l'environnement dont les souches ont été isolées. En effet, des souches issues d'une même niche écologique appartenaient à des groupes génotypiques distincts. Inversement, des isolats provenant de niches écologiques distinctes ont été retrouvés dans un même groupe. De ce fait, il n'y a pas d'écotypes au sein de la collection (l'écotype étant un groupe de souches ayant évolué dans une même niche écologique).

Parallèlement à cette étude de diversité génotypique, nous avons évalué la diversité du potentiel d'altération des souches via leur capacité à produire des molécules impliquées dans l'altération, comme l'acétoïne et le diacétyle, sur des milieux modèles (jus de viande/crevettes). Nous avons montré que toutes les souches testées produisaient ces deux composés sur les deux milieux et que les niveaux de production variaient en fonction des souches et des matrices alimentaires. Cependant, il ne semble pas y avoir un lien entre l'origine écologique des souches et leur capacité à produire l'acétoïne et le diacétyle. Deux couples de souches présentaient un profil particulièrement intéressant. Les souches EBP 3070 (isolée de filet de cabillaud altéré) et TAP 175 (isolée de cuisse de poulet non altérée) étaient parmi les plus fortement productrices d'acétoïne et de diacétyl sur le jus de crevette et parmi les plus faiblement productrices dans le jus de viande. Inversement, les souches CD 337 (isolée de crevettes altérées) et BSAS1 3 (isolée de l'environnement d'abattoir de bovins) étaient

parmi les plus fortement productrices d'acétoïne et de diacétyle sur jus de viande et parmi les plus faiblement productrices dans le jus de crevette.

Cette différence entre souches pourrait résulter de répertoires génétiques différents ou de différences plus subtiles influençant la régulation des gènes impliqués dans les voies métaboliques associées à la production de ces molécules. Nous avons donc choisi pour la suite de ces travaux, de sélectionner les quatre souches évoquées ci-dessus pour effectuer une analyse de génomique comparative.

Chapitre 3

Comparaison des génomes de *Brochothrix thermosphacta*

Nassima Illikoud
2018

Chapitre 3. Comparaison des génomes de *Brochothrix thermosphacta*

3.1. Préambule

Suite à l'étude de diversité génotypique et phénotypique de *B. thermosphacta* (chapitre 2), nous avons choisi quatre souches aussi diverses que possible CD 337, TAP 175, EBP 3070 et BSAS1 3. Elles ont été isolées de différentes niches écologiques, ont différentes capacités à produire des molécules malodorantes et appartiennent à des groupes Rep-PCR et PFGE distincts. Au début de ce travail, aucun génome complet n'était disponible pour cette espèce. Nous avons donc choisi de séquencer le génome complet de la souche CD 337 et d'effectuer des séquences « draft » pour les 3 autres souches. Le génome de la souche CD 337 a été annoté manuellement puis les annotations ont été transférées sur les trois autres génomes. Les séquences ont ensuite été comparées avec d'autres génomes de *B. thermosphacta* disponibles dans les bases de données publiques. Dans cette étude, l'accent a été mis sur les gènes et les voies métaboliques (i) associés aux capacités d'altération de *B. thermosphacta* et (ii) susceptibles d'assurer une meilleure survie et d'augmenter ses capacités d'adaptation à différentes niches écologiques (matrices alimentaires et environnement). Ces travaux ont fait l'objet d'un manuscrit soumis pour publication dans la revue *Standards in Genomic Sciences* (Référence : SIGS-D-18-00045).

3.2. One complete and three draft genome sequences of four *Brochothrix thermosphacta* strains, CD 337, TAP 175, BSAS1 3 and EBP 3070

Nassima Illikoud [1], Christophe Klopp [2], Alain Roulet [3], Olivier Bouchez [3], Nathalie Marsaud [4], Emmanuel Jaffrès [1], and Monique Zagorec [1*]

[1] UMR 1014 SECALIM, INRA, Oniris, Nantes, France

[2] Plateforme Bio-informatique, Toulouse Genopole, Institut National de la Recherche Agronomique, Castanet-Tolosan, France

[3] INRA, US 1426, GeT-PlaGe, Genotoul, Castanet-Tolosan, France

[4]LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France

3.2.1. Abstract

Brochothrix thermosphacta is one of the dominant bacterial species associated with spoilage of chilled meat and seafood products through the production of various metabolites responsible for off-odors. However, metabolic pathways leading to meat and seafood spoilage are not all well known. The production of spoiling molecules seems to depend both on strains and on food matrix. Several *B. thermosphacta* genome sequences have been reported, all issued from meat isolates. Here, we report four genome sequences, one complete and three as drafts. The four *B. thermosphacta* strains CD 337, TAP 175, BSAS1 3, and EBP 3070 were isolated from different ecological niches (seafood or meat products either spoiled or not and bovine slaughterhouse). These strains known as phenotypically and genetically different were selected to represent intraspecies diversity. CD 337 genome is 2,594,337 bp long, complete and circular, containing 2593 protein coding sequences and 28 RNA genes. TAP 175, BSAS1 3, and EBP 3070 genomes are arranged in 57, 83, and 71 contigs, containing 2515, 2668, and 2611 protein-coding sequences, respectively. These genomes were compared with two other *B. thermosphacta* complete genome sequences. The main genome content differences between strains are phages, plasmids, restriction/modification systems, and cell surface functions, suggesting a similar metabolic potential but a different niche adaptation capacity.

Keywords

Brochothrix thermosphacta; *Listeriaceae*; Spoilage; Chicken meat; Cooked shrimp; Bovine slaughterhouse, Smoked salmon

Abbreviations

VOCs, Volatile organic compounds

CFU, Colony forming unit

STAA, Streptomycin-thallous acetate-actidione

MALDI-TOF, Matrix assisted laser desorption ionization - time of flight

PFGE, Pulsed-field gel electrophoresis

Rep-PCR, repetitive element palindromic based-polymerase chain reaction

3.2.2. Introduction

Brochothrix and *Listeria* are the only two genera belonging to the *Listeriaceae* family. *Brochothrix thermosphacta* is a non-pathogenic, psychrotrophic, and ubiquitous bacterial species. It is responsible for the spoilage of chilled meat and seafood products stored aerobically or under modified atmosphere or vacuum packaging. Spoilage of these foodstuffs by *B. thermosphacta* results from the production of volatile organic compounds (VOCs) responsible for off-odors. Two VOCs, 3-hydroxy-2-butanone (acetoin) and 2,3-butanedione (diacetyl) have been associated with both meat and seafood products spoilage, whatever the packaging conditions (Illikoud et al., 2018). We recently showed that the concentration of acetoin and diacetyl produced in beef or shrimp juices was strain and matrix dependent although the differences in the production level did not depend on the ecological origin of the strains (Illikoud et al., *in press*). The presence of other VOCs associated to the presence of *B. thermosphacta* in food depends on the food product (meat or seafood) and on the storage conditions (Illikoud et al., 2018). Thus, it is yet unknown whether strains isolated from meat or from seafood have a similar spoilage potential and/or whether the food matrix or storage conditions can impact the expression of this potential. To date, 14 *B. thermosphacta* genome sequences, including two complete, are publicly available (Paoli et al., 2017; Stanborough et al., 2017). A comparative genomic analysis on 12 *B. thermosphacta* draft genome sequences showed a high degree of similarity between strains and a similar gene repertoire for the production of several malodorous molecules (Stanborough et al., 2017). Major gene repertoire differences among the genomes previously reported in the literature were characterized by phage related sequences (Paoli et al., 2017; Stanborough et al., 2017). However, all were isolated from meat products and thus may not represent exhaustive *B. thermosphacta* diversity since this species has also been isolated from other ecological niches (Stackebrandt and Jones, 2006). In addition, although *B. thermosphacta* has been reported to be involved in a wide variety of foodstuff spoilage, metabolic pathways responsible for most of the produced VOCs still remain unknown. In this study we chose four *B. thermosphacta* strains issued from diverse environments for a comparative genomic analysis. *B. thermosphacta* CD 337, TAP 175, EBP 3070, and BSAS1 3 were isolated from spoiled cooked and peeled shrimp, fresh chicken leg, spoiled smoked salmon, and from a bovine slaughterhouse environment (hide of a slaughtered animal), respectively (Illikoud et

al., *in press*). These strains were chosen to represent a larger diversity than previously explored in genome comparisons. Indeed we previously showed that the four chosen strains are genotypically distant and have different abilities to produce acetoin and diacetyl (Illikoud et al., *in press*). Gene content of these four strains was compared to those of 14 available *B. thermosphacta* genome sequences in order to screen for potential features associated to specific niche adaptation or fitness, and for putative differences in their spoilage potential.

3.2.3. Organism Information

3.2.3.1. Classification and features

B. thermosphacta CD 337, TAP 175, EBP 3070, and BSAS1 3 were isolated after plating on *B. thermosphacta* selective medium Streptomycin-thallous acetate-actidione (STAA, Oxoid) (Gardner, 1966, 1985). Strains belonging to *B. thermosphacta* species were described as Gram positive, non-spore forming, and non-motile regular unbranched rods (Table 3.1).

Table 3.1 Classification and general features of *B. thermosphacta* strains CD 337, TAP 175, BSAS1 13, and EBP 3070.

MIGS ID	Property	CD 337		TAP 175		BSAS1 3		EBP 3070	
		Term	**Evidence code[a]**	**Term**	**Evidence code[a]**	**Term**	**Evidence code[a]**	**Term**	**Evidence code[a]**
	Classification	Domain *Bacteria*	TAS [1]	Domain *Bacteria*	TAS [1]	Domain *Bacteria*	TAS [1]	Domain *Bacteria*	TAS [1]
		Phylum *Firmicutes*	TAS [2, 3]	Phylum *Firmicutes*	TAS [2, 3]	Phylum *Firmicutes*	TAS [2, 3]	Phylum *Firmicutes*	TAS [2, 3]
		Class *Bacilli*	TAS [4]	Class *Bacilli*	TAS [4]	Class *Bacilli*	TAS [4]	Class *Bacilli*	TAS [4]
		Order *Bacillales*	TAS [5]	Order *Bacillales*	TAS [5]	Order *Bacillales*	TAS [5]	Order *Bacillales*	TAS [5]
		Family *Listeriaceae*	TAS [6]	Family *Listeriaceae*	TAS [6]	Family *Listeriaceae*	TAS [6]	Family *Listeriaceae*	TAS [6]
		Genus *Brochothrix*	TAS [7]	Genus *Brochothrix*	TAS [7]	Genus *Brochothrix*	TAS [7]	Genus *Brochothrix*	TAS [7]
		Species *Brochothrix thermosphacta*	TAS [7]	Species *Brochothrix thermosphacta*	TAS [7]	Species *Brochothrix thermosphacta*	TAS [7]	Species *Brochothrix thermosphacta*	TAS [7]
		Strain CD 337	TAS [8]	Strain TAP 175	TAS [8]	Strain BSAS1 3	TAS [8]	Strain EBP 3070	TAS [8]
	Gram stain	Positive	IDA	Positive	IDA	Positive	IDA	Positive	IDA
	Cell shape	Rod	IDA	Rod	IDA	Rod	IDA	Rod	IDA
	Motility	Non-motile	NAS [9]	Non-motile	NAS [9]	Non-motile	NAS [9]	Non-motile	NAS [9]
	Sporulation	Non-sporulating	NAS [9]	Non-sporulating	NAS [9]	Non-sporulating	NAS [9]	Non-sporulating	NAS [9]
	Temperature range	0-30 °C	NAS [9]	0-30 °C	NAS [9]	0-30 °C	NAS [9]	0-30 °C	NAS [9]
	Optimum temperature	20-25 °C	NAS [9]	20-25 °C	NAS [9]	20-25 °C	NAS [9]	20-25 °C	NAS [9]
	pH range; Optimum	5–9; 7	NAS [9]	5–9; 7	NAS [9]	5–9; 7	NAS [9]	5–9; 7	NAS [9]
	Carbon source	Glucose, ribose, glycerol, mannose, mannitol, gluconate, glucosamine, fructose, maltose, sucrose, trehalose	NAS [10]	Glucose, ribose, glycerol, mannose, mannitol, gluconate, glucosamine, fructose, maltose, sucrose, trehalose	NAS [10]	Glucose, ribose, glycerol, mannose, mannitol, gluconate, glucosamine, fructose, maltose, sucrose, trehalose	NAS [10]	Glucose, ribose, glycerol, mannose, mannitol, gluconate, glucosamine, fructose, maltose, sucrose, trehalose	NAS [10]
MIGS-6	Habitat	Cooked and peeled spoiled shrimp	TAS [8]	Non-spoiled chicken leg	TAS [8]	Beef slaughterhouse environment	TAS [8]	Spoiled smoked salmon	TAS [8]

This table continues in the following page

MIGS ID	Property	CD 337		TAP 175		BSAS1 3		EBP 3070	
		Term	**Evidence code[a]**	**Term**	**Evidence code[a]**	**Term**	**Evidence code[a]**	**Term**	**Evidence code[a]**
MIGS-6.3	Salinity	Tolerate 8-10 % NaCl (w/v)	NAS [9]	Tolerate 8-10 % NaCl (w/v)	NAS [9]	Tolerate 8-10 % NaCl (w/v)	NAS [9]	Tolerate 8-10 % NaCl (w/v)	NAS [9]
MIGS-22	Oxygen requirement	Facultative anaerobe	NAS [9]	Facultative anaerobe	NAS [9]	Facultative anaerobe	NAS [9]	Facultative anaerobe	NAS [9]
MIGS-15	Biotic relationship	free-living	NAS	free-living	NAS	free-living	NAS	free-living	NAS
MIGS-14	Pathogenicity	Non-pathogenic	NAS [9]	Non-pathogenic	NAS [9]	Non-pathogenic	NAS [9]	Non-pathogenic	NAS [9]
MIGS-4	Geographic location	Pays de Loire, France	NAS	Pays de Loire, France	NAS	Pays de Loire, France	NAS	Pays de Loire, France	NAS
MIGS-5	Sample collection	2009	TAS[11]	February, 2014	TAS [8]	April, 2015	TAS [8]	June, 2011	NAS [8]
MIGS-4.1	Latitude	47.2173° N	NAS	47.2173° N	NAS	47.059° N	NAS	47.2173° N	NAS
MIGS-4.2	Longitude	1.5534° W	NAS	1.5534° W	NAS	0.876° W	NAS	1.5534° W	NAS
MIGS-4.4	Altitude	2-52; 20 m	NAS	2-52; 20 m	NAS	63-184; 100 m	NAS	2-52; 20 m	NAS

[a] Evidence codes - **IDA:** Inferred from Direct Assay; **TAS**: Traceable Author Statement (i.e., a direct report exists in the literature); **NAS:** Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project (Ashburner et al., 2000). When the evidence is IDA, the property was directly observed for a live isolate. **[1]**(Woese et al., 1990); **[2,3]** (De Vos et al., 2009; Gibbons and Murray, 1978); **[4]** (Ludwig et al., 2009a); **[5]** (Ludwig et al., 2009b); **[6]** (Ludwig et al., 2009c); **[7]** (Sneath, 2009); **[8]** (Illikoud et al., *in press*); **[9]** (Stackebrandt and Jones, 2006); **[10]** (Macaskie et al., 1984); **[11]** (Jaffrès et al., 2009).

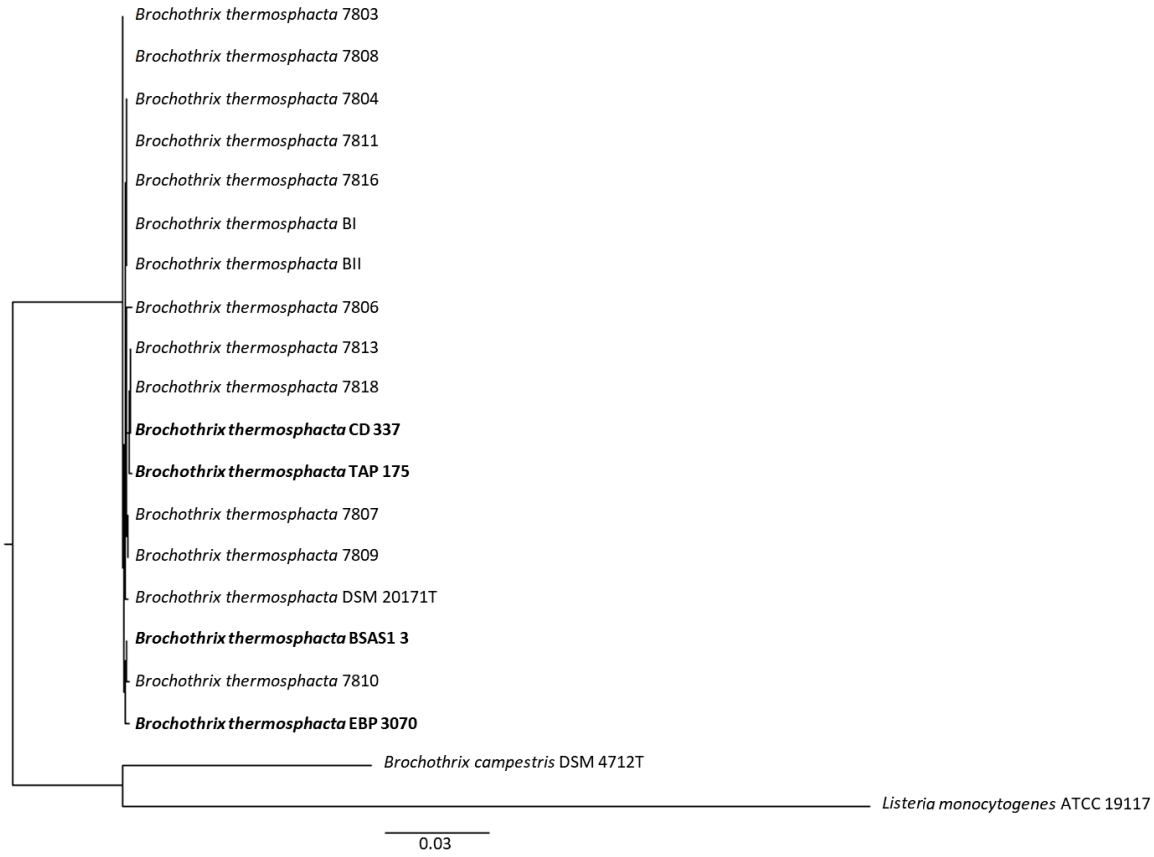

Figure 3.1 Phylogenetic tree showing the relationship of the four *B. thermosphacta* strains (shown in bold print) to other *B. thermosphacta* strains the genome of which is publicly available.

Brochothrix campestris, and *Listeria monocytogenes* type strains were used as outgroup. Tree is based on MAFFT (v7.309) (Katoh et al., 2002) aligned complete *rpoB* gene sequences. The tree was built using FastTree (v2.1.5) then visualized with FigTree (v1.4.3).

They are aerobe and facultative anaerobe, catalase positive and oxidase negative (Stackebrandt and Jones, 2006). Gram staining and catalase reaction of freshly-grown cells of *B. thermosphacta* CD 337, TAP 175, EBP 3070, and BSAS1 3 confirmed all to be Gram positive and catalase positive (Illikoud et al., *in press*). Phylogenetic analyses based on *rpoB* gene sequence alignments Figure 3.1 showed that *B. thermosphacta* strains CD 337, TAP 175, EBP 3070 and BSAS1 3 clustered within the *Brochothrix* genus. Based on these analyses, our four *B. thermosphacta* were also found to be closely related to *Brochothrix campestris* the only other species yet described in the *Brochothrix* genus and to *Listeria monocytogenes*.

Atomic force and scanning electron microscopies of fresh cultures showed that each strain population consisted mainly cells that were rod shaped with no flagella (Figure 3.2).

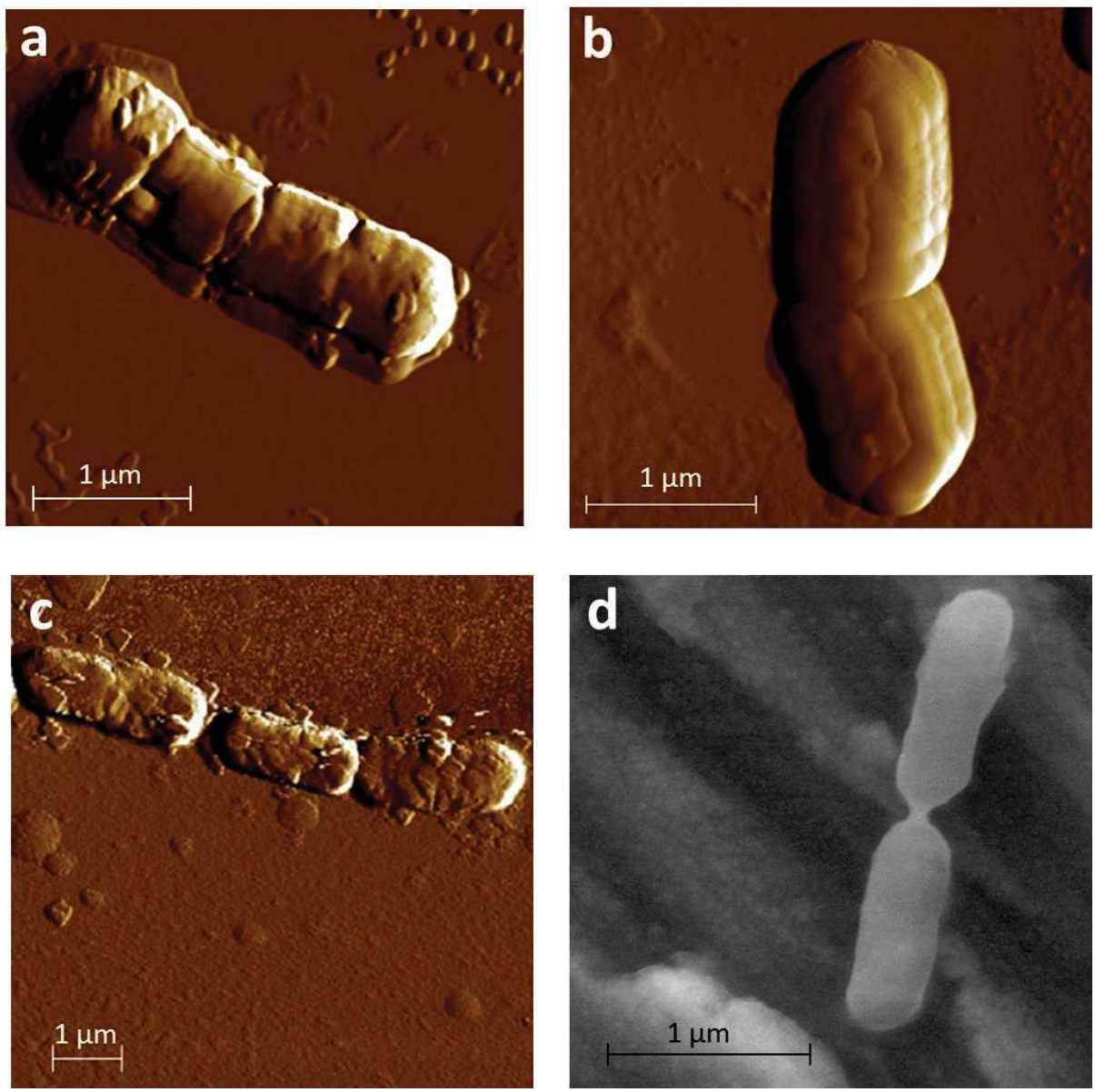

Figure 3.2 Electron microscopy pictures of liquid-grown *Brochothrix thermosphacta* cultures.

Brochothrix thermosphacta CD 337 **(a)**, *B. thermosphacta* EBP 3070 **(b)**, *B. thermosphacta* BSAS1 3 **(c)** were imaged by atomic force electron microscopy, and *B. thermosphacta* TAP 175 **(d)** was generated by scanning electron microscopy. The scale bars represent 1 µm.

3.2.3.2. Extended feature descriptions

The four strains were previously genetically and phenotypically characterized. These strains isolated from different ecological niches, belonged to different Rep-PCR, PFGE and MALDI-TOF clusters and present different abilities to produce diacetyl and acetoin in beef and cooked shrimp juices (Illikoud et al., *in press*). On the whole strain collection tested, CD 337 and BSAS1 3 were within the highest acetoin and diacetyl producers when grown in beef juice, and among the lowest after growth in cooked shrimp juice. Conversely TAP 175 and EBP 3070 produced the highest levels of acetoin and diacetyl in shrimp juice and the lowest ones after cultivation in beef juice (Illikoud et al., *in press*).

3.2.4. Genome sequencing information

3.2.4.1. Genome project history

In order to investigate *B. thermosphacta* species diversity, an initial study has been conducted on 159 isolates issued from various ecological niches. They were phenotyped on their ability to produce acetoin and diacetyl and were clustered, based on their genotypes (PFGE, Rep-PCR) and proteomic (MALDI-TOF) patterns (Illikoud et al., *in press*). Strains did not cluster based on their ecological origin nor on their spoilage compounds production ability. Therefore, to determine whether the spoilage potential of the strains was due to their gene repertoire and/or to the food matrix, we selected 4 strains, as diverse as possible, for a comparative genomic analysis. *B. thermosphacta* CD 337, TAP 175, BSAS1 3, and EBP 3070 have been isolated from a variety of food products and from environment. They have different abilities to produce spoiling molecules depending on the food matrix, and belong to different PFGE and Rep-PCR clusters (Illikoud et al., *in press*). Project information and associated MIGS are shown in Table 3.2.

Table 3.2 Project information.

MIGS ID	Property	Term, CD 337	Term, TAP 175	Term, BSAS1 3	Term, EBP 3070
MIGS 31	Finishing quality	Complete circular genome	Draft genome	Draft genome	Draft genome
MIGS-28	Libraries used	20-kb Template Preparation Using BluePippin Size-Selection System (15-kb size cutoff)	NEB Next Fast DNA fragmentation and library prep (NEB Biolabs)	NEB Next Fast DNA fragmentation and library prep (NEB Biolabs)	NEB Next Fast DNA fragmentation and library prep (NEB Biolabs)
MIGS 29	Sequencing platforms	PacBio RSII (Pacific BioSciences)	Ion S5 (Ion Torrent)	Ion S5 (Ion Torrent)	Ion S5 (Ion Torrent)
MIGS 31.2	Fold coverage	526.48	270.67	206.01	243.65
MIGS 30	Assemblers	CANU	SPAdes	SPAdes	SPAdes
		(version 1.3)	(version 3.9.0)	(version 3.9.0)	(version 3.9.0)
MIGS 32	Gene calling method	MicroScope Genoscope Plateform [a]	MicroScope Genoscope Plateform [a]	MicroScope Genoscope Plateform [a]	MicroScope Genoscope Plateform [a]
	Locus Tag	BTCD	BTTAP	BTBSAS	BTEBP
	Genbank ID	ERZ500814	ERZ500815	ERZ500816	ERZ500817
	GenBank Date of Release	25 May 2018	25 May 2018	25 May 2018	25 May 2018
	GOLD ID				
	BIOPROJECT	PRJEB25018	PRJEB25018	PRJEB25018	PRJEB25018
MIGS 13	Source Material Identifier				
	Project relevance	Food spoiler	Food spoiler	Food spoiler	Food spoiler

[a] (Vallenet et al., 2013)

3.2.4.2. Growth conditions and genomic DNA preparation

B. thermosphacta strains were grown overnight at 25 °C in l00 ml Luria-Bertani Broth (Invitrogen) containing 10 g/l NaCl. Cultures were shaken at 100 rpm. A cell pellet (about 2 x 10^{10} CFU) was obtained by centrifugation at 5,000 x *g* for 10 min. Genomic DNA was extracted using the Blood and Cell Culture DNA Midi Kit (Qiagen, France) according to the manufacturer's instructions for Gram positive bacteria with some modifications as previously described by (Falentin et al., 2016). Briefly, the cell pellet was resuspended in 3.5 ml buffer B1 containing 0.2 mg/ml RNAse A. Bacterial cells were lysed by the addition of 220 mg lysozyme

powder (Euromedex, France) followed by incubation for 2 h at 37 °C. High molecular weight genomic DNA was purified by gravity flow and anion exchange chromatography, eluted in 5 ml QF buffer (Qiagen) and precipitated with 3.5 ml isopropanol. DNA was collected by centrifugation for 15 min at 4 °C and 10,000 x *g* and then air dried for 10 min. DNA was resuspended in 100 µl TE buffer (10 Mm Tris-HCl, 1 mM EDTA, pH 8.0) for two hours at 55 °C. DNA integrity was checked on a 0.8 % agarose gel. DNA concentration and purity were checked using Nanodrop spectrophotometer 2000 (Thermo Scientific). The ratio 260 nm and 280 nm was assessed to be 1.9.

3.2.4.3. Genome sequencing and assembly

B. thermosphacta CD 337 sequence reads were generated at GeT-PlaGe (Plateforme Génomique), INRA Auzeville, France with a single-molecule-real-time (SMRT) using Pacific Biosciences RS II sequencing technology (Table 3.2). A total of 113,824 reads was produced. *De-novo* assembly was carried out using CANU version 1.3 with standard parameters (Koren et al., 2017). Raw data were aligned then polished with pbalign and quiver de smrtshell-2.3.0, respectively. The resulting contig was circularized with circlator (version 1.3.0) (Hunt et al., 2015). For the three other strains TAP 175, BSAS1 3, and EBP 3070, library preparation and genome sequencing were carried out at GeT-Biopuces platform (INSA, Toulouse, France) using S5 sequencer from Ion Torrent technology (Table 3.2). The resulted reads, approximately 2.60, 2.26, and 2.88 million for TAP 175, BSAS1 3 and EBP 3070, respectively, were *de-novo* assembled using SPAdes (version 3.9.0) with default parameters (Bankevich et al., 2012). The assembly resulted in 57, 83, and 71 contigs, respectively.

3.2.4.4. Genome annotation

The new complete and draft genome sequences were integrated in the MicroScope platform hosted in the Genoscope for automatic annotation (Vallenet et al., 2013). This tool uses multiple databases: TrEMBL, SwissProt, FigFam, PubMed, InterPro, etc. The Microscope platform also provides links to databases as PkGDB, MicroCyc, KEGG for extracting genomic and metabolic data from the pathway genome database (Vallenet et al., 2013). Expert annotation was performed for all the genes of *B. thermosphacta* CD 337 genome using the gene annotation editor. Expert manual annotations were then transferred from CD 337 on

close orthologs (*i.e.* >90% identity on >80% length or >85% identity when syntheny was observed) of the draft genomes from the three other *B. thermosphacta* strains.

3.2.5. Genome Properties

The circular genome of *B. thermosphacta* CD 337 is 2,594,337 nucleotides with a 36.46 % GC content (Table 3) and contains one finalized chromosome (Figure 3.3). This genome contains 2943 protein coding sequences (CDS).

The draft genomes of strains TAP 175, BSAS1 3, and EBP 3070 consist in 57, 83, and 71 contigs, respectively. The genome of *B. thermosphacta* TAP 175 has an estimated size of 2,506,748 bp, with a 36.28% GC content. That of *B. thermosphacta* BSAS1 3 encompasses 2,617,996 bp (36.20% G+C), and that of the strain EBP 3070 is 2,541,668 bp long (36.22% G+C) (Figure 3.3). The three genomes contain 2515, 2668, and 2611 CDS, respectively. The genome properties and statistics are summarized in Table 3.3, and the number of genes assigned to COG functional categories in Table 3.4.

A high degree of genomic sequence similarity among the four *B. thermosphacta* strains was observed by the calculation of Average Nucleotide Identity (ANI) using OrthoANIu, an orthologous ANI algorithm (Edgar, 2010). Strain to strain genomic comparisons showed orthoANI (Orthologous Average Nucleotide Identity) values varying from 98.94% to 99.11%, correlating thus with previous observations on other *B. thermosphacta* genome sequences (Paoli et al., 2017; Stanborough et al., 2017).

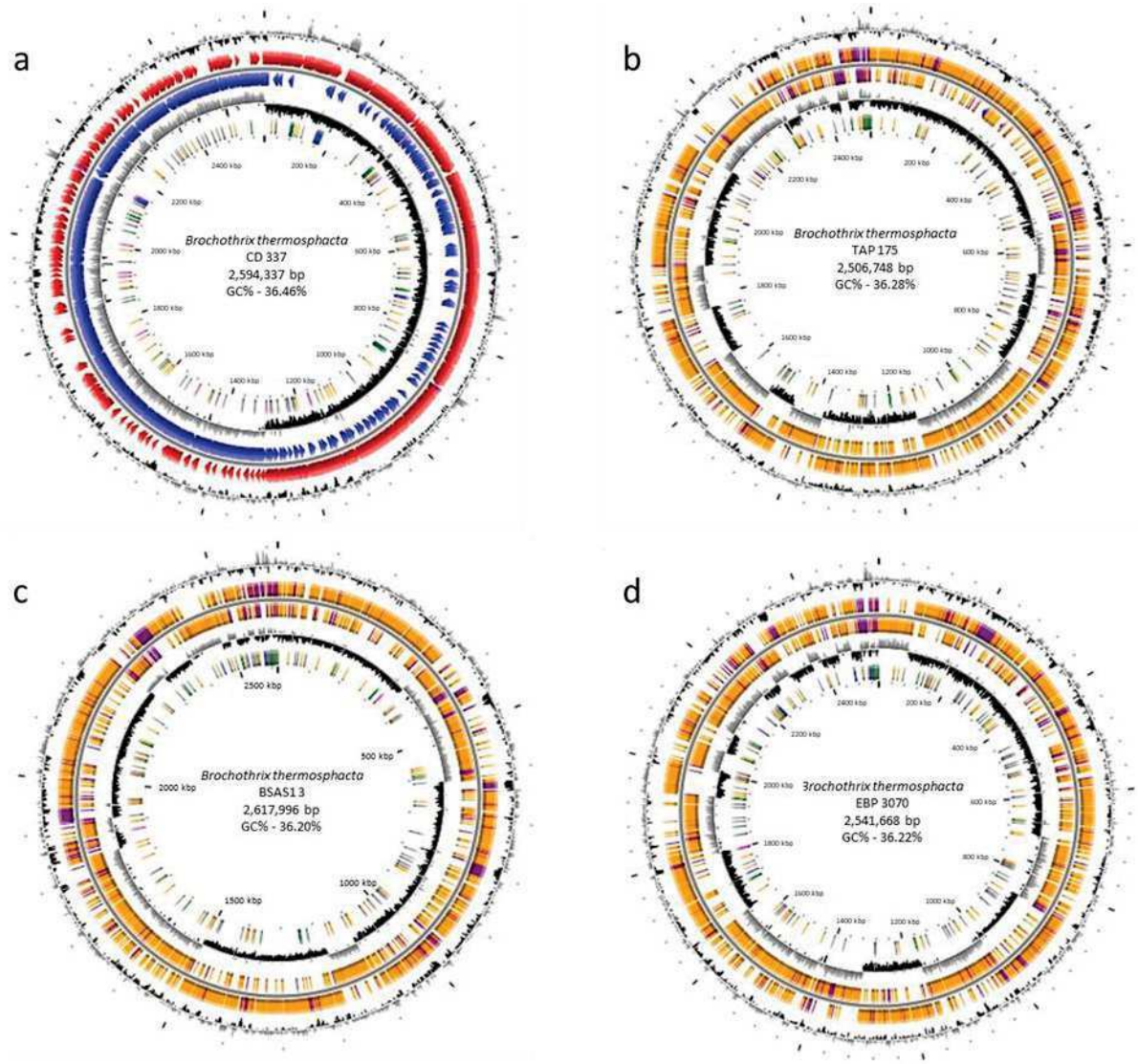

Figure 3.3 Circular views of genome sequences of *B. thermosphacta* strains.

B. thermosphacta CD 337 **(a)**, *B. thermosphacta* TAP 175 **(b)**, *B. thermosphacta* BSAS1 3 **(c)**, and *B. thermosphacta* EBP 3070 **(d)**. The circular display shows, from outside to inside: (i) GC percentage; (ii) Predicted CDSs transcribed in the clockwise direction; (iii) Predicted CDSs transcribed in the counterclockwise direction. In (ii) and (iii), red and blue colors represent MaGe validated annotations, orange color represents the MicroScope automatic annotation with a reference genome, and the purple color represents Primary/Automatic annotations; (iv) GC skew (G + C/G-C) and (v) color-code representing rRNA (blue), tRNA (green), miscellaneous RNA (orange), Transposable elements (pink) and pseudogenes (grey).

Table 3.3 Genome statistics.

Attribute	CD 337		TAP 175		BSAS1 3		EBP 3070	
	Value	% of Total	Value	% of Total	Value	% of Total	Value	% of Total
Genome size (bp)	2,594,337	100	2,506,748	100	2,617,996	100	2,541,668	100
DNA coding (bp)	2,182,130	84.11	2,136,251	85.22	2,213,516	84.55	2,118,751	83.36
DNA G+C (bp)	945,895	36.46	909,448	36.28	947,714	36.20	920,592	36.22
DNA scaffolds	1	100	57	100	83	100	71	100
Total genes	2,743	100	2,638	100	2,796	100	2,740	100
Protein coding genes	2,593	94.53	2,515	95.3	2,668	95.4	2,611	95.3
RNA genes	28	1.0	6	0.2	8	0.3	9	0.3
Pseudo genes	38	1.4	43	1.6	57	2.0	102	3.7
Genes in internal clusters	N/D	-	N/D	-	N/D	-	N/D	-
Genes with function prediction	2,031	74.04	1,928	73.1	1,957	70.0	1,954	71.3
Genes assigned to COGs	2,486	90.6	2,429	92.1	2,472	88.4	2,432	88.7
Genes with Pfam domains	2,026	73.9	1,982	75.1	2,040	73.0	2,002	73.1
Genes with signal peptides	85	3.1	83	3.1	83	3.0	85	3.1
Genes with transmembrane helices	392	14.3	384	14.5	386	13.8	389	14.2
CRISPR repeats	1	0.04	2	0.07	1	0.03	0	0

Table 3.4 Number of genes associated with general COG functional categories.

Code	CD 337		TAP 175		BSAS1 3		EBP 3070		Description
	Value	%age	Value	%age	Value	%age	Value	%age	
J	158	6.09	161	6.40	159	5.96	164	6.28	Translation, ribosomal structure and biogenesis
A	ND	-	ND	-	ND	-	ND	-	RNA processing and modification
K	237	9.14	235	9.34	242	9.07	234	8.96	Transcription
L	131	5.05	125	4.97	139	5.21	130	4.98	Replication, recombination and repair
B	ND	-	ND	-	ND	-	ND	-	Chromatin structure and dynamics
D	35	1.35	35	1.39	36	1.35	34	1.30	Cell cycle control, Cell division, chromosome partitioning
V	63	2.43	55	2.19	52	1.95	57	2.18	Defense mechanisms
T	100	3.86	95	3.74	98	3.67	99	3.79	Signal transduction mechanisms
M	117	4.51	106	4.21	119	4.46	109	4.17	Cell wall/membrane biogenesis
N	16	0.62	12	0.48	17	0.64	15	0.57	Cell motility
U	33	1.27	27	1.07	29	1.09	28	1.07	Intracellular trafficking and secretion
O	71	2.74	69	2.74	66	2.47	69	2.64	Posttranslational modification, protein turnover, chaperones
C	106	4.09	106	4.21	107	4.01	105	4.02	Energy production and conversion
G	233	8.98	232	9.22	227	5.81	221	8.46	Carbohydrate transport and metabolism
E	244	9.41	247	9.82	246	9.22	242	9.27	Amino acid transport and metabolism
F	72	2.78	71	2.82	71	2.66	71	2.72	Nucleotide transport and metabolism
H	76	2.93	76	3.02	75	2.81	80	3.06	Coenzyme transport and metabolism
I	60	2.31	62	2.46	60	2.25	58	2.22	Lipid transport and metabolism
P	159	6.13	159	6.32	158	5.92	156	5.97	Inorganic ion transport and metabolism
Q	40	1.54	39	1.55	38	1.42	37	1.42	Secondary metabolites biosynthesis, transport and catabolism
R	320	12.34	310	12.33	318	11.92	307	11.76	General function prediction only
S	211	8.14	206	8.19	215	8.06	215	8.23	Function unknown
-	107	4.14	86	3.49	196	10.05	179	6.89	Not in COGs

The total is based on the total number of protein coding genes in the genome.

3.2.6. Insights from the genome sequence

Comparative genomics of the pan genome was based on MicroScope gene/protein families (MICFAMs). This tool classifies proteins in homolog groups of proteins sharing at least 80% amino-acid identity and 80% alignment coverage (Vallenet et al., 2016). The core genome includes MICFAMs associated with at least one gene from every compared genomes. The variable-genome includes MICFAM present in at least two compared genomes. Specific genome includes genes that are singletons and present in only one genome.

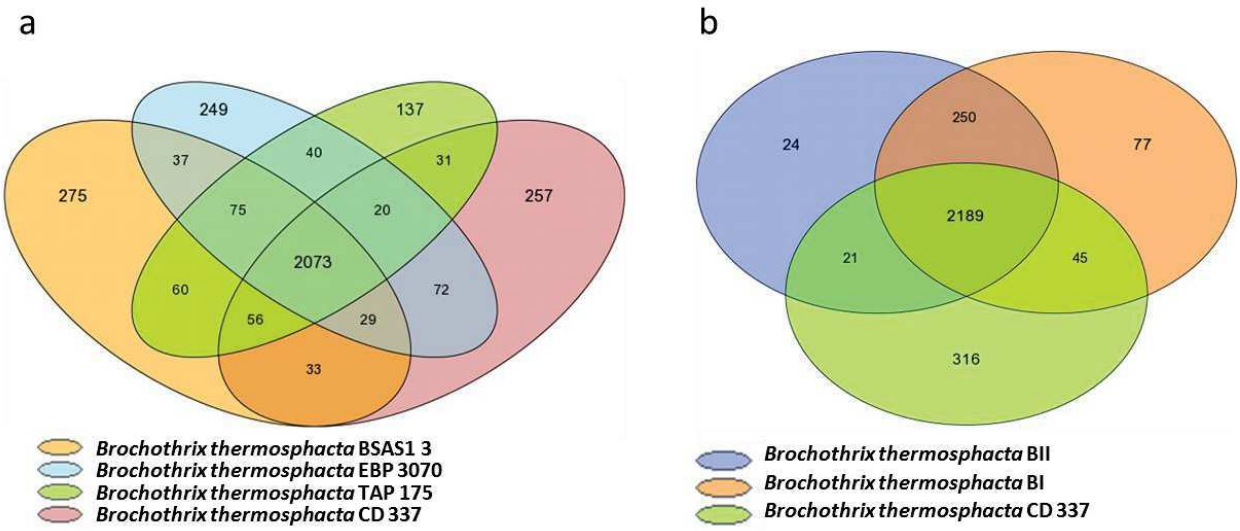

Figure 3.4 Core and pan-genome analysis.

Venn diagram analysis of **(a)** *Brochothrix thermosphacta* CD 337, *B. thermosphacta* TAP 175, *B. thermosphacta* BASA1 3, and *B. thermosphacta* EBP 3070; and **(b)** *B. thermosphacta* CD 337, *B. thermosphacta* BI, and *B. thermosphacta* BII. Values on diagram represent the numbers of MICFAM families for each organism intersections.

The pan genome of strains CD337, TAP 175, BSAS1 3, and EBP 3070 comprised 10,373 genes. Among them, 8339 genes, corresponding to 2,073 MICFAMs were shared by all strains and therefore represent the core genome. The variable genome contained 2,034 genes grouped into 1371 MICFAMs. This analysis revealed that the four strains contain 10.19%, 5.46%, 10.66% and 9.59% strain-specific coding sequences, respectively (Figure 3.4a). The same analysis was performed on the 3 complete genomes, *i.e.* the newly sequenced genome CD337 and the two publicly available genomes of *B. thermosphacta* BI and BII, two strains isolated from ground chicken meat (Figure 3.4b) (Paoli et al., 2017). From these 3 complete genomes,

the pan genome comprised 7,746 genes (2922 MICFAMs), with 6667 genes (2,189 MICFAMs) constituting the core genome, and 1,079 genes (733MICFAMs) the variable genome. Strain specific genome of *B. thermosphacta* BI, BII, and CD 337 comprised 3.21%, 0.94%, 12.06%, and genes, respectively. Strain-specific genes included some proteins involved in regulatory functions, cell surface composition, use of various carbon sources, or bacteriocin production. In particular we observed a four gene cluster, unique to *B. thermosphacta* CD 337 putatively encoding at least part of the machinery for the production of a type 2 lantibiotic. It encompasses a gene for putative lantibiotic modifying enzyme, lanthionine synthetase C-like, and lantibiotic leader peptide-processing serine protease, and a small 70 amino acid peptide similar to lichenicidin, a lantibiotic produced by *Bacillus licheniformis* (Dischinger et al., 2009). However, a large proportion of these strain-specific genes encode proteins of unknown functions or are fragmented genes. The list of the specific genes of *B. thermosphacta* CD 337, BI, and BII is given in additional file 1 (Table 3.5, Table 3.6, and Table 3.7, respectively).

Bacteriophage prediction results using PHAST (PHAge Search Tool) (Zhou et al., 2011) showed that the four genomes contained at least one bacteriophage region. Similarities to phages previously described were provided based on the highest number of proteins most similar to those in the region. A schematic representation of the phage content of the four strains is presented in Figure 3.5.

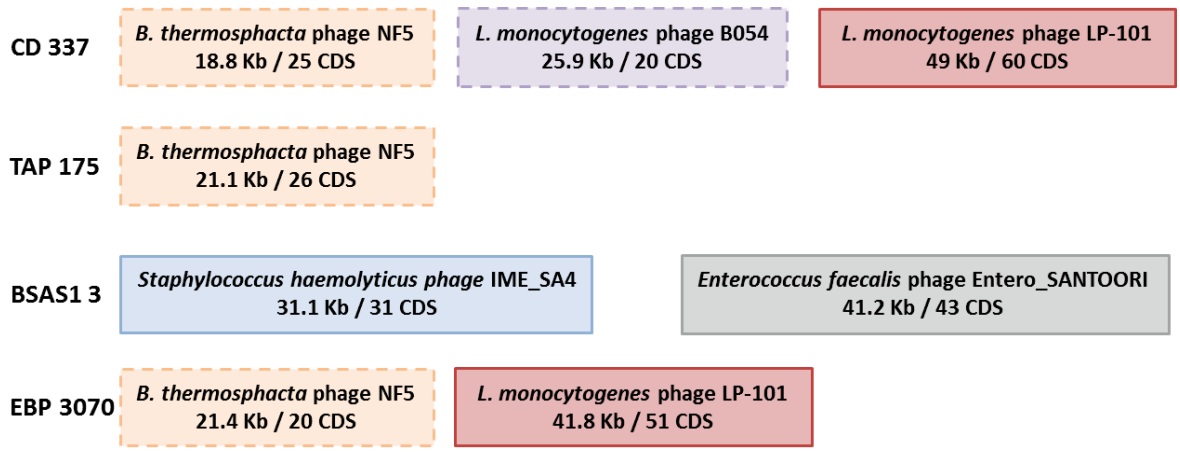

Figure 3.5 Schematic representation of phage content of the four *B. thermosphacta* strains.

The phage identification was given by PHAST program (Zhou et al., 2011). It refers to the phage with the highest number of proteins most similar to those in the region. The phages were represented by boxes surrounded by solid line (intact phages) or dashed line (incomplete phages). The phage size and the number of CDS were also given.

Both CD 337 and EBP 3070 harbored a complete phage (about 49 Kb) similar to LP-101 of *L. monocytogenes* (Denes et al., 2014) and an incomplete NF5 bacteriophage (18.8 Kb) previously described in *B. thermosphacta* (Kilcher et al., 2010). CD 337 comprised a third region (18.8 Kb) similar to *L. monocytogenes* B054 phage (Dorscht et al., 2009). TAP 175 genome comprised only one bacteriophage region consisting in a fraction of the *B. thermosphacta* NF5 bacteriophage. Strain BSAS1 3 harbored two complete bacteriophages similar to IME_SA4 (31.1 Kb) and Entero_SANTOORI (41.2 Kb) described in *Staphylococcus haemolyticus* (http://www.genome.jp/dbget-bin/www_bget?refseq:NC_029025 direct submission) and *Enterococcus faecalis* ATCC 19433 (http://www.genome.jp/dbget-bin/www_bget?refseq:NC_031051 direct submission), respectively.

Since clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins constitute an adaptive immune system against bacteriophages and other foreign genetic elements in bacteria and archaea (Hille et al., 2018), we investigate the occurrence of CRISPR-Cas systems in the four genomes of the present study. Interestingly, we found a diversity between strains regarding CRISPR/Cas System (additional file 1, Table 3.8). Indeed, in the genome of the strain EBP 3070 we do not found CRISPR/Cas System, in the genome of the strain CD 337 we found only one putative CRISPR-associated endoribonuclease, while in the genome of the strain BSAS1 3 we found a CRISPR-Cas system composed by three CRISPR-associated endoribonucleases (Cas1, Cas2, and Cas9) and a Type II-A CRISPR-associated protein Csn2. Finally, in the genome of the strain TAP 175 we found a CRISPR-Cas system composed by at least 5 CRISPR-associated endoribonucleases (Cas1, Cas2, Cas4, Cas5d and Cas9) and a Type II-A CRISPR-associated protein Csn2. This diversity in four strains could participate to explain their adaptation and survival to various ecological niches, as CRISPR/Cas System provides bacterial immunity against lytic bacteriophages, which occur in food and food environments (Greer and Dilts, 2002; Stout et al., 2017).

B. thermosphacta CD 337 complete genome contained no plasmid. However, putative plasmids were found in the three draft genomes as shown in (Figure 3.6). Indeed, these contained contigs harboring genes related to plasmid proteins (*ie: repB* involved in plasmid replication or *mob/pre* genes involved in recombination and conjugative mobilization). Such a 4557 bp long contig was found in both TAP 175 and BSAS1 3 with a high similarity degree

between the two strains. This plasmid encoded also a protein annotated as a quaternary ammonium compound-resistance protein. The genome of EBP 3070 harbored two putative plasmids (8624 bp; 5011 bp). One of these may confer tetracycline resistance as it carried a gene encoding a multifunctional tetracycline antiporter which was 81% identical to the *tetB(L)* gene of *Bacillus subtilis* (Cheng et al., 1994).

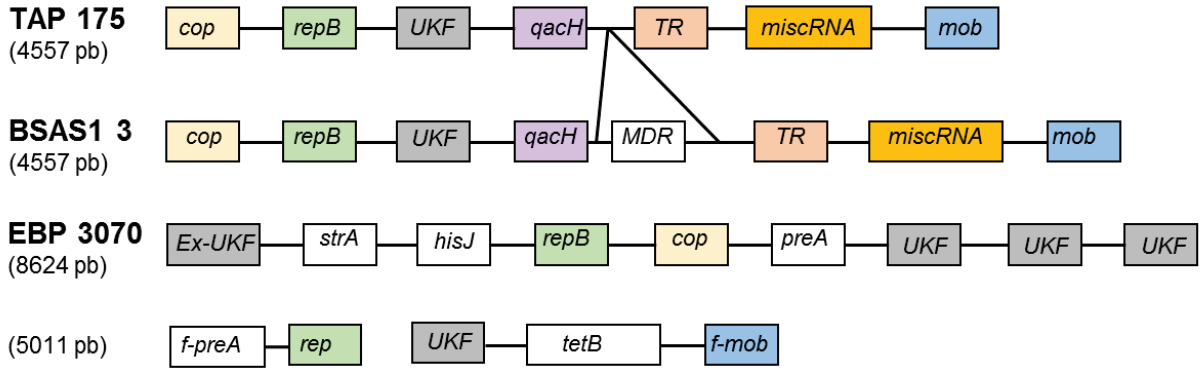

Figure 3.6 Schematic representation of putative plasmids content of three *B. thermosphacta* strains.

The plasmids size and the CDS content were given. Abbreviations: ***cop*****:** protein Cop*;* ***repB:*** replication protein RepB; ***qacH:*** quaternary ammonium compound-resistance protein QacH; ***mob/preA:*** Mob/Pre recombination and conjugative mobilization; ***strA:*** sortase A; ***hisJ:*** histidinol phosphatase; **MDR:** small multidrug resistance protein; **TR:** transcriptional regulator; **UKF:** protein of unknown function; ***Ex-UKF:*** exported protein of unknown function; ***miscRNA:*** miscellaneous RNA described on *Bacillus* plasmids; ***f-***: gene fragment.

Extended insights

Functions putatively involved in the niche adaptation and in the spoilage properties were searched in the genomes of the four strains. We found a large repertoire of substrate specific genes from the phosphoenolpyruvate dependent phosphotransferase system (PTS). The genomes of the four strains contained genes for glucose, maltose, fructose, mannose, trehalose, cellobiose, mannitol, beta-glucosides, and N-acetylglucosamine transport and phosphorylation. Genes encoding transporters for ribose, glycerol-3-phosphate, maltose, and *myo*-inositol were also present, attesting the large capacity of carbon sources used by *B. thermosphacta*.

No major difference for sugar utilization was noticed between the four strains. In addition predicted metabolic pathways of our strains were very similar to those previously described in other *B. thermosphacta* strains (Stanborough et al., 2017). Briefly, all genes required for

glycolysis and the pentose phosphate pathway were present in all strains. The citrate cycle was incomplete since only four of the eight enzymes were detected. The genes coding for alpha-ketoglutarate dehydrogenase (EC 1.2.4.2), succinate thiokinase (EC 6.2.1.4), succinate dehydrogenase (EC 1.3.5.1), and malate dehydrogenase (EC 1.1.1.37) were absent. Moreover, the gene coding the pyruvate carboxylase (EC 6.4.1.1) was present while that of the fumarate reductase (EC 1.3.5.4) was absent.

Genes involved in the production of molecules associated to meat or seafood spoilage, as acetoin, diacetyl, and 2.3-butanediol (Figure 3.7), lactate, ethanol, and acetate were identified.

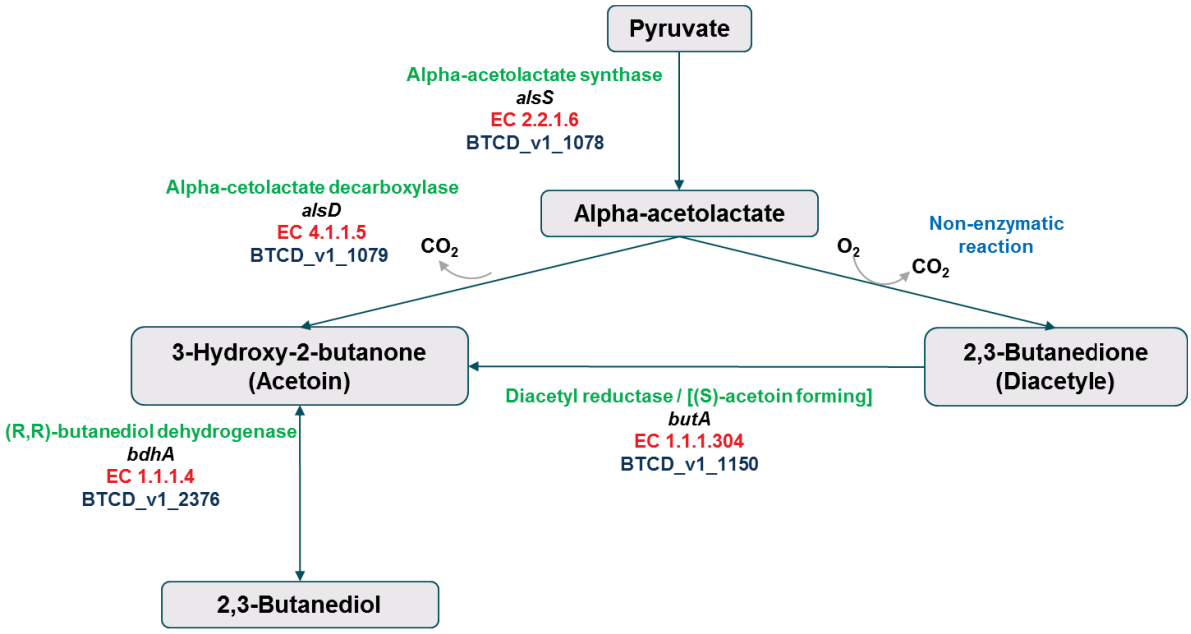

Figure 3.7 Metabolic pathway for the production of acetoin and diacetyl from pyruvate degradation.
All genes encoding the necessary enzymes were found.

Although we had previously observed differences between strains in their ability to produce acetoin, the *butA* gene encoding diacetyl reductase [(S)-acetoin forming], as well as its 300 bp upstream region was 100% identical in all strains. Conversely, the *bdhA* gene encoding (R,R)-2,3 butanediol dehydrogenase able to convert (R,R)- 2,3-butanediol or diacetyl to acetoin presented 100% identity between BSAS1 3 and CD 337 but, amino-acid at position 201 (alanine) was replaced by glutamic acid in both EBP 3070 and TAP 175 enzymes, thus introducing a negative charge. Interestingly replacing aspartic acid by alanine in glycerol

dehydrogenase of *Escherichia coli* improved its activity toward 1,3-butanediol (Zhang et al., 2010). As EBP 3070 and TAP 175 had a similar pattern for acetoin and diacetyl production (they were among the highest producers of acetoin and diacetyl in shrimp juice, and among the lowest in beef) we can hypothesize that the alanine/glutamate replacement may be involved in the different acetoin production levels we previously observed.

Moreover, the upstream region of the *bdhA* gene of EBP 3070 and TAP 175 were identical and showed differences with that of BSAS1 3 at positions -141, -137, -110, -67, and -43 upstream from the start codon suggesting transcriptional regulation of *bdhA* might be different. However, CD 337 presented the same differences as EBP 3070 and TAP 175 plus an additional one at position -46.

In addition, genes involved in the production of isovaleric, isobutyric, and 2-methylbutyric branched-chain fatty acids were found in the genome of the four strains. These compounds, associated with off-odors, were suggested to be produced from the degradation of branched-chain amino acids leucine, valine, and isoleucine, respectively (Holley, 2014). The catabolism of leucine can also lead further to the synthesis of 3-methylbutanal and 3-methylbutanol. 3-methybutanal, a branched-chain aldehyde has been described as associated to the production of desirable aroma in many cheeses (Afzal et al., 2017), while it is responsible for off-odors in meat and seafood products (Casaburi et al., 2015b; Jaffrès et al., 2011). The pathway of 3-methylbutanal production from leucine catabolism has been described in lactic acid bacteria. The first step of this pathway is the transamination of leucine to α-ketoisocaproate which is the central metabolite in leucine degradation (Smit et al., 2004). Then the formation of 3-methylbutanal may occur in two possible metabolic pathways: directly via the non-oxidative decarboxylation by an α-ketoacid decarboxylase or indirectly through an oxidative decarboxylation by α-ketoacid dehydrogenase (Afzal et al., 2017). Investigation of *B. thermosphacta* genomes showed that all the genes encoding enzymes required for isovalerate, 3-methylbutanal and 3-methylbutanol production from leucine were present (Figure 3.8). Interestingly, the α-ketoacid decarboxylase from BSAS1 3, TAP 175, and EBP 3070 was 100% identical (except glutamine 311 replaced by a histidine in EPB 3070) but was mutated in CD 337 resulting in a fragmented gene and 2 mutations on amino acids 200 and 205. We also noticed that the gene encoding the E1 component, alpha subunit of the alpha-

keto acid dehydrogenase was 100% identical in CD 337, EPB 3070, and TAP 175 but fragmented in BSAS1 3. This suggests that production of isovalerate, 3-methylbutanol, and 3-methylbutanal may differ between strains and use different pathways, possibly impacting the spoilage potential of the strains.

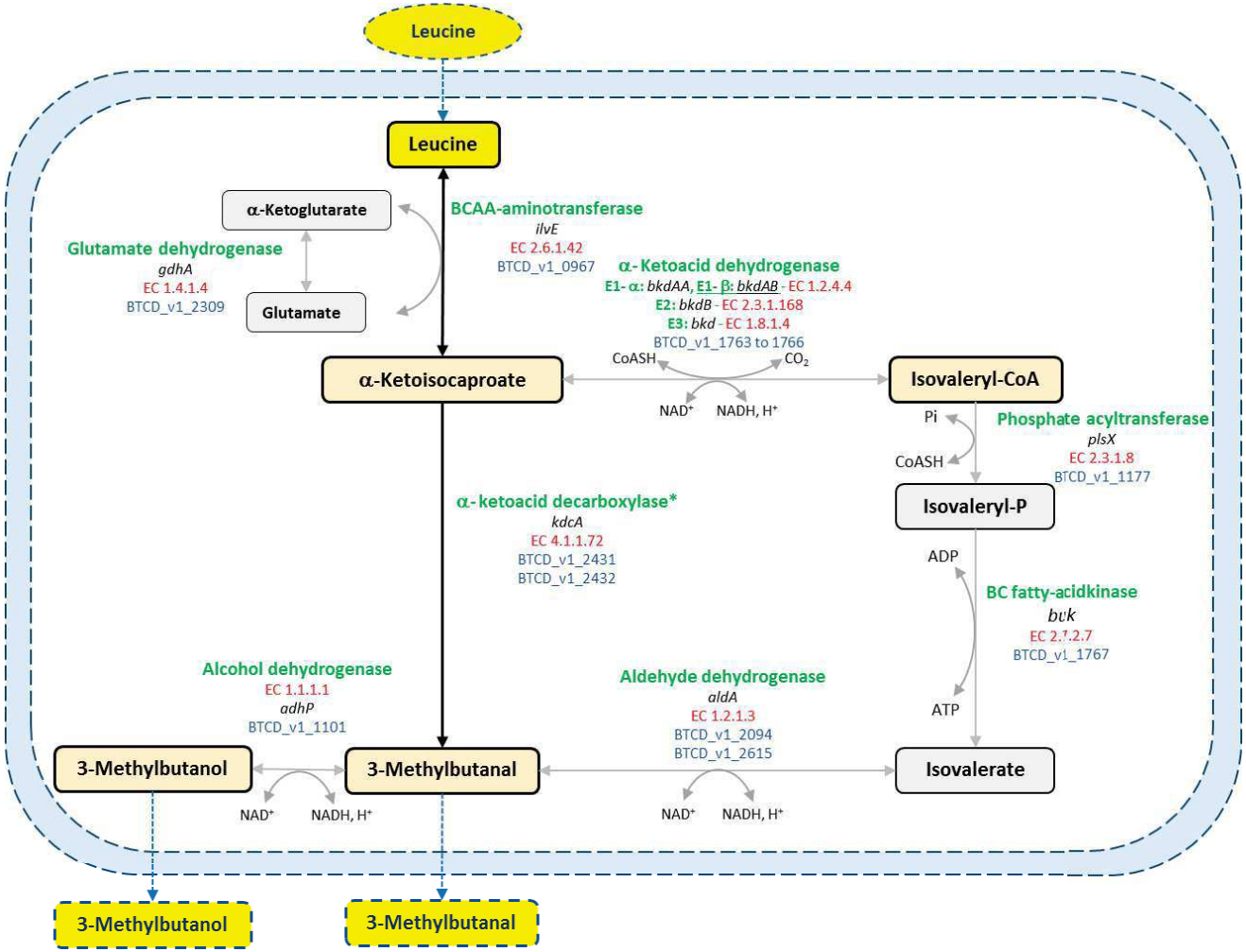

Figure 3.8 Probable metabolic pathway for the biosynthesis of 3-methylbutanal from L-leucine degradation by *B. thermosphacta.*
All the genes encoding the necessary enzymes for the direct (dark line) and the indirect (gray line) pathways were present. Alcohol dehydrogenase was also present. BCAA refers to branched-chain amino acids. * The *kdcA* gene is fragmented in *B. thermosphacta* CD 337. The underlined gene *bkdAB* is fragmented in BSAS1 3.

Since *B. thermosphacta* has been associated to the production of biogenic amines (Paleologos et al., 2004) the presence of amino acid decarboxylases was searched in the genomes. Genes encoding histidine decarboxylase and tyrosine decarboxylase responsible for the production of histamine and tyramine, respectively, were not found. Nevertheless, all the genomes harbored the genes encoding the ABC transporter complex PotABCD involved in the import of spermidine and putrescine, two polyamines present in meat and fish.

A putative cell-wall associated adhesin was found in CD 337 genome, which was absent or truncated in other strains. Adhesins may promote substrate adhesion and survival in the environment, and the presence of adhesin in only some strains may contribute to their different niche adaptation. Nine proteins annotated as containing an LPxTG-like motif were detected in CD 337. In addition, among the genes encoding exported proteins of unknown functions two CDS (BTCD_v1_656 and BTCD_v1_1960) also harbored an LPxTG motif and a signal peptide, as well as a protein annotated as a putative fimbrial isopeptide formation D2 domain-containing protein (BTCD_v1_1958). Such proteins are surface proteins covalently linked to the bacterial cell wall by a sortase. All were well conserved in the four strains, except three (BTCD_v1_1958 to 1960) which were unique to CD 337 and located in a genomic island of 24,132 bp. This island also encompassed genes encoding putative recombinases and transposases suggesting its acquisition through horizontal gene transfer. Putative sortase genes were also present, showing this island may indeed encode functions for specifically linking the three LPxTG proteins to the cell surface. It also hosted genes encoding proteins involved in polysaccharide metabolism as a putative polysaccharide deacetylase, a putative polysialyltransferase, and proteins resembling the NeuBCDA enzymes involved in the amino sugar N-acetyl neuraminic acid (sialic acid) metabolism. Futhermore, a putative O-acetyltransferase EpsM (BTCD_v1_0680) most probably involved in biofilm formation was also found in CD 337 genome, conserved in *B. thermosphacta* BI and BII genomes, but absent in the three other strain genomes of the present study (EPB 3070, TAP 175 and BSAS1 3). It has been shown in *B. subtilis* a similar O-acetyltransferase (EpsM), which is a member of the eps operon, involved in the production of the exopolysaccharide (EPS) component of the extracellular matrix during biofilm formation (Nagorska et al., 2010).

All these proteins may have an important role in the survival and the persistence of bacteria in the food-processing environment. Such differences in the gene repertoire between the four strains might correlate to the different substrates our strains were isolated from.

3.2.7. Conclusions

The four strains we selected for comparative genomics were chosen as diverse as possible (different ecological origin, different ability to produce some spoilage molecules, and belonging to different PFGE and Rep-PCR clusters). However, a high genome content similarity was observed as previously reported by other authors on different meat product issued strains. The major differences we observed in the gene content were represented by phages or plasmids, restriction/modification systems, cell surface functions, or use of various carbon sources. These could participate to their fitness or adaptation to various niches, in particular the functions involved in carbon sources utilization or those associated to cell surface or adhesion that may help to colonize specific environment. Most of the strain specific genome encompasses proteins of unknown function.

The simple comparison of the variable genome could not explain the differences we observed in the ability to produce acetoin and diacetyl. Nevertheless, we showed that mutations (fragmentation or point mutation) in genes encoding enzymes involved in the production of VOCs and differences in the DNA sequences located upstream from start codons, thus potentially in the promoter region of these genes may lead to different efficacies to produce such VOCs and therefore to spoil meat or seafood products.

Therefore, the diversity of spoilage potential of *B. thermosphacta* on various foods reported in the literature may result from i) a strain dependent specificity to adapt to different ecological niches, characterized by strain specific genome content; ii) a strain dependent capacity to produce malodorous molecules driven by the presence/absence/mutations of enzymes involved in the catabolism of branched chain amino acids and pyruvate; and iii) a strain dependent capacity to express the corresponding genes.

Funding

This work was financed by “Région Pays de la Loire” (grant to MZ). NI was the recipient of a PhD fellowship from the French Ministry of Higher Education and Research.

Acknowledgements

We thank Angélina D’Orlando and INRA BIBS plateform (composante Microscopie) for microscopy imaging. We would like to thank the platform GeT-Biopuces at the Genopole in Toulouse for the S5 sequencing experiments and bioinformatics advices. This work was performed in collaboration with the GeT (Genome and Transcriptome) core facility, Toulouse, France (http://get.genotoul.fr), and was supported by France Génomique National infrastructure, funded as part of “Investissement d’avenir” program managed by Agence Nationale pour la Recherche (contract ANR-10-INBS-09) and by the GET-PACBIO program (« Programme operationnel FEDER-FSE MIDI-PYRENEES ET GARONNE 2014-2020 »). The LABGeM (CEA/IG/Genoscope & CNRS UMR8030) and the France Génomique National infrastructure (funded as part of Investissement d'avenir program managed by Agence Nationale pour la Recherche, contract ANR-10-INBS-09) are acknowledged for support within the MicroScope annotation platform.

Authors' contributions

NI participated to conceive the study, performed laboratory experiments (bacterial cultures, DNA extraction), assembled the draft *B. thermosphacta* genomes, participated to the *B. thermosphacta* CD 337 genome annotation, performed comparative genomic analysis, interpreted the results, and wrote the manuscript. OB and AR sequenced the complete genome of CD 337. CK assembled the complete genome of CD 337 and participated to write the manuscript. NM sequenced the draft genomes. MZ conceived the study, participated to the *B. thermosphacta* CD 337 genome annotation and to the comparative genomic analysis, and wrote the manuscript. EJ participated to conceive the study, participated to the genome annotation and wrote the manuscript.

All authors read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interest.

Additional file 1

Table 3.5 List of *B. thermosphacta* CD 337 specific genes.

	Label	Type	Gene	Product	Mutation		Label	Type	Gene	Product	Mutation
1	BTCD_v1_0020	CDS	_	protein of unknown function	no	22	BTCD_v1_0363	CDS	_	putative transcriptional regulator (TetR/AcrR family) fragment	pseudo
2	BTCD_v1_0094	CDS	_	protein of unknown function	no	23	BTCD_v1_0364	CDS	_	conserved membrane protein of unknown function	
3	BTCD_v1_0147	CDS	_	Putative transcriptional regulator, AraC family protein	no	24	BTCD_v1_0365	CDS	_	putative NAD(P)H oxidoreductase	
4	BTCD_v1_0148	CDS	_	putative thermostable beta-glucosidase AB (modular protein)	no	25	BTCD_v1_0367	CDS	_	conserved exported protein of unknown function	
5	BTCD_v1_0227	CDS	_	Putative major facilitator family protein	no	26	BTCD_v1_0368	CDS	_	conserved protein of unknown function	
6	BTCD_v1_0228	CDS	_	protein of unknown function	no	27	BTCD_v1_0369	CDS	_	putative SAM-dependent methyltransferase (fragment)	no
7	BTCD_v1_0229	CDS	_	protein of unknown function	no	28	BTCD_v1_0370	CDS	_	putative SAM-dependent methyltransferase (fragment)	no
8	BTCD_v1_0261	CDS	_	hypothetical protein	no	29	BTCD_v1_0382	CDS	_	protein of unknown function (fragment)	no
9	BTCD_v1_0270	CDS	_	hypothetical protein	no	30	BTCD_v1_0405	CDS	_	protein of unknown function	no
10	BTCD_v1_0271	CDS	_	hypothetical protein	no	31	BTCD_v1_0415	CDS	*ydcL*	putative phage integrase	no
11	BTCD_v1_0272	CDS	_	protein of unknown function	no	32	BTCD_v1_0416	CDS	*immR*	putative HTH-type transcriptional regulator	no
12	BTCD_v1_0286	CDS	_	protein of unknown function	no	33	BTCD_v1_0417	CDS	_	putative HTH type transcriptionnel regulator	no
13	BTCD_v1_0300	CDS	_	exported protein of unknown function	no	34	BTCD_v1_0431	CDS	_	putative restriction endonuclease subunit S	no
14	BTCD_v1_0317	CDS	_	conserved membrane protein of unknown function	no	35	BTCD_v1_0434	CDS	_	protein of unknown function	no
15	BTCD_v1_0318	CDS	_	putative response regulator of the LytR/AlgR family	no	36	BTCD_v1_0455	CDS	_	putative maltodextrin import ATP-binding protein MsmX	no
16	BTCD_v1_0319	CDS	_	protein of unknown function	no	37	BTCD_v1_0456	CDS	*msmX*	maltodextrin import ATP-binding protein	no
17	BTCD_v1_0343	CDS	_	protein of unknown function	no	38	BTCD_v1_0464	CDS	_	protein of unknown function	no
18	BTCD_v1_0344	CDS	_	protein of unknown function	no	39	BTCD_v1_0490	CDS	_	protein of unknown function	no
19	BTCD_v1_0346	CDS	_	protein of unknown function	no	40	BTCD_v1_0491	CDS	_	conserved exported protein of unknown function	no
20	BTCD_v1_0348	CDS	_	protein of unknown function	no	41	BTCD_v1_0505	CDS	_	protein of unknown function	no
21	BTCD_v1_0362	CDS	_	putative transcriptional regulator (TetR/AcrR family) fragment	no	42	BTCD_v1_0507	CDS	_	protein of unknown function	no

43	BTCD_v1_0552	CDS	_	protein of unknown function	no	64	BTCD_v1_0885	CDS	_	protein of unknown function	no
44	BTCD_v1_0556	CDS	_	protein of unknown function	no	65	BTCD_v1_0913	CDS	*gntT*	gluconate transporter, high-affinity GNT I system	no
45	BTCD_v1_0577	fCDS	*ytoI*	fragment of conserved hypothetical protein (part 1)	pseudo	66	BTCD_v1_0914	CDS	_	putative 4-carbon acid sugar kinase	no
46	BTCD_v1_0578	fCDS	*ytoI*	fragment of conserved hypothetical protein (part 2)	pseudo	67	BTCD_v1_0915	CDS	*garR*	2-hydroxy-3-oxopropionate reductase	no
47	BTCD_v1_0626	CDS	_	conserved exported protein of unknown function	no	68	BTCD_v1_0916	CDS	_	putative HTH-type transcriptional regulator	no
48	BTCD_v1_0658	CDS	_	putative two-component sensor histidine kinase	no	69	BTCD_v1_0917	CDS	_	protein of unknown function	no
49	BTCD_v1_0659	CDS	_	membrane protein of unknown function	no	70	BTCD_v1_0945	CDS	_	protein of unknown function	no
50	BTCD_v1_0662	CDS	_	conserved protein of unknown function	no	71	BTCD_v1_0957	CDS	_	protein of unknown function	no
51	BTCD_v1_0663	CDS	_	putative regulator	no	72	BTCD_v1_0963	CDS	_	conserved exported protein of unknown function	no
52	BTCD_v1_0664	CDS	_	protein of unknown function	no	73	BTCD_v1_0964	CDS	_	conserved protein of unknown function	no
53	BTCD_v1_0665	CDS	_	protein of unknown function	no	74	BTCD_v1_0994	CDS	_	membrane protein of unknown function	no
54	BTCD_v1_0669	CDS	_	putative UDP-glucose/GDP-mannose dehydrogenase (fragment)	no	75	BTCD_v1_0996	CDS	_	protein of unknown function	no
55	BTCD_v1_0670	CDS	_	putative UDP-glucose/GDP-mannose dehydrogenase CapL (fragment)	no	76	BTCD_v1_0997	CDS	_	protein of unknown function	no
56	BTCD_v1_0772	CDS	_	protein of unknown function	no	77	BTCD_v1_1013	CDS	_	putative transcriptional antiterminator, BglG family	no
57	BTCD_v1_0786	CDS	_	protein of unknown function	no	78	BTCD_v1_1032	CDS	_	protein of unknown function	no
58	BTCD_v1_0787	CDS	_	protein of unknown function	no	79	BTCD_v1_1105	CDS	_	protein of unknown function	no
59	BTCD_v1_0806	CDS	_	protein of unknown function	no	80	BTCD_v1_1137	CDS	_	conserved protein of unknown function	no
60	BTCD_v1_0812	CDS	_	protein of unknown function	no	81	BTCD_v1_1142	CDS	_	protein of unknown function	no
61	BTCD_v1_0874	CDS	_	putative heme-iron transport system permease protein IsdF (fragment)	no	82	BTCD_v1_1165	CDS	_	putative transposase (fragment)	no
62	BTCD_v1_0875	CDS	_	putative heme-iron transport system permease protein IsdF (fragment)	no	83	BTCD_v1_1167	CDS	_	putative type 2 lantibiotic biosynthesis protein LanM	no
63	BTCD_v1_0884	CDS	_	conserved protein of unknown function	no	84	BTCD_v1_1168	CDS	_	putative lanthionine synthetase C-like proteins (LanC)	no

This table continues in the following pages

	Label	Type	Gene	Product	Mutation		Label	Type	Gene	Product	Mutation
85	BTCD_v1_1169	CDS	_	putative lantibiotic lichenicidin	no	105	BTCD_v1_1219	CDS	_	putative restriction endonuclease subunit S	no
86	BTCD_v1_1170	CDS	_	putative lantibiotic leader peptide-processing serine protease	no	106	BTCD_v1_1220	CDS	_	putative type I site-specific deoxyribonuclease, HsdR (fragment)	no
87	BTCD_v1_1186	CDS	_	membrane protein of unknown function	no	107	BTCD_v1_1221	CDS	_	putative type I restriction-modification system, restriction subunit R (fragment)	no
88	BTCD_v1_1187	CDS	_	membrane protein of unknown function	no	108	BTCD_v1_1223	CDS	_	conserved protein of unknown function (fragment)	no
89	BTCD_v1_1188	CDS	_	putative permease of the major facilitator superfamily (MFS)	no	109	BTCD_v1_1227	CDS	_	protein of unknown function	no
90	BTCD_v1_1189	CDS	_	putative permease of the major facilitator superfamily (MFS)	no	110	BTCD_v1_1237	fCDS	_	protein of unknown function	no
91	BTCD_v1_1191	CDS	_	putative restriction endonuclease, type I, HsdS	no	111	BTCD_v1_1241	CDS	_	protein of unknown function	no
92	BTCD_v1_1192	CDS	_	protein of unknown function	no	112	BTCD_v1_1270	fCDS	_	protein of unknown function	no
93	BTCD_v1_1193	CDS	_	protein of unknown function	no	113	BTCD_v1_1271	CDS	_	conserved protein of unknown function (fragment)	no
94	BTCD_v1_1194	CDS	_	membrane protein of unknown function	no	114	BTCD_v1_1272	CDS	_	conserved protein of unknown function	no
95	BTCD_v1_1195	CDS	_	putative ABC transporter (ATP-binding subunit)	no	115	BTCD_v1_1273	CDS	_	conserved protein of unknown function	no
96	BTCD_v1_1196	CDS	_	membrane protein of unknown function	no	116	BTCD_v1_1274	CDS	_	conserved protein of unknown function	no
97	BTCD_v1_1197	CDS	_	membrane protein of unknown function	no	117	BTCD_v1_1283	CDS	_	putative HTH-type transcriptional regulator	no
98	BTCD_v1_1198	CDS	_	protein of unknown function	no	118	BTCD_v1_1284	CDS	_	PTS system, beta-glucoside-specific IIBC component	no
99	BTCD_v1_1199	CDS	*hxlR*	HTH-type transcriptional activator HxlR	no	119	BTCD_v1_1285	CDS	_	PTS system beta-glucoside-specific, enzyme IIA component	no
100	BTCD_v1_1200	CDS	_	putative nitroreductase	no	120	BTCD_v1_1286	CDS	*bglH*	aryl-phospho-beta-D-glucosidase	no
101	BTCD_v1_1215	CDS	_	conserved protein of unknown function	no	121	BTCD_v1_1287	fCDS	_	protein of unknown function	no
102	BTCD_v1_1216	CDS	_	conserved protein of unknown function	no	122	BTCD_v1_1288	CDS	_	putative HTH-type transcriptional regulator	no
103	BTCD_v1_1217	CDS	_	conserved protein of unknown function	no	123	BTCD_v1_1289	CDS	_	conserved protein of unknown function	no
104	BTCD_v1_1218	CDS	_	putative type I restriction-modification system, M subunit	no	124	BTCD_v1_1290	CDS	_	putative 4-carboxymuconolactone decarboxylase	no

This table continues in the following pages

125	BTCD_v1_1291	CDS	_	putative short-chain dehydrogenase/reductase, NAD(P) dependent	no	146	BTCD_v1_1466	fCDS	*gmuE*	fragment of ROK fructokinase; glucomannan utilization protein E (part 1)	pseudo
126	BTCD_v1_1294	fCDS	*alkA*	fragment of DNA-3-methyladenine glycosylase (part 2)	pseudo	147	BTCD_v1_1475	CDS	_	phosphotransferase system (PTS) mannose-specific enzyme IIC component	no
127	BTCD_v1_1303	CDS	_	protein of unknown function	no	148	BTCD_v1_1490	CDS	_	protein of unknown function	no
128	BTCD_v1_1304	CDS	_	exported protein of unknown function	no	149	BTCD_v1_1514	CDS	_	exported protein of unknown function	no
129	BTCD_v1_1305	CDS	_	conserved protein of unknown function	no	150	BTCD_v1_1520	CDS	_	conserved protein of unknown function	no
130	BTCD_v1_1306	CDS	_	protein of unknown function	no	151	BTCD_v1_1522	CDS	_	protein of unknown function	no
131	BTCD_v1_1308	CDS	_	putative transposase (fragment)	no	152	BTCD_v1_1523	CDS	_	protein of unknown function	no
132	BTCD_v1_1338	CDS	_	protein of unknown function	no	153	BTCD_v1_1524	CDS	_	putative C-5 cytosine methyltransferase	no
133	BTCD_v1_1341	fCDS	*polC*	fragment of DNA polymerase III (alpha subunit) (part 2)	pseudo	154	BTCD_v1_1538	CDS	_	protein of unknown function	no
134	BTCD_v1_1343	CDS	_	protein of unknown function	no	155	BTCD_v1_1544	CDS	_	protein of unknown function	no
135	BTCD_v1_1346	CDS	_	protein of unknown function	no	156	BTCD_v1_1554	fCDS	_	protein of unknown function	no
136	BTCD_v1_1365	CDS	_	protein of unknown function	no	157	BTCD_v1_1560	CDS	_	putative glycoside hydrolase	no
137	BTCD_v1_1370	fCDS	_	Putative nuclease SbcCD subunit D, C-terminal domain (fragment)	no	158	BTCD_v1_1561	CDS	_	conserved exported protein of unknown function	no
138	BTCD_v1_1371	CDS	_	Putative nuclease SbcCD subunit D (fragment)	no	159	BTCD_v1_1572	fCDS	_	protein of unknown function	no
139	BTCD_v1_1377	CDS	_	protein of unknown function	no	160	BTCD_v1_1573	CDS	_	conserved protein of unknown function	no
140	BTCD_v1_1393	CDS	_	protein of unknown function	no	161	BTCD_v1_1643	CDS	_	protein of unknown function	no
141	BTCD_v1_1397	CDS	_	protein of unknown function	no	162	BTCD_v1_1644	CDS	_	protein of unknown function	no
142	BTCD_v1_1403	CDS	_	membrane protein of unknown function	no	163	BTCD_v1_1645	CDS	_	protein of unknown function	no
143	BTCD_v1_1425	CDS	_	conserved protein of unknown function	no	164	BTCD_v1_1655	CDS	_	conserved protein of unknown function	no
144	BTCD_v1_1426	CDS	_	putative phosphoglycerate mutase family protein (fragment)	no	165	BTCD_v1_1656	CDS	_	protein of unknown function	no
145	BTCD_v1_1452	CDS	_	putative ribosome biogenesis GTPase RsgA (fragment)	no	166	BTCD_v1_1657	CDS	_	protein of unknown function	no

This table continues in the following pages

	Label	Type	Gene	Product	Mutation
167	BTCD_v1_1658	CDS	_	protein of unknown function	no
168	BTCD_v1_1686	fCDS	*ykrA*	fragment of putative hydrolase (part 2)	pseudo
169	BTCD_v1_1687	fCDS	*ykrA*	fragment of putative hydrolase (part 1)	pseudo
170	BTCD_v1_1721	CDS	_	protein of unknown function	no
171	BTCD_v1_1735	CDS	_	protein of unknown function	no
172	BTCD_v1_1739	CDS	_	protein of unknown function	no
173	BTCD_v1_1741	CDS	_	Protein of unknown function	no
174	BTCD_v1_1752	CDS	_	protein of unknown function	no
175	BTCD_v1_1753	CDS	_	protein of unknown function	no
176	BTCD_v1_1774	CDS	_	putative endopeptidase (serine protease activity)	no
177	BTCD_v1_1788	CDS	_	putative CRISPR-associated endoribonuclease	no
178	BTCD_v1_1842	CDS	_	putative holin; skin element	no
179	BTCD_v1_1845	CDS	_	putative surface protein of unknown function	no
180	BTCD_v1_1846	CDS	_	conserved protein of unknown function	no
181	BTCD_v1_1847	CDS	_	putative phage related protein	no
182	BTCD_v1_1864	CDS	_	phage protein Gp4	no
183	BTCD_v1_1865	CDS	_	putative phage related protein	no
184	BTCD_v1_1866	CDS	_	protein of unknown function	no
185	BTCD_v1_1867	CDS	_	protein of unknown function	no
186	BTCD_v1_1869	CDS	_	protein of unknown function	no
187	BTCD_v1_1870	CDS	_	protein of unknown function	no
188	BTCD_v1_1871	CDS	_	putative phage related protein	no
189	BTCD_v1_1872	CDS	_	phage related protein	no
190	BTCD_v1_1871	CDS	_	putative phage related protein	no
191	BTCD_v1_1875	CDS	_	conserved protein of unknown function	no
192	BTCD_v1_1879	CDS	_	putative phage related protein	no
193	BTCD_v1_1881	CDS	_	protein of unknown function	no
194	BTCD_v1_1894	CDS	_	protein of unknown function	no
195	BTCD_v1_1900	CDS	_	protein of unknown function	no
196	BTCD_v1_1918	fCDS	_	putative rod shape-determining protein RodA (fragment)	no
197	BTCD_v1_1919	CDS	_	membrane protein of unknown function	no
198	BTCD_v1_1921	fCDS	*lysC*	fragment of aspartokinase II alpha subunit (aa 1->408) and beta subunit (aa 246->408) (part 1)	pseudo
199	BTCD_v1_1944	CDS	_	conserved membrane protein of unknown function	no
200	BTCD_v1_1947	CDS	_	protein of unknown function	no
201	BTCD_v1_1948	CDS	_	protein of unknown function	no
202	BTCD_v1_1949	CDS	_	protein of unknown function	no
203	BTCD_v1_1950	CDS	_	protein of unknown function	no
204	BTCD_v1_1962	CDS	_	protein of unknown function	no
205	BTCD_v1_1963	CDS	_	protein of unknown function	no
206	BTCD_v1_1964	CDS	_	conserved protein of unknown function	no
207	BTCD_v1_1965	CDS	_	Putative polysaccharide deacetylase	no
208	BTCD_v1_1966	CDS	_	conserved protein of unknown function	no
209	BTCD_v1_1967	CDS	_	conserved protein of unknown function	no
210	BTCD_v1_1968	CDS	_	putative polysialyltransferase	no
211	BTCD_v1_1969	CDS	*neuA*	N-acylneuraminate cytidylyltransferase	no
212	BTCD_v1_1970	CDS	_	putative sugar O-acyltransferase, sialic acid O-acetyltransferase NeuD family	no

This table continues in the following pages

213	BTCD_v1_1971	CDS	*neuC*	UDP-N-acetylglucosamine 2-epimerase	no	234	BTCD_v1_2151	fCDS	*luxS*	fragment of S-ribosylhomocysteinase (part 1)	pseudo
214	BTCD_v1_1972	CDS	_	protein of unknown function	no	235	BTCD_v1_2177	fCDS	*hemY*	fragment of promiscuous protoporphyrinogen IX and coproporphyrinogen III oxidase (part 2)	pseudo
215	BTCD_v1_1973	CDS	*neuB*	N-acylneuraminate-9-phosphate synthase	no	236	BTCD_v1_2178	fCDS	*hemY*	fragment of promiscuous protoporphyrinogen IX and coproporphyrinogen III oxidase (part 1)	pseudo
216	BTCD_v1_1974	CDS	_	protein of unknown function	no	237	BTCD_v1_2184	CDS	_	protein of unknown function	no
217	BTCD_v1_1984	CDS	_	protein of unknown function	no	238	BTCD_v1_2194	CDS	_	protein of unknown function	no
218	BTCD_v1_1991	fCDS	*ktrD*	fragment of K+-transporting ATPase (part 2)	pseudo	239	BTCD_v1_2199	CDS	_	protein of unknown function	no
219	BTCD_v1_1992	fCDS	*ktrD*	fragment of K+-transporting ATPase (part 1)	pseudo	240	BTCD_v1_2200	CDS	_	putative peptidase (fragment)	no
220	BTCD_v1_1993	CDS	_	protein of unknown function	no	241	BTCD_v1_2201	CDS	_	putative acetylornithine deacetylase (fragment)	no
221	BTCD_v1_2014	CDS	_	conserved protein of unknown function	no	242	BTCD_v1_2213	CDS	_	protein of unknown function	no
222	BTCD_v1_2022	CDS	_	protein of unknown function	no	243	BTCD_v1_2252	CDS	_	protein of unknown function	no
223	BTCD_v1_2041	CDS	_	protein of unknown function	no	244	BTCD_v1_2257	CDS	_	protein of unknown function	no
224	BTCD_v1_2043	CDS	_	membrane protein of unknown function	no	245	BTCD_v1_2261	CDS	_	protein of unknown function	no
225	BTCD_v1_2099	CDS	_	conserved protein of unknown function	no	246	BTCD_v1_2302	CDS	_	protein of unknown function	no
226	BTCD_v1_2104	CDS	_	putative endoribonuclease L-PSP/chorismate mutase-like (fragment)	no	247	BTCD_v1_2303	CDS	_	exported protein of unknown function	no
227	BTCD_v1_2105	CDS	_	conserved protein of unknown function (fragment)	no	248	BTCD_v1_2307	CDS	_	conserved membrane protein of unknown function	no
228	BTCD_v1_2121	CDS	_	putative zinc-containing alcohol dehydrogenase superfamily (fragment)	no	249	BTCD_v1_2321	CDS	*licA*	phosphotransferase system (PTS) lichenan-specific enzyme IIA component	no
229	BTCD_v1_2122	CDS	_	putative zinc-containing alcohol dehydrogenase superfamily (fragment)	no	250	BTCD_v1_2322	CDS	*celA*	phosphotransferase system (PTS) lichenan-specific enzyme IIB component	no
230	BTCD_v1_2126	CDS	_	putative transporter, N-terminal fragment	no	251	BTCD_v1_2327	CDS	_	protein of unknown function	no
231	BTCD_v1_2127	CDS	_	putative transporter, C-terminal fragment	no	252	BTCD_v1_2349	CDS	*galE*	UDP-glucose 4-epimerase	no
232	BTCD_v1_2135	fCDS	_	protein of unknown function	no	253	BTCD_v1_2350	CDS	_	putative mannosyl-glycoprotein endo-beta-N-acetylglucosamidase/muramidase	no
233	BTCD_v1_2150	fCDS	*luxS*	fragment of S-ribosylhomocysteinase (part 2)	pseudo	254	BTCD_v1_2351	CDS	_	protein of unknown function	no

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	Label	Type	Gene	Product	Mutation
255	BTCD_v1_2352	fCDS	_	protein of unknown function	no
256	BTCD_v1_2353	CDS	_	putative CDP-glycerol:glycerophosphate glycerophosphotransferase	no
257	BTCD_v1_2354	CDS	_	putative glycosyltransferases involved in cell wall biogenesis	no
258	BTCD_v1_2356	CDS	_	putative CDP-glycerol:glycerophosphate glycerophosphotransferase TarF	no
259	BTCD_v1_2358	CDS	_	putative glycosyl/glycerophosphate transferases involved in teichoic acid biosynthesis	no
260	BTCD_v1_2360	fCDS	_	protein of unknown function	no
261	BTCD_v1_2367	fCDS	_	protein of unknown function	no
262	BTCD_v1_2371	CDS	*bglP*	beta-glucoside-specific PTS enzyme IIC component	no
263	BTCD_v1_2372	CDS	*bglP*	beta-glucoside-specific PTS enzymes IIBC component	no
264	BTCD_v1_2380	fCDS	_	protein of unknown function	no
265	BTCD_v1_2392	CDS	_	protein of unknown function	no
266	BTCD_v1_2411	CDS	_	protein of unknown function	no
267	BTCD_v1_2423	CDS	_	putative ABC-type Na+ efflux pump, permease component	no
268	BTCD_v1_2424	CDS	*yhaQ*	Na+-efflux ABC transporter, ATP-binding protein	no
269	BTCD_v1_2431	CDS	_	putative pyruvate decarboxylase/indolepyruvate decarboxylase (N terminal fragment)	no
270	BTCD_v1_2432	CDS	_	putative pyruvate decarboxylase /indolepyruvate decarboxylase (C terminal fragment)	no
271	BTCD_v1_2449	CDS	_	conserved protein of unknown function (fragment)	no
272	BTCD_v1_2450	CDS	_	conserved protein of unknown function (grament)	pseudo
273	BTCD_v1_2460	fCDS	*licT*	fragment of transcriptional antiterminator (BglG family) (part 1)	no
274	BTCD_v1_2481	CDS	_	protein of unknown function	no
275	BTCD_v1_2484	CDS	_	putative cell wall surface LXPTG-anchor family protein	no
276	BTCD_v1_2485	CDS	_	conserved protein of unknown function	no
277	BTCD_v1_2494	CDS	_	protein of unknown function	no
278	BTCD_v1_2495	fCDS	_	protein of unknown function	no
279	BTCD_v1_2496	CDS	_	exported protein of unknown function	no
280	BTCD_v1_2497	CDS	_	conserved exported protein of unknown function	no
281	BTCD_v1_2498	CDS	_	protein of unknown function	no
282	BTCD_v1_2501	CDS	_	protein of unknown function	no
283	BTCD_v1_2502	CDS	_	conserved protein of unknown function	no
284	BTCD_v1_2505	CDS	_	conserved protein of unknown function	no
285	BTCD_v1_2506	CDS	_	putative type I restriction-modification system (HsdR)	no
286	BTCD_v1_2507	CDS	_	putative type I restriction endonuclease subunit S, HsdS	no
287	BTCD_v1_2508	CDS	_	putative type I restriction-modification system, DNA-methyltransferase subunit M (HsdM) (fragment)	no
288	BTCD_v1_2509	CDS	_	putative type I restriction-modification system DNA-methyltransferase subunit M (HsdM) (fragment)	no

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	Label	Type	Gene	Product	Mutation		Label	Type	Gene	Product	Mutation
289	BTCD_v1_2510	CDS	_	putative transcriptional regulator	no	303	BTCD_v1_2565	CDS	_	protein of unknown function	no
290	BTCD_v1_2511	CDS	_	protein of unknown function	no	304	BTCD_v1_2572	CDS	_	protein of unknown function	no
291	BTCD_v1_2512	CDS	_	conserved protein of unknown function	no	305	BTCD_v1_2577	CDS	_	putative LPXTG-domain-containing cell wall protein	no
292	BTCD_v1_2513	CDS	_	conserved protein of unknown function	no	306	BTCD_v1_2578	CDS	_	putative collagen binding protein	no
293	BTCD_v1_2516	CDS	_	putative transcriptional regulator	no	307	BTCD_v1_2579	CDS	_	conserved protein of unknown function	no
294	BTCD_v1_2541	CDS	_	conserved protein of unknown function	no	308	BTCD_v1_2585	CDS	_	conserved protein of unknown function	no
295	BTCD_v1_2542	CDS	_	putative VanZ-like protein	no	309	BTCD_v1_2586	CDS	_	putative glycoside hydrolase	no
296	BTCD_v1_2558	CDS	_	conserved protein of unknown function	no	310	BTCD_v1_2591	CDS	_	membrane protein of unknown function	no
297	BTCD_v1_2559	CDS	_	protein of unknown function	no	311	BTCD_v1_2597	CDS	_	protein of unknown function	no
298	BTCD_v1_2560	CDS	_	protein of unknown function	no	312	BTCD_v1_2598	CDS	_	protein of unknown function	no
299	BTCD_v1_2561	CDS	_	protein of unknown function	no	313	BTCD_v1_2599	CDS	_	protein of unknown function	no
300	BTCD_v1_2562	CDS	_	protein of unknown function	no	314	BTCD_v1_2602	CDS	*mtlD*	mannitol-1-phosphate dehydrogenase	no
301	BTCD_v1_2563	CDS	_	protein of unknown function	no	315	BTCD_v1_2603	CDS	_	putative transcriptional regulator MtlR with PTS EIIAB components	no
302	BTCD_v1_2564	CDS	_	protein of unknown function	no	316	BTCD_v1_2604	CDS	*mtlA*	mannitol-specific PTS enzyme IIABC component	no

Table 3.6 List of *B. thermosphacta* BI specific genes.

	Label	Type	Gene	Product	Mutation		Label	Type	Gene	Product	Mutation
1	BTBI0206	CDS	_	conserved protein of unknown function	no	26	BTBI2529	CDS	_	protein of unknown function	no
2	BTBI0208	CDS	_	conserved protein of unknown function	no	27	BTBI2644	CDS	_	protein of unknown function	no
3	BTBI0214	CDS	_	conserved protein of unknown function	no	28	CNY62_00830	CDS	_	protein of unknown function	no
4	BTBI0271	CDS	_	conserved protein of unknown function	no	29	CNY62_00835	CDS	_	conserved protein of unknown function	no
5	BTBI0532	CDS	_	protein of unknown function	no	30	CNY62_00840	CDS	_	conserved protein of unknown function	no
6	BTBI0539	CDS	_	conserved protein of unknown function	no	31	CNY62_00845	CDS	_	putative holin	no
7	BTBI0635	CDS	_	conserved protein of unknown function	no	32	CNY62_00850	CDS	_	Lysozyme	no
8	BTBI0822	CDS	_	conserved protein of unknown function	no	33	CNY62_00860	CDS	_	conserved protein of unknown function	no
9	BTBI0828	CDS	_	conserved protein of unknown function	no	34	CNY62_00865	CDS	_	conserved protein of unknown function	no
10	BTBI1028	CDS	_	protein of unknown function	no	35	CNY62_00870	CDS	_	conserved protein of unknown function	no
11	BTBI1215	CDS	_	protein of unknown function	no	36	CNY62_00875	CDS	_	conserved membrane protein of unknown function	no
12	BTBI1396	CDS	_	protein of unknown function	no	37	CNY62_00880	CDS	_	conserved protein of unknown function	no
13	BTBI1410	CDS	_	conserved protein of unknown function	no	38	CNY62_00885	CDS	_	conserved protein of unknown function	no
14	BTBI1416	CDS	_	conserved protein of unknown function	no	39	CNY62_00890	CDS	_	conserved protein of unknown function	no
15	BTBI1421	CDS	_	conserved protein of unknown function	no	40	CNY62_00895	CDS	_	conserved protein of unknown function	no
16	BTBI1513	CDS	_	protein of unknown function	no	41	CNY62_00900	CDS	_	conserved protein of unknown function	no
17	BTBI1550	CDS	_	protein of unknown function	no	42	CNY62_00905	CDS	_	conserved protein of unknown function	no
18	BTBI1858	CDS	_	conserved protein of unknown function	no	43	CNY62_00910	CDS	_	conserved protein of unknown function	no
19	BTBI1910	CDS	_	conserved protein of unknown function	no	44	CNY62_00915	CDS	_	conserved protein of unknown function	no
20	BTBI1988	CDS	_	conserved protein of unknown function	no	45	CNY62_00920	CDS	_	Phage portal protein	no
21	BTBI2101	CDS	_	protein of unknown function	no	46	CNY62_00925	CDS	_	conserved protein of unknown function	no
22	BTBI2174	CDS	_	protein of unknown function	no	47	CNY62_00930	CDS	_	Phage terminase, small subunit	no
23	BTBI2217	CDS	_	conserved protein of unknown function	no	48	CNY62_00935	CDS	_	conserved protein of unknown function	no
24	BTBI2378	CDS	_	conserved protein of unknown function	no	49	CNY62_00940	CDS	_	conserved protein of unknown function	no
25	BTBI2413	CDS	_	conserved protein of unknown function	no	50	CNY62_00945	CDS	_	conserved protein of unknown function	no

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	Label	Type	Gene	Product	Mutation
51	CNY62_00950	CDS	_	conserved protein of unknown function	no
52	CNY62_00955	CDS	_	conserved protein of unknown function	no
53	CNY62_00825	CDS	_	protein of unknown function	no
54	CNY62_00975	CDS	_	conserved protein of unknown function	no
55	CNY62_00980	CDS	_	conserved protein of unknown function	no
56	CNY62_00985	CDS	_	conserved protein of unknown function	no
57	CNY62_00990	CDS	_	Prophage LambdaBa03, site-specific recombinase, phage integrase family protein	no
58	CNY62_02360	CDS	_	conserved protein of unknown function	no
59	CNY62_02365	CDS	_	conserved protein of unknown function	no
60	CNY62_02370	CDS	_	conserved protein of unknown function	no
61	CNY62_02375	CDS	_	protein of unknown function	no
62	CNY62_02380	CDS	_	conserved protein of unknown function	no
63	CNY62_02390	CDS	_	Phage holin	no
64	CNY62_02395	CDS	_	conserved protein of unknown function	no
65	CNY62_02410	CDS	_	conserved protein of unknown function	no
66	CNY62_02415	CDS	_	conserved protein of unknown function	no
67	CNY62_02420	CDS	_	conserved protein of unknown function	no
68	CNY62_02500	CDS	_	conserved protein of unknown function	no

	Label	Type	Gene	Product	Mutation
69	CNY62_00960	CDS	_	conserved protein of unknown function	no
70	CNY62_00965	CDS	_	conserved protein of unknown function	no
71	CNY62_00970	CDS	_	Programmed cell death toxin YdcE	no
72	CNY62_02515	CDS	_	Phage protein (fragment)	no
73	CNY62_02520	CDS	_	conserved protein of unknown function	no
74	CNY62_02525	CDS	_	conserved protein of unknown function	no
75	CNY62_02530	CDS	_	Phage DNA binding protein	no
76	CNY62_02540	CDS	_	protein of unknown function	no
77	CNY62_02545	CDS	_	conserved protein of unknown function	no
78	CNY62_02565	CDS	_	conserved protein of unknown function	no
79	CNY62_02605	CDS	_	conserved protein of unknown function	no
80	CNY62_02615	CDS	_	conserved protein of unknown function	no
81	CNY62_02620	CDS	_	protein of unknown function	no
82	CNY62_04705	CDS	_	conserved protein of unknown function	no
83	CNY62_06765	fCDS	_	fragment of Glyoxalase family protein (part 1)	pseudo
84	CNY62_06770	fCDS	_	fragment of Glyoxalase family protein (part 2)	pseudo

Table 3.7 List of *B. thermosphacta* BII specific genes.

	Label	Type	Gene	Product	Mutation
1	BTBII0122	CDS	_	protein of unknown function	no
2	BTBII0537	CDS	_	conserved protein of unknown function	no
3	BTBII0655	CDS	_	conserved protein of unknown function	no
4	BTBII0941	CDS	_	protein of unknown function	no
5	BTBII1677	CDS	_	protein of unknown function	no
6	BTBII1729	CDS	_	protein of unknown function	no
7	BTBII1730	CDS	_	protein of unknown function	no
8	BTBII2082	CDS	_	conserved protein of unknown function	no
9	BTBII2099	CDS	_	protein of unknown function	no
10	BTBII2141	CDS	_	conserved protein of unknown function	no
11	BTBII2541	CDS	_	conserved protein of unknown function	no
12	BTBII2551	CDS	_	protein of unknown function	no
13	BTBII2553	CDS	_	protein of unknown function	no
14	CPF12_03555	CDS	_	conserved exported protein of unknown function	no
15	CPF12_03560	CDS	_	conserved protein of unknown function	no
16	CPF12_03565	CDS	_	protein of unknown function	no
17	CPF12_04275	CDS	_	protein of unknown function	no
18	CPF12_04965	CDS	_	conserved protein of unknown function	no
19	CPF12_07660	fCDS	*ppdK*	fragment of Pyruvate, phosphate dikinase (part 1)	pseudo
20	CPF12_08235	CDS	_	DNA-binding protein	no
21	CPF12_08240	CDS	_	conserved protein of unknown function	no
22	CPF12_08285	CDS	_	conserved protein of unknown function	no
23	CPF12_11935	fCDS	_	fragment of putative transcriptional regulator, MerR family (part 1)	pseudo
24	CPF12_11940	fCDS	_	fragment of putative transcriptional regulator, MerR family (part 2)	pseudo

Table 3.8 List of CRISPR genes found in CD 337, BSAS1 3, and TAP 175.

Label	**Strain**	**Type**	**Gene**	**Product**
BTCD_v1_1788	*B. thermosphacta* CD337	CDS	_	Putative CRISPR-associated endoribonuclease
BTBSAS_v1_190007	*B. thermosphacta* BSAS13	CDS	_	Type II-A CRISPR-associated protein Csn2
BTBSAS_v1_190008	*B. thermosphacta* BSAS13	CDS	*cas*	CRISPR-associated endoribonuclease Cas2
BTBSAS_v1_190009	*B. thermosphacta* BSAS13	CDS	*cas*	CRISPR-associated endonuclease Cas1
BTBSAS_v1_190010	*B. thermosphacta* BSAS13	CDS	*cas*	CRISPR-associated endonuclease Cas9
BTTAP_v1_20211	*B. thermosphacta* TAP175	CDS	*cas*	CRISPR-associated endonuclease Cas2
BTTAP_v1_20214	*B. thermosphacta* TAP175	CDS	_	CRISPR-associated protein Cas4
BTTAP_v1_20217	*B. thermosphacta* TAP175	CDS	_	CRISPR-associated protein Cas5d
BTTAP_v1_20229	*B. thermosphacta* TAP175	CDS	_	Type II-A CRISPR-associated protein Csn2
BTTAP_v1_20230	*B. thermosphacta* TAP175	CDS	*cas*	CRISPR-associated endoribonuclease Cas2
BTTAP_v1_20231	*B. thermosphacta* TAP175	CDS	*cas*	CRISPR-associated endonuclease Cas1
BTTAP_v1_20232	*B. thermosphacta* TAP175	CDS	*cas*	CRISPR-associated endonuclease Cas9

3.3. Résultats complémentaires

Les génomes des 4 souches étudiées précédemment ont été également comparés avec l'ensemble des génomes de *B. thermosphacta* disponibles pour rechercher s'il existait des différences notables au niveau de la taille des génomes (Figure 3.9), et dans le pourcentage de gènes appartenant au core-génome, au génome accessoire et au génome spécifique (Figure 3.10).

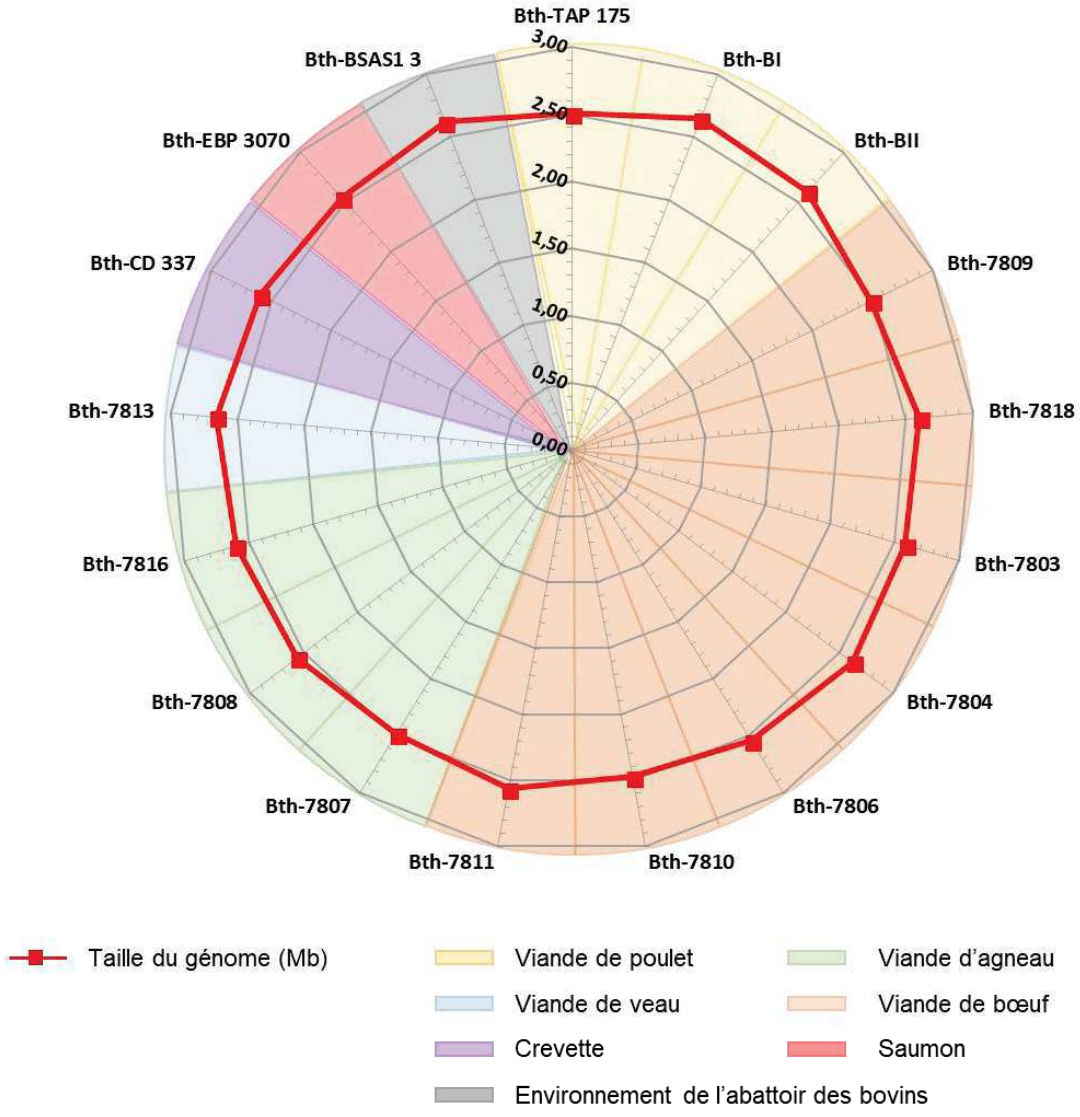

Figure 3.9 Origine de souches et taille des génomes séquencés de *B. thermosphacta*.

Les génomes des souches BI, BII et CD 337 sont complets. Les génomes des autres souches sont en état de « draft ».

Il apparaît que la taille globale des génomes est très conservée dans les 18 souches. Elle est en moyenne de 2,5 Mb, avec un minimum de 2,49 Mb pour la souche Bth-7807et un maximum de 2,66 Mb pour la souche Bth-7813. En revanche, le génome accessoire varie suivant les souches (de 22 % chez Bth-7810 à 27 % chez BSAS1 3). Cependant, cette variabilité n'a pas de lien avec taille des génomes.

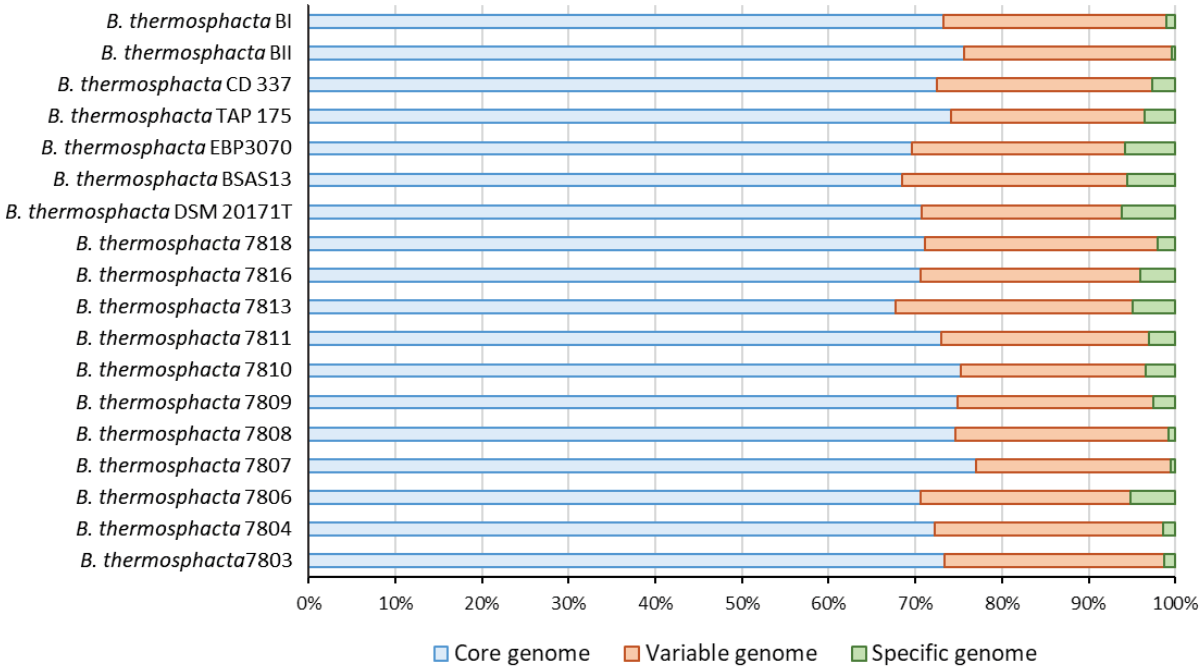

Figure 3.10 Représentation de la taille du core-génome et du génome accessoire chez les souches de *B. thermosphacta*.

3.4. Ce qu'il faut retenir de ce chapitre

Au cours de cette étude, les séquences génomiques de quatre souches de *B. thermosphacta* (CD 337, TAP 175, BSAS1 3 et EBP 3070) ont été determinées. Ces souches ont été isolées de différentes niches écologiques et étaient phénotypiquement et génétiquement différentes.

Nous avons choisi de séquencer le génome d'une souche (CD 337) par la technologie PacBio, ce qui a permis de générer un génome complet et circulaire avec 2 594 337 pb, contenant 2593 séquences codant des protéines (CDS) et 28 gènes d'ARN ribosomique. Le génome des trois autres souches, TAP 175, BSAS1 3 et EBP 3070, a été séquencé par la technologie Ion Torrent générant ainsi des « drafts » de 57, 83 et 71 contigs, contenant 2515, 2668 et 2611 CDS, respectivement.

Les quatre génomes nouvellement séquencés ont été comparés entre eux, et avec les génomes des souches de *B. thermosphacta* BI et BII, dont les séquences complètes ont été récemment publiées. Nous avons noté que le répertoire de gènes des différentes souches est globalement similaire. Les différences majeures observées résident dans le contenu en phages, en CRISPR et en plasmides. Les fonctions qui varient entre les souches sont principalement des systèmes de restriction/modification, des protéines d'adhésion ou liées à la surface cellulaire et à la capacité à utiliser diverses sources de carbone. Ces fonctions pourraient conférer aux souches des capacités d'adaptation différentes leur permettant de coloniser diverses matrices alimentaires et d'y survivre. De plus, des différences ponctuelles ont été observées au niveau des promoteurs ou des séquences codantes de gènes impliqués dans la production des molécules volatiles liées à l'altération. Ces résultats laissent supposer que la diversité du potentiel altérant de *B. thermosphacta* pourrait être liée à des capacités d'adaptation ou de production de molécules malodorantes ou encore à une régulation différentielle de l'expression des gènes correspondants qui seraient souche-dépendantes.

Afin de vérifier ces hypothèses, nous avons étudié l'effet « souche » et l'effet « matrice » sur la production des molécules volatiles et sur l'expression différentielle des gènes. Nous avons également cherché à faire un lien entre les gènes différentiellement exprimés et les métabolites différentiellement produits afin d'identifier les gènes clés impliqués dans le processus d'altération chez *B. thermosphacta.*

Chapitre 4

Analyse fonctionnelle de la diversité du potentiel altérant de *Brochothrix thermosphacta*

Nassima Illikoud
2018

Chapitre 4. Analyse fonctionnelle de la diversité du potentiel altérant de *Brochothrix thermosphacta*

4.1. Préambule

Nous avons constaté que le potentiel d'altération des souches de *B. thermosphacta* est variable en fonction des souches et des matrices alimentaires et ce malgré la similarité du répertoire génomique des souches. Ce chapitre est consacré à l'étude de l'effet souche et l'effet matrice sur la production des molécules altérantes et l'expression différentielle des gènes. Pour ce faire, nous avons utilisé deux souches (CD 337 et TAP 175) qui sont représentatives de la diversité génotypique et phénotypique (chapitre 2) et dont la séquence génomique a été déterminée (chapitre 3). *B. thermosphacta* CD 337 a été isolée de crevette altérée et produit plus d'acétoïne et de diacétyle sur jus de viande que sur jus de crevette. En revanche, *B. thermosphacta* TAP 175, isolée de cuisse de poulet non altérée, est plus altérante sur jus de crevette que sur jus de viande. Ces deux souches ont été inoculées sur deux milieux modèles stériles (jus de viande et jus de crevette) puis stockés à 8 °C durant 7 jours. Un contrôle non inoculé a été réalisé pour chaque matrice. La démarche expérimentale est schématisée dans la Figure 4.1.

Durant le stockage la croissance bactérienne et le pH ont été suivis et des échantillons ont été récoltés pour l'analyse du volatilome. Les ARNs ont été extraits à J4 pour l'analyse du transcriptome. Pour chaque souche, les volatilomes et les transcriptomes produits sur chaque matrice ont été comparés, puis nous avons comparé les deux souches entre elles. Enfin nous avons tenté de relier les voies métaboliques potentiellement exprimées à la synthèse de molécules volatiles.

Cette étude est présentée sous forme d'un manuscrit en préparation.

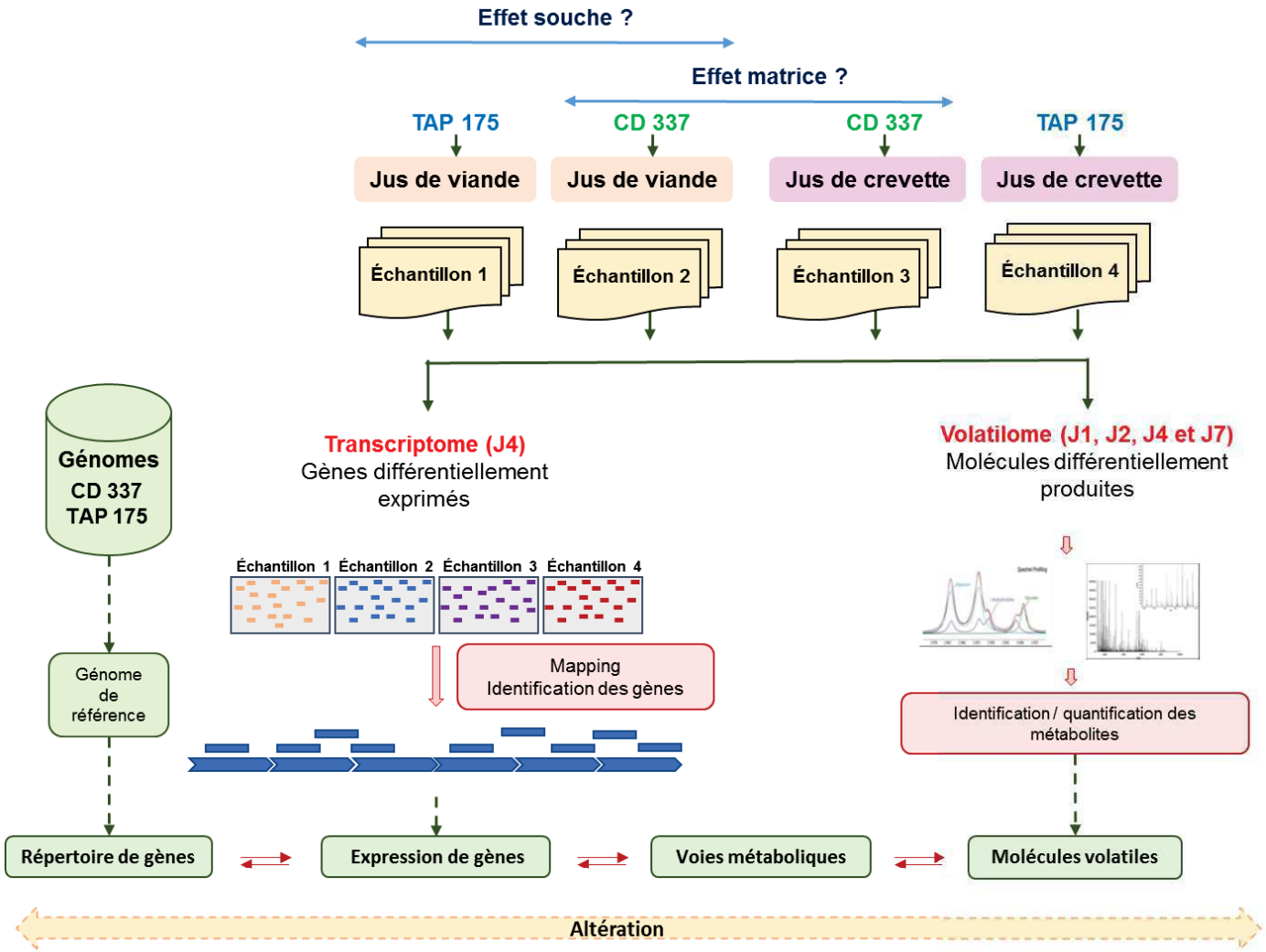

Figure 4.1 Démarche expérimentale.

4.2. Transcriptomic and volatilome analysis of food-spoilage associated bacterium *Brochothrix thermosphacta*

Nassima Illikoud[1], Rodérick Gohier[2], Dalal Werner[2], Célia Barrachina[3], David Roche[4], Emmanuel Jaffrès[1], and Monique Zagorec[1#]

[1] SECALIM, INRA, Oniris, Université Bretagne Loire, Nantes, France

[2] Aérial, ITAI-CRT, 250 rue Laurent Fries, Illkirch, France

3 MGX, CNRS, INSERM, Univ. Montpellier, Montpellier, France

4 Génomique Métabolique, Génoscope, Institut François Jacob, CEA, CNRS, Université d'Evry, Université Paris-Saclay, Evry, France

[#] Corresponding author: Monique Zagorec, monique.zagorec@oniris-nantes.fr, Oniris - INRA, UMR 1014 SECALIM - Route de Gachet-CS40706 -44307 Nantes cedex 3, France

Key word

Shrimp; Meat; Gene differential expression; Volatile Organic Compounds (VOCs); Diacetyl; Acetoin

4.2.1. Introduction

Brochothrix thermosphacta is commonly found as belonging to the microbiota of a wide range of food matrices including various raw and processed meat and seafood (Chaillou et al., 2015; Remenant et al., 2015). This results from its ubiquitous nature and its ability to grow at refrigerated temperatures used for such foodstuff storage (Stackebrandt and Jones, 2006). *B. thermosphacta* has been frequently associated to food spoilage through the production of off-odors as a result of volatile organic compounds (VOCs) production (Casaburi et al., 2014; Casaburi et al., 2015b; Odeyemi et al., 2018). The production of such molecules seems to be food matrix dependent (Illikoud et al 2018). For example acetoin, diacetyl, and 3-methylbutanol associated with cheesy and creamy dairy off-odors have been reported in beef meat (Casaburi et al., 2014; Dainty et al., 1989), while in cooked and peeled shrimp diacetyl and 3-methyl-1-butanal associated with strong butter, buttermilk-like, sour, and nauseous off-odors were produced (Jaffrès et al., 2011). Recently, genotypic and phenotypic analysis of a

B. thermosphacta strain collection revealed significant genetic diversity, not related to their ecological origin (Illikoud et al., *in press*). However, levels of acetoin and diacetyl production were variable depending on strains. Hence, these results suggest that the spoilage potential of *B. thermosphacta* may be strain- and matrix-dependent. Genome comparison showed high degree of similarity in the gene repertoire of *B. thermosphacta* strains (Illikoud et al., Submitted; Stanborough et al., 2017). The major differences reside in phages and plasmids, cell surface proteins and adhesins, which could not explain the different spoilage abilities of the strains but rather variable adaptation or fitness for different ecological niches. Nevertheless, slight differences in or upstream from genes encoding enzymes required for the production of spoilage molecules were noticed (Illikoud et al., Submitted), that may suggest different spoilage abilities associated to differences in gene expression or enzyme activity.

The characterization of the genes expressed and the metabolites produced by *B. thermosphacta* strains on different food matrices may contribute to better understand the *B. thermosphacta* spoilage mechanisms and the reasons for spoilage potential differences. Therefore, our strategy for the present study was to compare volatilomes and transcriptomes of two different strains inoculated on two model matrices (meat and shrimp juices). The objectives are to discriminate the strain effect from the matrix effect, and to attempt identify and elucidate metabolic functions involved in spoilage potential of *B. thermosphacta*.

4.2.2. Materiel and methods

4.2.2.1. Bacterial strains and growth conditions

B. thermosphacta CD 337 and TAP 175 (Illikoud et al., *in press*) isolated from spoiled peeled shrimp and non-spoiled chicken cuts, respectively, were used in this study. The strains were stored at -80 °C in Brain Heart Infusion (BHI) broth (VWR, France) added with 20% (v/v) glycerol and were routinely grown at 25 °C in BHI medium.

After serial dilutions of meat and shrimp juices, total viable counts were enumerated on Plate Count Agar (PCA) (Biomerieux, France) and *B. thermosphacta* on selective STAA agar base containing STAA selective supplement (Oxoid, France), after incubation for 48 h at 30 °C and 25 °C, respectively.

4.2.2.2. Meat and shrimp juice preparation

Meat and shrimp juices were prepared as previously described (Illikoud et al., *in press*) with slight modifications. Ground beef (25 g) purchased from a local supermarket was stomached in 100 ml Ringer's solution (Oxoid, France), the resulted juice was centrifuged at 6000 *xg* for 20 min at 4 °C. The supernatant was filtered through a 0.45 µm membrane filter before sterilization with a 0.2 µm membrane filter. Shrimp juice was prepared by crushing 100 g frozen raw peeled shrimp from Ecuador (91/100 without sulfite, purchased from industry, Nantes) in 300 ml sterile distilled water. The mixture was heated (100 °C; 2 min), centrifuged at 600 *xg* for 20 min, and the resulting supernatant was autoclaved (100 °C; 30 min). The resulting juices, mimicking raw ground beef and cooked peeled shrimp, were aliquoted by 20 ml in 45 ml sterile glass vials and then stored frozen at -80 °C until use.

4.2.2.3. Challenge tests

B. thermosphacta overnight cultures were harvested by centrifugation (13,400 *xg*, 5 min, 4 °C) and washed with 1 ml Ringer's solution or sterile distilled water, centrifuged with the same parameters, re-suspended in 1 ml of meat or shrimp juice, and inoculated in 20 ml food juice at an initial concentration of 10^6 CFU/ml. Glass vials were closed and inoculated juices were incubated for 7 days at 8 °C. Non-inoculated juice samples were included as controls. Bacterial enumeration, volatilome analysis and pH measurement (Crison pH-meter) were performed after 0, 1, 2, 4, and 7 days whereas transcriptome analysis was performed at day 4. Challenge-tests were performed in triplicate.

4.2.2.4. Volatile Organic Compound (VOCs) analysis

At each sampling time, vials were immediately frozen at -20°C without opening the vials, and stored frozen until analysis. Samples were thawed over night at room temperature. Nine milliliters of sample extract were transferred into a 20-ml headspace vial and sealed with a screwcap with silicone rubber septum. Analyses were carried out in triplicate.

VOCs were determined by HS–GC–MS. All analyses were performed on a Varian 450 gas chromatograph (Varian, Palo Alto, CA, USA) coupled to a Varian 225-IT mass spectrometer (Varian), equipped with a CTC Combi PAL (CTC Analytics AG, Zwingen, Switzerland). Samples were equilibrated by agitation at 60 °C for 20 min prior to injection. The HS-GC–MS conditions

were as follow: capillary column: DB-624 UI (30m x 0.25 mm I.D x 1.4 mm film thickness) (Agilent Technologies); Carrier gas: Helium with a flow rate of 1.4 ml/min; Injection port mode: splitless; Needle temperature: 60 °C; Injection temperature: 220°C, The oven temperature was programmed from an initial temperature of 40 °C (7 min holding), rising to 50 °C at 4 °C/min (1 min holding), to 70 °C at 4 °C/min (1 min holding), to 120°C at 3 °C/min (2 min holding) and to 245 °C at 30 °C/min (4 min holding). Transfer line temperature: 250 °C. The temperatures of the manifold and the ion trap were kept constant at 150 °C and 40 °C respectively. Results were obtained in scan mode at 4 scans/s in the mass range (m/z) of 35–350 atomic mass units. VOCs were identified by comparison of GC retention times and mass spectra with those of the standard compounds. Peak area (in UA) was used as quantitative data to monitor the relative changes of VOCs over storage and, where appropriate, to correlate these findings, with bacterial strain as well as with the studied food matrices.

4.2.2.5. RNA preparation and sequencing

At day 4, 1 ml cultures were sampled and bacteria were harvested by centrifugation at 10,000 *xg* for 5 min at 4 °C. The cell pellet was immediately resuspended in 200 µl RNA Protect Cell Reagent (Qiagen) and frozen at -80 °C, as recommended by the manufacturer, to prevent alteration of the gene expression profile. Total RNA was extracted from the pellet using the All Prep Qiagen Kit following manufacturer's instructions: bacteria were first chemically lysed in phenol/chloroform and Qiagen lysis buffer containing β-mercaptoethanol and mechanical lysis was carried out using a FastPrep (MP Biomedicals) for 40 s at a frequency of 5.5 m/s. RNA were then washed and eluted on spin membrane. Total RNAs were quantified using a Nanodrop 2000 spectrophotometer (Thermo Scientific) and quality was checked by Experion™ Automated Electrophoresis System (Biorad).

rRNA depletion was performed on 1 µg total RNA with the Ribo-Zero rRNA Removal Kit dedicated to bacteria (Illumina). Purified mRNA quality was validated by capillary electrophoresis on a Fragment Analyzer (Advanced Analytical, Ankeny, IA).

RNA-Seq libraries were constructed with the Truseq stranded mRNA sample preparation (Low throughput protocol) kit from Illumina. Purified mRNA (10 ng) were cleaved into small fragments using divalent cations under elevated temperature. The cleaved RNA fragments

were converted into a first strand cDNA using SuperScript II reverse transcriptase (Invitrogen), actinomycin D and random hexamer primers. A second strand cDNA was synthesized by replacing dTTP with dUTP. These cDNA fragments then have the addition of a single 'A' base and subsequent ligation of the adapter. The products were then purified and enriched with 15 cycles of PCR. The final cDNA libraries were validated with a Fragment Analyzer (Advanced Analytical, Ankeny, IA) and quantified with a KAPA qPCR kit (Kapa Biosystems, Wilmington, MA).

On 1 sequencing lane of a flowcell V4, the 12 libraries (2 strains x 2 juices x 3 replicates) were pooled in equal proportions, denatured with NaOH and diluted to 12 pM before clustering. Cluster formation, primer hybridization and single-end read, 50 cycles sequencing were performed on cBot and HiSeq2500 (Illumina, San Diego, CA), respectively.

Image analysis and base calling were performed using the HiSeq Control Software and Real-Time Analysis component. Demultiplexing was performed using Illumina's sequencing analysis software. The quality of the data was assessed using FastQC from the Babraham Institute and the Illumina software SAV (Sequence Analysis Viewer). Potential contaminants were searched with the FastQ Screen software from the Babraham Institute.

4.2.2.6. Transcriptome analysis

Transcriptomic high throughput sequencing data were analyzed using a bioinformatic pipeline implemented in the Microscope platform (Médigue et al., 2017). The current pipeline is a “Master” shell script that launches the various parts of the analysis (i.e. a collection of Shell/Perl/R scripts) and controls for all tasks having been completed without errors. In a first step, the RNA-Seq data quality was assessed by including option like reads trimming or merging/split paired-end reads. In a second step, reads were mapped onto the corresponding genome sequence, CD 337 (ERZ500814) or TAP 175 (ERZ500814), using the SSAHA2 package (Ning et al., 2001) that combines the SSAHA searching algorithm (sequence information is encoded in a perfect hash function) aiming at identifying regions of high similarity, and the cross-match sequence alignment program (Ewing et al., 1998), which aligns these regions using a banded Smith-Waterman-Gotoh algorithm (Smith and Waterman, 1981). An alignment score equal to at least half of the read is required for a hit to be retained. To lower false

positives discovery rate, the SAMtools (v.0.1.8) (Li et al., 2009a) are then used to extract reliable alignments from SAM formatted files. The number of reads matching each genomic object harbored by the reference genome is subsequently computed with the Bioconductor-GenomicFeatures package (Carlson et al., 2011). If reads matching several genomic objects, the count number is weighted in order to keep the same total number of reads. Finally, the Bioconductor-DESeq package (Anders and Huber, 2010) with default parameters is used to analyze raw counts data and test for differential expression between conditions.

4.2.3. Results and discussion

4.2.3.1. Bacterial growth in food juices

Growth of *B. thermosphacta* CD 337 and TAP 175 in beef and shrimp juices was monitored during storage at 8 °C for 7 days. Total aerobic mesophilic and *B. thermosphacta* counts were similar. The absence of bacteria detection (< 1CFU/ml) in the controls (non-inoculated juices) throughout the storage confirmed that both juices were sterile before inoculation (data not shown). As observed in Figure 4.2, the two strains grew in both juices, reaching stationary phase after 2-4 days of storage. In shrimp juice, *B. thermosphacta* population increased rapidly during the first two days and reached a final cell density of 7.8-8.0 log CFU/ml after 4 days of storage. In meat juice, the stationary phase was reached at day 4 but did not exceed 7.5 log CFU/ml for both strains. Initial growth rate of *B. thermosphacta* TAP 175 was slightly higher than that of strain CD 337 in both media.

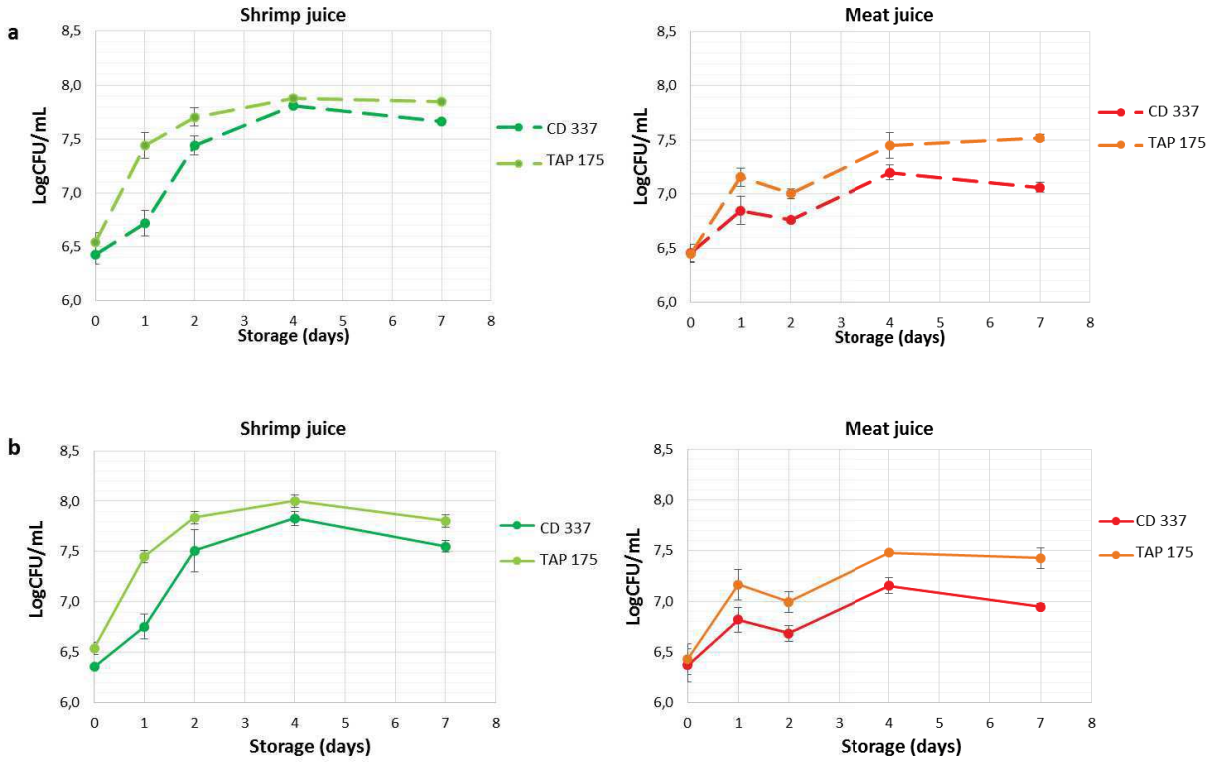

Figure 4.2 Growth kinetics of *B. thermosphacta* strains (CD 337 and TAP 175) in meat and shrimp juices monitored on STAA Specific medium **(a)** and PCA **(b).**

Total aerobic mesophilic bacteria and *B. thermosphacta* were enumerated at day 0, 1, 2, 4, and 7.

4.2.3.2. pH evolution during storage

The initial pH values were equivalent in inoculated and non-inoculated juices, around 6.8 and 5.8 in shrimp and meat juices, respectively (Figure 4.3). For the control samples, pH was constant, but decreased during storage when juices were inoculated. No significant difference was observed between strains. However, the pH of shrimp juice decreased from 6.8 at day 0 to 5.2-5.4 after 4 days of storage, whereas that of meat juice decreased from 5.8 to 5.4-5.6 at day 4. In all cases, pH remained stable or slightly increased between day 4 and day 7.

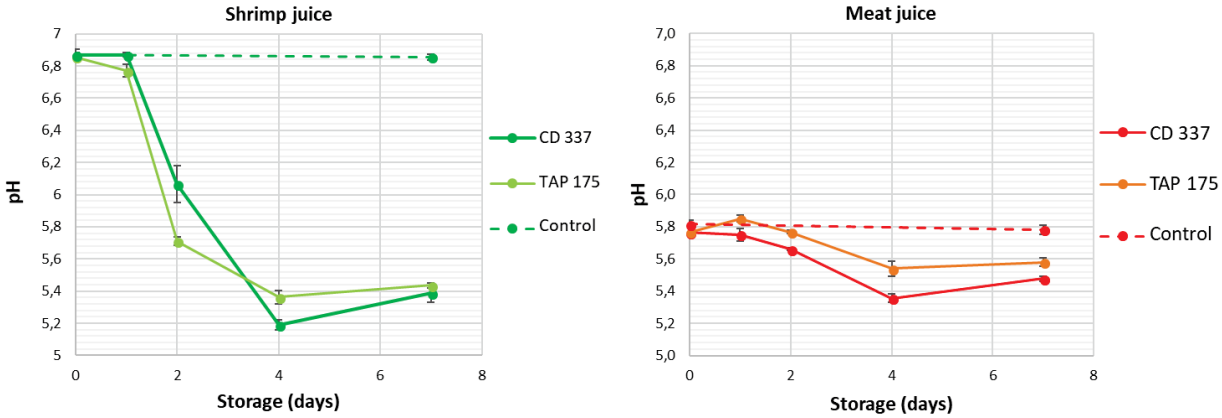

Figure 4.3 Evolution of pH during storage at 8 °C of meat and shrimp juices inoculated by *B. thermosphacta* strains CD 337 and TAP 175.

4.2.3.3. Volatile organic compounds analysis

VOCs production was monitored by SPME/GC-MS analysis afer 1, 3, 4, and 7 days of storage. In total for each matrix juice, seven compounds were detected and identified (Table 4.1) among which the 2-ethyl-1-hexanol present at similar levels in non-inoculated meat juice and in all inoculated samples, and therefore was considered as issued from beef meat.

VOC production varied depending the matrix juice. In meat juice, only ethanol and acetoin were detected after inoculation of *B. thermosphacta*, whereas six compounds (ethanol, isobutyraldehyde, 2-methylbutyraldehyde, 3-methylbutyraldehyde acetoin, and 3-methyl-1-butanol) were produced in inoculated shrimp juices. Slight differences between strains were observed, mostly in the production kinetics. For example, 3-methyl-1-butanol was detected at day 4 and 7 in shrimp juice inoculated with CD 337 while it was detected only at day 7 in the juice inoculated by TAP 175.

Table 4.1 VOCs identified in control (non-inoculated) meat and shrimp juices and after growth of *B. thermosphacta* CD 337 and TAP 175.

Values for control were obtained. In inoculated juices, values are given after 1, 2, 4, and 7 days of storage à 8 °C. For a given juice, differences between strains are highlighted in red.

	VOCs	Control[a]	CD 337				TAP 175			
			D1	D2	D4	D7	D1	D2	D4	D7
Shrimp juice	Ethanol	-	-	+	++	++	-	+	++	++
	Isobutyraldehyde	-	-	-	+	-	-	+	-	-
	3-methylbutyraldehyde	-	+	++	+++	+	+	++	++	+
	2-methylbutyraldehyde	-	+	++	++	-	+	++	+/++	-
	Acetoin	-	-	-	-	+	-	-	-	-
	3-methyl-1-butanol	-	-	-	+	++	-	-	-	+
	2-ethyl-1-hexanol	-	-	-	-	-	-	-	-	-
Meat juice	Ethanol	-	-	-	+	+	-	-	+	++
	Isobutyraldehyde	-	-	-	-	-	-	-	-	-
	3-methylbutyraldehyde	-	-	-	-	-	-	-	-	-
	2-methylbutyraldehyde	-	-	-	-	-	-	-	-	-
	Acetoin	-	-	-	+	+	-	-	+	+/++
	3-methyl-1-butanol	-	-	-	-	-	-	-	-	-
	2-ethyl-1-hexanol	+	+	+	+	+	+	+	+	+

[a] controls (non-inoculated juices): VOCs were determined at day 0, 1, 2, 4, 7.

4.2.3.4. Characterization of differentially expressed genes

RNAs were extracted at day 4, *i.e.* approximately when strains reached the stationary phase and produced various VOCs. Cells were then considered metabolically active.

A total of 248,189,013 reads were generated using HiSeq 2500 single-end (Illumina, San Diego, CA). For each transcriptome, more than 98% of the generated reads had a PHRED quality score higher than 30 meaning that the reads were of high quality (i.e. base call accuracy higher than 99.9%). The reads of each sample were mapped against the corresponding *B. thermosphacta* genome (Illikoud et al., Submitted). Summary of data is given in Table 4.2.

Table 4.2 Summary of RNA-seq data.

	Samples	Total number of reads	Reads mapped on rRNA	Analyzed reads	% of analyzed reads
Shrimp juice	CD 337-Replicate-1	17,994,011	656,829	16,104,771	89,5
	CD 337-Replicate-2	27,868,431	43,583	26,341,984	94,52
	CD 337-Replicate-3	16,591,350	27,217	15,977,713	96,3
	Average CD 337	20,817,931	242,543	19,474,823	93,55
	TAP 175-Replicate-1	22,909,377	96,881	21,736,762	94,88
	TAP 175-Replicate-2	21,142,474	95,607	19,315,917	91,36
	TAP 175-Replicate-3	20,486,747	122,987	18,834,959	91,94
	Average TAP 175	21,512,866	105,158	19,962,546	92,79
Meat juice	CD 337-Replicate-1	17,057,863	91,325	15,849,566	92,92
	CD 337-Replicate-2	22,063,054	20,768	20,643,613	93,57
	CD 337-Replicate-3	19,036,542	7,452	16,392,076	86,11
	Average CD 337	19,385,820	39,848	17,628,418	90,93
	TAP 175-Replicate-1	21,983,405	104,026	20,865,072	94,91
	TAP 175-Replicate-2	19,054,166	14,970	17,882,940	93,85
	TAP 175-Replicate-3	22,001,593	624,111	18,188,678	82,67
	Average TAP 175	21,013,055	247,702	18,978,897	90,32
	Total	**248,189,013**	**1,905,756**	**228,134,051**	

In a first investigation, we searched for genes that were differentially expressed by both strains on both matrix juices [log2 fold change (LFC) < | 2 |; adjusted p value (FDR) < 0.05]. We identified 205 and 312 such genes in CD 337 and TAP 175, respectively, whose expression was matrix dependent. Among those, 136 genes were common to both strains. Then, for each strain, we searched for genes that were constitutively expressed on both juices and those that were specifically upregulated either in meat or in shrimp juice (Figure 4.4a). From the 205 differentially expressed genes in CD 337, 97 were upregulated in meat juice and 108 were upregulated in shrimp juice. Among the 312 genes that were differentially expressed in TAP 175, 161 were upregulated in meat juice and 151 were upregulated in shrimp juice.

Finally, for each food juice, we searched for genes that were common to both strains or that were differentially expressed in only one of the two strains (Figure 4.4b). This enabled to discriminate the strain effect from the matrix effect on the differential expression of genes.

Figure 4.4 Differential gene expression analysis.

Venn diagrams illustrating the distribution of differentially expressed (DE) genes in *B. thermosphacta* strains CD 337 and TAP 175 inoculated in meat and shrimp juices **(a)**; The matrix **(a)** and the strain **(b)** effects on the differential gene expression were represented. (≡) refers to constitutively expressed genes.

4.2.3.5. Genes specifically upregulated in meat juice

Among the genes specifically upregulated in meat juice (N=97 in CD 337 and N=161 in TAP 175), 63 were upregulated in both strains (Figure 4.4) Out of those 8 encoded proteins of unknown function, 9 were annotated as encoding putative, enzymes, transporters or regulators of unknown specificity, and 2 were related to ribosomal proteins or RNAs. Interestingly 44 genes could be correlated to functions that are associated to growth on meat substrate.

Myo-inositol

For example, the 10 out of 12 gene cluster *iolKRABCDETGHIJ* involved in *myo*-inositol utilization were upregulated. The genes *iolAB* were upregulated in CD 337, in TAP 175, the Log Fold Change (LFC) values.of the two genes *iolAB* 1.42 and 1.96, respectively were below the used cutoff (LFC2). For this reason, they are not included in the list of the common genes upregulated in meat juice.

This gene cluster encodes the transcriptional regulator IolR, the transporter IolT, and the enzymes responsible for catabolism of inositol as a carbon source. This compound is present in foods of vegetable origin (Clements and Darnell, 1980), but also in meat (Lilyblade and Peterson, 1962). Moreover, *myo*-inositol was first purified from meat juice (Hoffmann-Ostenhof and Pittner, 1982).

Several microorganisms can grow *myo*-inositol as carbon source. These include Gram-negative bacteria such as *Salmonella*, Serratia, and *Klebsiella* (Gutnick et al., 1969; Kröger and Fuchs, 2009; Legakis et al., 1976) and Gram-positive bacteria as *B. subtilis* (Yoshida et al., 1999; Yoshida et al., 2004; Yoshida et al., 2002; Yoshida et al., 1997). In this species, *iolR* acts as a transcriptional repressor in the absence of inositol. When *myo*-inositol is present, it acts as an inducer antagonizing IolR binding enabling then the expression of the *iol* genes (Yoshida et al., 1999; Yoshida et al., 2002).The same regulation might thus also exist in *B. thermosphacta*

Fist, *myo*-inositol is taken into the cell by IolT then metabolized by IolGEDBCJ enzymes to malonic semialdehyde and/or dihydroxyacetone phosphate (Figure 4.5). Malonic semialdehyde could be metabolized into acetyl-CoA and/or acetaldehyde by IolA and IolK, respectively. Dihydroxyacetone phosphate could be converted into glyceraldehyde-3-phosphate by triose phosphate isomerase encoded by *tpiA* gene encoding. However, this enzyme was downregulated in meat juice. This suggests that *B. thermosphacta* catabolizes *myo*-inositol, via malonic semialdehyde, preferably into acetaldehyde and in le lesser extend to acetyl-CoA in TAP 175. Acetyl -CoA could be integrated in tricarboxylic (TCA) cycle.

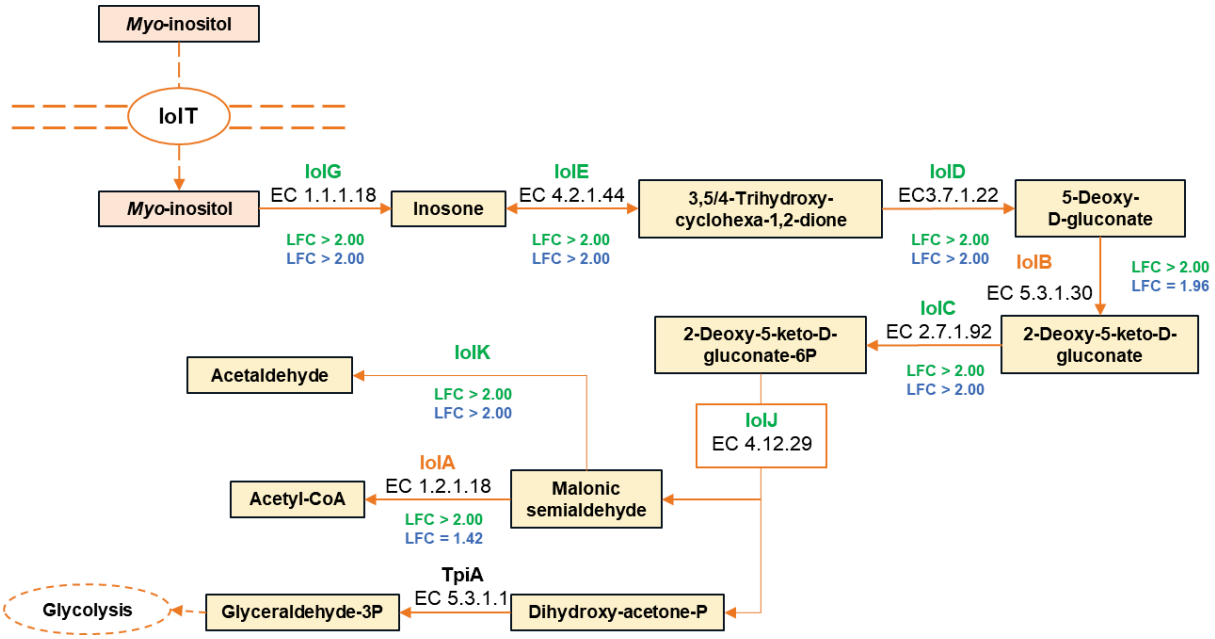

Figure 4.5 Predicted pathway of *myo*-inositol catabolism by *B. thermosphacta* strains CD 337 and TAP 175 in meat juice. LFC values are represented with a color code depending on strain: CD 337 (green); TAP 175 (blue). The gene encoding TpiA was downregulated in meat juice.

Ethanolamine

Part of the *eut* gene cluster (*eut GCLKEMDNHQ)* involved in ethanolamine utilization was also upregulated in meat juice. Ethanolamine is a major constituent of lipids of eukaryotic cells especially in beef meat. It is also present in fish but at lower levels (Fogerty et al., 1991). This may explain the specific upregulation of this set of genes in meat juice. Several bacteria can use ethanolamine as a unique source of carbon and/or nitrogen, such as species from genera *Salmonella, Pseudomonas,* and *Clostridium* (Garsin, 2010). However, the gene content, organization and regulation of the *eut* operon were highly variable between bacterial species (Del Papa and Perego, 2008; Pitts et al., 2012; Tsoy et al., 2009). The major reactions of ethanolamine catabolism are represented in Figure 4.6. Ethanolamine, after transport by EutH is converted to acetyl-CoA in two sequential reactions. It is first converted into acetaldehyde and ammonia by EutBC (cobalamin-dependent ethanolamine ammonia) then into acetyl-CoA by EutE (acetaldehyde dehydrogenase) (Garsin, 2010). Acetaldehyde can also be converted to ethanol by EutG (alcohol dehydrogenase) (Del Papa and Perego, 2008; Pitts et al., 2012). However, this enzyme was downregulated by *B. thermosphacta* strains in meat juice,

suggesting that *B. thermosphacta* catabolizes ethanolamine into acetyl-CoA rather than in ethanol.

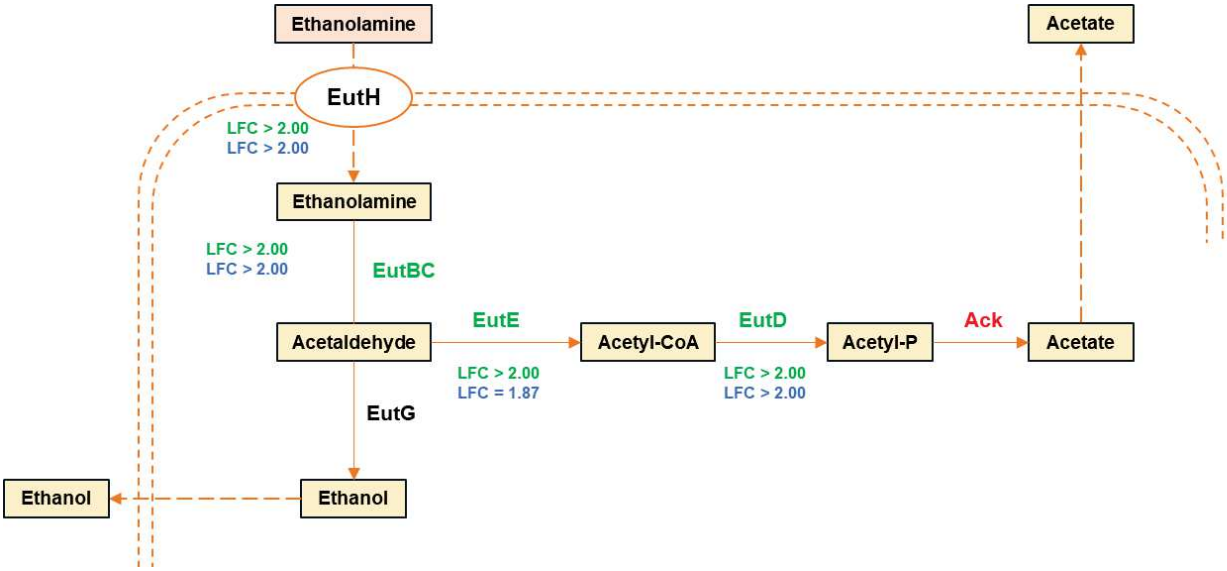

Figure 4.6 Proposed pathway of ethanolamine utilization by *B. thermosphacta* strains CD 337 and TAP 175 in meat juice.
LFC values are represented with a color code depending on strain: CD 337 (green); TAP 175 (blue). The gene encoding EutG was downregulated in meat juice. The gene encoding acetate kinase (Ack) was constitutively expressed.

Sulfur compounds utilization

Five genes involved in sulfur compound utilization were found to be upregulated. Among these, *tcyJLM* involved in cysteine uptake, *snaA* encoding N-acetyltransferase acting on sulfur compounds and its regulator AscR, and a putative FMN reductase related with sulfur starvation. In *B. subtilis*, the expression of *snaA* was reported to be strongly increased in the presence of glutathione (Coppée et al., 2001). This later has been reported to be present at high concentrations in beef, chicken and pork meats but less concentration in raw fish (Rose et al., 1999). This most probably explain the upregulation of this set of genes to cope with sulfur starvation and/or presence of glutathione in beef juice.

pot operon

All genes of the *pot* operon (*potRABCD)* involved in spermidine/putrescine uptake were upregulated in meat juice. Seiler (1999) reported that these polyamines and their intermediates are found in a wide range of organisms including bacteria, plants, and animals.

They are essential for bacterial growth. Several studies indicate that polyamine transporters are highly conserved in many Gram-positive and Gram-negative bacteria such as *Escherichia coli, Salmonella enterica*, *and S. aureus* (Igarashi and Kashiwagi, 1999). These systems were described to be involved in adaption and/or survival of microorganisms and in biofilm formation (Shah et al., 2011; Shah and Swiatlo, 2008; Wortham et al., 2007). In bacteria, polyamine uptake from the environment may provide resistance to various environmental stresses including reactive oxygen species, temperature changes, osmotic pressure or other toxic compounds (Gevrekci, 2017). This suggests that these polyamines may be transported by *B. thermosphacta* and contributed to its fitness and survival in meat. However, as in *S. aureus* the preferred substrates of the Pot system were spermidine and spermine, but not putrescine (Yao and Lu, 2014), we cannot ensure which substrate is transported by the PotABCD transporter in *B. thermosphacta*.

Pyruvate uptake

A pyruvate uptake transporter encoded by *pftAB* genes was upregulated. Pyruvate has been reported to be one of the substrates used by spoilage bacteria in meat. This precursor of various spoilage molecules such as acetoin, diacetyl, and acetic acid (Casaburi et al., 2015b; Nychas et al., 2007). *B. thermosphacta* could therefore transport it from meat and convert it to acetoin and diacetyl for example, two molecules resulting from meat spoilage by *B. thermosphacta*.

Purine and pyrimidine metabolism

Part of the *pur* operon (*purBCSQLF*) involved in purine biosynthesis was upregulated. Purine biosynthetic pathway has been reported to be conserved in bacteria (Kappock et al., 2000; Zhang et al., 2008). Inosine monophosphate (IMP) is the first nucleotide formed from purine biosynthesis. Then, IMP may be converted in adenosine monophosphate (AMP) and/or guanosine monophosphate (GMP) (Figure 4.7A). The *purHD* genes encoding PurH and PurD enzymes were not found among the DE genes in CD 337. In TAP175, only *purD* was not found among DE genes. One can hypothesize that since these two genes are at the end of the operon (Figure 4.7B), their RNAs might be degraded and thus not appearing as upregulated in our experiments. In addition, *xapB-pupG* and *pyrRP* encoding permeases for xanthine and uracil

and their corresponding phosphoribosyltransferase converting them in Xanthosine monophosphate (XMP) and uridine monophosphate (UMP), respectively. This may suggest that uracil and xanthine are available from meat juice, but other nucleotides could be rather synthetized by *B. thermosphacta*.

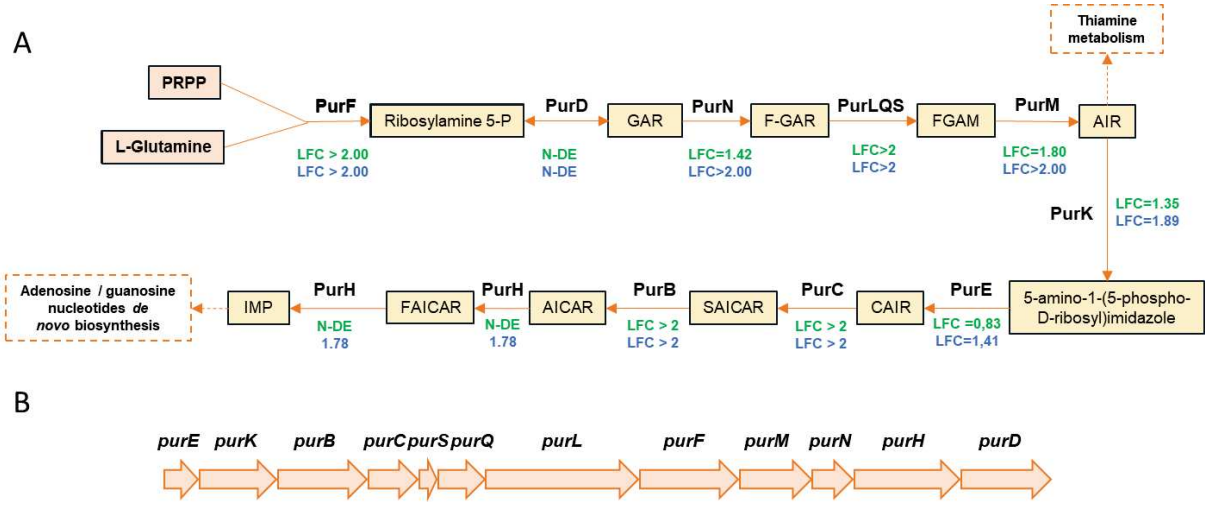

Figure 4.7 Proposed pathway of purine biosynthesis in meat juice by *B. thermosphacta* strains CD 337 and TAP 175 **(A)**, and *pur* operon organization **(B).**

LFC values were represented with a color code depending on strain: CD 337 (green); TAP 175 (blue). **Abbreviations:** N-DE, not differentially (constitutively) expressed; PRPP, 5-phosphoribosylpyrophosphate; GAR, glycinamide ribonucleotide; FGAR, formylglycinamide ribonucleotide; FGAM, formylglycinamidine ribonucleotide; AIR, aminoimidazole ribonucleotide; CAIR, carboxyaminoimidazole ribonucleotide; SAICAR, N-succinocarboxamide-5-aminoimidazole ribonucleotide; AICAR, aminoimidazole-4-carboxamide ribonucleotide; FAICAR, 5-formamido-4-imidazolecarboxamide ribonucleotide; IMP, inosine monophosphate.

Ferric iron transport system

Finally, five genes encoding a putative Fe^{3+} (ferric ion) transport system were also upregulated by both *B. thermosphacta* strains in meat juice. This iron form is available in raw meat (Carpenter and Clark, 1995). Intracellular iron could be used in biological reactions such as oxygen transport, gene regulation, DNA biosynthesis and reparation (Andrews et al., 2003). Moreover, *B. thermosphacta* has been reported to require iron for growth under aerobic conditions (Thomson and Collins-Thompson, 1986). This explain its uptake from meat juice.

4.2.3.6. Genes specifically upregulated in shrimp juice

Among the 73 genes, common to TAP175 and CD337 and upregulated on shrimp, 10 encoded proteins of unknown function, 5 were tRNA, and 4 encoded miscellaneous RNA including the 6S RNA. This later may play a role in transcriptional regulation. Indeed, in *E. coli* for example 6S RNA has been reported as accumulated during stationary phase (Cavanagh et al., 2012) , downregulating transcription, and important for modulating stress and survival during nutrient limitation (Cavanagh and Wassarman, 2014). This correlate with the sampling time of the present study for RNA extraction (at day 4), when bacterial growth ceased in shrimp juice. Two putative transporters ComEA and ComF, similar to DNA transport system, DNA binding and uptake, were upregulated. In *Thermus thermophilus,* the *comEA* operon is upregulated by nutrient limitation and low temperature (Salzer et al., 2016). In addition, 3 putative oxidoreductases, 1 putative dehydrogenase, 1 putative NAD binding enzyme, 1 putative esterase, 1 putative acetyltransferase and 1 transporter *(vlmR*), all of unknown specificity, were upregulated in shrimp juice.

Interestingly, the 2 genes *copA* and *copZ* encoding for copper export were also upregulated in shrimp juice. Copper is present in crustaceans as these organisms contain hemocyanin (a copper-containing respiratory protein dissolved in the hemolymph). Hemocyanin is a hexamer in which each subunit contains two Cu^{2+} ions, which are stabilized by six histidine residues (Marxen et al., 2013; Olmedo et al., 2013). Copper content was measured in meat and it ranges between 0.19 and 1.09 mg/kg, depending on the type of cuts (Cabrera et al., 2010). Concentrations of copper were much higher in brine shrimp, ranging 5.4-126 mg/kg (Adams et al., 2015).

Copper is essential for bacterial growth since it is a required cofactor for a number of enzymes with oxidase and oxygenase activities, detoxication of oxygen-derived free radicals, and electron transfer (Frausto da Silva and Williams, 2001). However at high concentrations, it can be toxic due to its interaction with proteins, enzymes, nucleic acids and metabolites (Trevors and Cotter, 1990). In *B. subtilis*, CopZ contributes to the copper sequestration and interacts with CopA for copper export (Radford et al., 2003). Although the copper content and availability in our juices have not been measured, one can hypothesize that an excess of

copper in shrimp juice, compared to meat juice, most probably induced its export out of the cells.

Six genes encoding proteins related to stress response or chaperoning activity were also up-regulated: GroEL, HrcA, GrpE, DnaK, a Gls24 homolog and ClpB. ClpB, a stress-induced multi-chaperone system with DnaK, DnaJ and GrpE has been described to be involved in the processing of protein aggregates and/or damaged proteins in *B. subtilis* (Mogk et al., 1997) and *L. monocytogenes* (Liu et al., 2002). This system has been also found in lactic acid bacteria (Papadimitriou et al., 2016). It seems that the machinery for refolding injured proteins is upregulated in *B. thermosphacta* in shrimp juice.

Eight genes of the gene cluster *hisIFAHBDGZ* involved in histidine biosynthesis were upregulated (Figure 4.8). However, the gene *hisC* was not upregulated (end of the gene cluster, may be degraded).

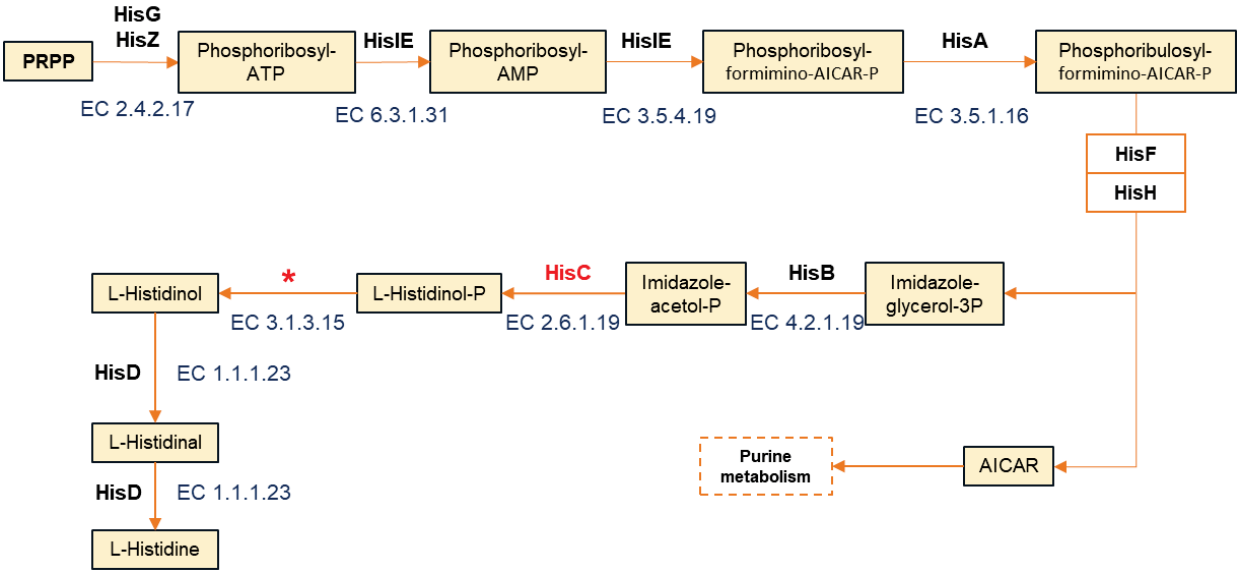

Figure 4.8 Probable histidine biosynthetic pathway by *B. thermosphacta* strains in shrimp juice.
The gene encoding HisC was not upregulated; *refers to a putative histidinol-phosphate phosphatase encoded by gene out of the *his* gene cluster.
Abbreviations: PRPP, phosphoribosyl pyrophosphate; ATP; adenosine triphosphate; AMP, adenosine monphosphate; P, phosphate; AICAR, 1-(5'-phosphoribosyl)-5-amino-4-imidazolecarboxamide.

Sixteen genes encoding proteins involved in various sugar transport and/or utilization were upregulated in shrimp juice. These genes include: i) 4 genes *levDEFG* encoding a complete mannose/fructose specific enzyme IIABCD; ii) 3 genes encoding a complete Enzyme IIABC specific for beta-glucosides (BglP); iii) 2 genes encoding BglH, the phospho-betaglucosidase; iv) 2 genes *mtlRA* for a phosphotransferase system (PTS) dependent utilization of mannitol/glucitol; and v) 1 gene *mdxK* encoding a maltose phosphorylase. Sixgenes involved in carbon source catabolism were also upregulated. These genes encoded: triose phosphate isomerase (EC 5.3.1.1), phosphoglycerate mutase (EC 5.4.2.12), Glycerate 2-kinase (EC 2.7.1.165), phosphoglyceromutase (EC 5.4.2.1), N-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25) and succinate-semialdehyde dehydrogenase (EC 1.2.1.24). This suggests an active carbon catabolism in shrimp juice and use of variety of sugars through the PTS systems.

The 2 genes *pdxT* and *pdxS*, encoding glutamine amidotransferase subunit PdxT (EC 3.5.1.2, 4.3.3.6) and glutamine amidotransferase PdxS synthase subunit (EC 4.3.3.6), respectively, which are involved in the pyridoxal 5'-phosphate biosynthesis and glutamine degradation.

Other genes encoding various functions were also upregulated but could not be associated to the regulation of whole pathways.

Besides the differential expression, shared by the two strains, genes showed a strain-dependent regulation. In meat juice, CD 337 upregulated 34 genes that were not upregulated in TAP 175. Conversely, TAP 175 upregulated 98 genes that were not upregulated in CD 337. Similarly, in shrimp juice CD 337 upregulated 35 genes that were not upregulated by TAP 175. Conversely, TAP 175 upregulated 78 that are not upregulated by CD 337 (Figure 4.4b).

It would be interesting to discriminate among these genes those which are present in the genome of only one strain (strain specific genes). These genes may be involved in the niche adaptation (meat or shrimp juice), and/or explain the difference in spoilage abilities between strains. Similarly, genes that are present in the genome of both strains but differently regulated in CD 337 and TAP 175 may also provide such information.

4.2.4. Conclusion

This study provided insights into the relative difference in gene expression and VOCs production by *B. thermosphacta* during growth in meat and shrimp juices. We early observed an obvious matrix effect on *B. thermosphacta* metabolism. The catabolism of meat or shrimp specific substrates may lead to different metabolisms depending on the food matrix. This may suggest that the food matrix drives the VOCs production. Moreover, *B. thermosphacta* strains produced six VOCs in shrimp juice, while it produced only two in meat juice (ethanol and acetoin). This suggests that *B. thermosphacta* alone most probably exerts a moderate effect on meat spoilage. Nevertheless, as it has been many associated with spoilage of natural meat products in several previous studies, one can hypothesize that *B. thermosphacta* may need interactions with other bacterial species within the meat microbiota, to increase its involvement in the spoilage process of meat. Conversely, in shrimp juice *B. thermosphacta* alone can produce various molecules associated with the spoilage. However, this do not exclude that interaction of this species with other microbiota member could intensify its spoilage potential. Nevertheless, we could not directly link the regulated functions to the volatile molecules produced.

Table 4.3 Genes upregulated in meat juice by both CD 337 and TAP 175

	Label (CD 337)	Name	EC number	Product	LFC (CD 337)	LFC (TAP175)	Fonction
1	BTCD_v1_0023	*guaC*	1.7.1.7	GMP reductase (NADP-dependent)	-2,21	-3,61	putative enzymes with uncertain function
2	BTCD_v1_0159	-		putative biotin transporter BioY	-3,89	-2,7	Putative transport systems of unknown or uncertain specificity
3	BTCD_v1_0177	-		conserved protein of unknown function	-2,24	-3,41	Unknown
4	BTCD_v1_0287	-		conserved membrane protein of unknown function	-2,08	-2,83	Unknown
5	BTCD_v1_0373	-		conserved protein of unknown function	-2,6	-3,63	Unknown
6	BTCD_v1_0512	*fruA*	2.7.1.69 / 2.7.1.202	phosphotransferase system (PTS) fructose-specific enzyme IIABC component	-2,92	-2,09	PTS transporter
7	BTCD_v1_0523	-		putative transcriptional regulator of the potABCD operon	-2,59	-3,09	Spermidine/putrescine transport system + regulator
8	BTCD_v1_0524	*potA*		Spermidine/putrescine import ATP-binding protein PotA	-2,5	-2,69	
9	BTCD_v1_0525	*potB*		Spermidine/putrescine transport system permease protein PotB	-2,84	-3,16	
10	BTCD_v1_0526	*potC*		spermidine/putrescine transport system permease protein PotC	-3,07	-3,15	
11	BTCD_v1_0527	*potD*		spermidine/putrescine-binding periplasmic protein	-2,72	-3	
12	BTCD_v1_0608	-		putative ABC-type transport system, permease component	-3,81	-2,33	Putative transport systems of unknown or uncertain specificity
13	BTCD_v1_0632	*purB*	4.3.2.2	adenylosuccinate lyase	-2,12	-2,48	Purine metabolism
14	BTCD_v1_0633	*purC*	6.3.2.6	phosphoribosylaminoimidazole succinocarboxamide synthetase	-3,9	-5,13	
15	BTCD_v1_0634	*purS*		factor required for phosphoribosylformylglycinamidine synthetase activity	-3,77	-5,27	
16	BTCD_v1_0635	*purQ*	6.3.5.3	phosphoribosylformylglycinamidine synthetase I	-3,5	-4,54	
17	BTCD_v1_0636	*purL*	6.3.2.6	phosphoribosylformylglycinamidine synthetase II	-2,88	-3,53	
18	BTCD_v1_0637	*purF*	2.4.2.14	glutamine phosphoribosylpyrophosphate amidotransferase	-2,15	-2,6	

This table continues in the following pages

19	BTCD_v1_1012	-		membrane protein of unknown function	-3,44	-4,04	Unknown
20	BTCD_v1_1052	-		putative oligopeptide ABC transporter, periplasmic oligopeptide-binding, AppA	-2,3	-2,38	Putative transport systems of unknown or uncertain specificity
21	BTCD_v1_1061	*yrdN*	5.3.2.-	putative tautomerase (IolK)	-2,94	-2,98	Myo-inositol utilization
22	BTCD_v1_1062	*iolR*		transcriptional regulator (DeoR family)	-2,33	-2,39	
23	BTCD_v1_1065	*iolC*	2.7.1.92	2-deoxy-5-keto-D-gluconic acid kinase	-4,34	-2,4	
24	BTCD_v1_1066	*iolD*	3.7.1.22	3D-(3,5/4)-trihydroxycyclohexane-1,2-dione hydrolase	-4,68	-2,79	
25	BTCD_v1_1067	*iolE*	4.2.1.44	2-keto-myo-inositol dehydratase	-5,17	-3,84	
26	BTCD_v1_1068	*iolT*		*myo*-inositol transporter	-4,92	-4	
27	BTCD_v1_1069	*iolG*	1.1.1.18	*myo*-inositol 2-dehydrogenase/D-chiro-inositol 3-dehydrogenase	-5,34	-3,89	
28	BTCD_v1_1070	*iolH*	5.3.-.-	sugar-phosphate epimerase/isomerase	-5,26	-4,31	
29	BTCD_v1_1071	*iolI*	5.3.99.11	inosose isomerase	-5,1	-4,37	
30	BTCD_v1_1072	*iolJ*	4.1.2.29	2-deoxy-5-keto-D-gluconic acid 6-phosphate aldolase	-4,23	-3,72	
31	BTCD_v1_1127	*pyrR*	2.4.2.9	Bifunctional protein PyrR: transcriptional attenuator and uracil phosphoribosyltransferase activity	-2,11	-3,03	Uracil transport
32	BTCD_v1_1128	*pyrP*		uracil permease	-2,12	-2,56	
33	BTCD_v1_1143	-		Protein of unknown function	-3,39	-5	Unknown ?
34	BTCD_v1_1209	*rpsP*		ribosomal protein S16 (BS17)	-2,31	-2,37	Ribosomal proteins and tRNA
35	BTCD_v1_1292	-		protein of unknown function	-2,97	-3,07	Unknown
36	BTCD_v1_1428	*eutQ*	factor	Ethanolamine utilization protein EutQ	-2,24	-2,41	Ethanolamine utilization
37	BTCD_v1_1429	*eutH*	transport	putative ethanolamine permease, EutH	-2,32	-2,25	
38	BTCD_v1_1430	*eutN*		putative ethanolamine utilization protein EutN	-2,32	-2,44	
39	BTCD_v1_1432	*eutD*	2.3.1.8	putative phosphotransacetylase EutD, similar to pduL	-2,36	-2,56	
40	BTCD_v1_1433	*eutM*		Ethanolamine carboxysome structural protein, BMC family	-3,22	-2,51	
41	BTCD_v1_1508	*pftA*		pyruvate uptake system subunit A	-5,19	-5,51	Pyruvate transporter (uptake)
42	BTCD_v1_1509	*pftB*		pyruvate import system subunit B	-4,12	-4,04	

	Label (CD 337)	Name	EC number	Product	LFC (CD 337)	LFC (TAP175)	Fonction
43	BTCD_v1_1750	*rpmGA*		ribosomal protein L33	-2,14	-2,93	Ribosomal proteins and tRNA
44	BTCD_v1_2045	-		conserved membrane protein of unknown function	-2,08	-2,24	Unknown
45	BTCD_v1_2225	*cutR*		putative HTH-type transcriptional repressor YcnK	-2,04	-2,05	Ptative regulator with unknown function
46	BTCD_v1_2241	-		protein of unknown function	-3,51	-2,46	Unknown
47	BTCD_v1_2248	-		conserved membrane protein of unknown function	-2,31	-2,38	Unknown
48	BTCD_v1_2310	-		putative ABC transporter permease	-3,19	-2,89	Putative transport systems of unknown or uncertain specificity
49	BTCD_v1_2311	*yxdL*		ABC transporter (ATP-binding protein); efflux of cationic peptides; bacitracin ABC efflux transporter	-3,06	-2,66	Putative transport systems of unknown or uncertain specificity
50	BTCD_v1_2338	*xapB*		xanthosine transporter	-3	-2,22	Purine metabolism
51	BTCD_v1_2339	*pupG*	2.4.2.1	purine nucleoside phosphorylase	-2,74	-2,32	
52	BTCD_v1_2384	-		putative transcriptional regulator containing CheY-like receiver domain and AraC-type DNA-binding domain	-3,01	-2,02	Fe^{3+} transport system + regulator
53	BTCD_v1_2386	-		putative ABC-type Fe3+ transport system, permease component	-6,78	-4,48	
54	BTCD_v1_2387	-	3.6.3.-	putative ABC-type Fe3+ transport system, ATP-binding protein	-5,62	-3,32	
55	BTCD_v1_2388	-		putative ABC-type Fe3+ transport system, periplasmic component	-5,39	-3,56	
56	BTCD_v1_2389	*nhaC*		Na(+)/H(+) antiporter NhaC	-2,33	-2,14	
57	BTCD_v1_2396	*ascR*		transcriptional regulator of operon snaA-ytnM degrading cysteine-containing compounds (LysR family)	-3,44	-5,11	Sulrur compounds utilization + regulator
58	BTCD_v1_2397	-	1.5.1.29	putative FMN reductase NADPH-dependent	-3,16	-2,65	
59	BTCD_v1_2398	*snaA*		N-acetyltransferase acting on sulfur compounds	-2,88	-2,41	
60	BTCD_v1_2399	*tcyJ*		sulfur containing amino acid ABC transporter binding lipoprotein	-4,01	-2,3	
61	BTCD_v1_2401	*tcyL*		sulfur-containing amino acid ABC transporter (permease)	-2,21	-2,35	
62	BTCD_v1_2402	*tcyM*		sulfur-containing amino acid ABC transporter (permease)	-2,43	-2,07	
63	BTCD_v1_2549	-		putative ECF transporter, substrate-specific component	-2,15	-2,94	Putative transport systems of unknown or uncertain specificity

Table 4.4 Genes upregulated in shrimp juice by both CD337 and TAP 175

	Label	Gene name	EC number	Product	LFC (CD 337)	LFC (TAP175)	Function
1	BTCD_v1_0235	-		putative ComF operon protein A, helicase competence protein	2,01	2,51	putative transporter
2	BTCD_v1_0267	*gabD*	1.2.1.24	succinate-semialdehyde dehydrogenase	3,18	4,71	Succinate synthesis
3	BTCD_v1_0268	-		protein of unknown function	2,48	3,74	unknown
4	BTCD_v1_0292	*tpiA*	5.3.1.1	triose phosphate isomerase	2,05	2,53	Glycolysis
5	BTCD_v1_0293	*pgm*	5.4.2.12	phosphoglycerate mutase	2,14	2,67	Glycolysis
6	BTCD_v1_0345	-		protein of unknown function	2,3	2,81	unknown
7	BTCD_v1_0398	*copZ*		copper insertion chaperone and transporter component	2,57	3,27	copper export
8	BTCD_v1_0399	*copA*		copper transporter ATPase	4,32	6,24	
9	BTCD_v1_0400	-		conserved protein of unknown function	3,24	3,24	unknown
10	BTCD_v1_0531	-		protein of unknown function	3,3	3,64	unknown
11	BTCD_v1_0723	-	1.6.5.5	putative Zn-dependent oxidoreductase	2,51	2,79	putative oxidoreductase
12	BTCD_v1_0727	*groEL*		chaperonin large subunit	2,74	2,8	adaptation for various stress
13	BTCD_v1_0753	-		conserved protein of unknown function	4,04	3,83	unknown
14	BTCD_v1_0755	*hrcA*		transcriptional regulator of heat-shock genes	2,53	2,29	adaptation for various stress
15	BTCD_v1_0756	*grpE*		nucleotide exchange factor for DnaK activity	2,26	2,32	adaptation for various stress
16	BTCD_v1_0757	*dnaK*		molecular chaperone, ATP-dependent	2,43	2,68	adaptation for various stress
17	BTCD_v1_0851	*comEA*		membrane bound high-affinity DNA-binding receptor	2,34	3,35	putative transporter
18	BTCD_v1_0869	-		protein of unknown function	2,21	2,23	unknown
19	BTCD_v1_0891	-		putative nucleotide- or NAD- binding protein (epimerase family protein YfhF)	2,87	3,61	putative enzyme
20	BTCD_v1_1031	*garK*	2.7.1.165	Glycerate 2-kinase	2,12	2,36	
21	BTCD_v1_1042	-	1.2.1.76	putative succinate-semialdehyde dehydrogenase (acetylating)	4,28	3	utanoate metabolism reaction: succinate semialdehyde + CoA + NADP+ = succinyl-CoA + NADPH + H+

22	BTCD_v1_1058	*nagA*	3.5.1.25	N-acetylglucosamine-6-phosphate deacetylase	2,21	2,47	Amin-osugar and nucleotide-sugar metabolism NAG degradation vers F6P et glycolyse
23	BTCD_v1_1077	-		conserved protein of unknown function	2,1	2,86	unknown
24	BTCD_v1_1133	*pyrK*	1.3.98.1	dihydroorotate dehydrogenase (electron transfer subunit)	2,46	2,54	pyrimidine metabolism
25	BTCD_v1_1134	*pyrD*	1.3.1.14	dihydroorotate dehydrogenase (catalytic subunit)	2,76	2,87	
26	BTCD_v1_1135	*pyrF*	4.1.1.23	orotidine 5'-phosphate decarboxylase	2,92	3,04	
27	BTCD_v1_1136	*pyrE*	2.4.2.10	orotate phosphoribosyltransferase	3,35	3,48	
28	BTCD_v1_1296	*pdxT*	3.5.1.2, 4.3.3.6	glutamine amidotransferase subunit PdxT	3,86	5,32	
29	BTCD_v1_1297	*pdxS*	4.3.3.6	glutamine amidotransferase , pdxS synthase subunit	3,53	4,65	
30	BTCD_v1_1300	-	3.1.1.-	putative Esterase/lipase	2,09	2,03	putative esterase
31	BTCD_v1_1326	-		putative acetyltransferase	3,51	3,13	putative acetyltransferase
32	BTCD_v1_1339	-		Stress response regulator gls24 homolog	2,12	2,54	adaptation for various stresses
33	BTCD_v1_1344	*vmlR*		ATP-binding cassette efflux transporter	2,91	4,27	putative transporter
34	BTCD_v1_1410	*hisI*	3.5.4.19, 3.6.1.31	phosphoribosyl-AMP cyclohydrolase	2,41	3,76	histidine biosynthesis
35	BTCD_v1_1411	*hisF*	2.4.2.-	imidazole glycerol phosphate synthase subunit	2,3	4,68	
36	BTCD_v1_1412	*hisA*	5.3.1.16	phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	2,51	5,2	
37	BTCD_v1_1413	*hisH*	transport	imidazole glycerol phosphate synthase, glutamine amidotransferase subunit	2,44	4,58	
38	BTCD_v1_1414	*hisB*	4.2.1.19	imidazoleglycerol-phosphate dehydratase [Mn(II)-dependent]	2,5	4,07	
39	BTCD_v1_1415	*hisD*	1.1.1.23	histidinol dehydrogenase	2,67	4,57	
40	BTCD_v1_1416	*hisG*	2.4.2.17	ATP phosphoribosyltransferase	2,46	3,61	
41	BTCD_v1_1417	*hisZ*	2.4.2.17	ATP phosphoribosyltransferase regulatory subunit	2,27	2,36	

This table continues in the following pages

	Label	Gene name	EC number	Product	LFC (CD 337)	LFC (TAP175)	Function
42	BTCD_v1_1483	-		conserved protein of unknown function	2,45	3,24	putative oxidoreductase
43	BTCD_v1_1593	*mdxK*	2.4.1.8	maltose phosphorylase	2,37	3,57	maltose utilization
44	BTCD_v1_1648	-		protein of unknown function	2,45	2,8	unknown
45	BTCD_v1_1700	*nnrD*	4.2.1.136	putative ADP-dependent (S)-NAD(P)H-hydrate dehydratase	2,5	2,09	*
46	BTCD_v1_1922	*argD*	2.6.1.11	N-acetylornithine aminotransferase	2,7	3,19	Ornithine biosynthesis from glu to N-acetyl-ornithin
47	BTCD_v1_1923	*argB*	2.7.2.8	Acetylglutamate kinase	3,29	3,42	Ornithine biosynthesis from glu to N-acetyl-ornithin
48	BTCD_v1_2004	*yvgN*	1.1.1.283	methylglyoxal reductase (NADPH-dependent).	2,63	3,89	detoxification
49	BTCD_v1_2039	*gpmA*	5.4.2.1	phosphoglyceromutase	2,97	3,2	2P-glycerate <=> 3P-glycerate glycolyse vers PEP
50	BTCD_v1_2040	*clpB*		Chaperone protein ClpB	2,67	2,75	adaptation for various stresses
51	BTCD_v1_2108	-	1.14.14.3	putative FMN-dependentoxidoreductase	2,56	2,61	putative oxidoreductase
52	BTCD_v1_2121	-		putative zinc-containing alcohol dehydrogenase superfamily (fragment)	2,85	3,42	putative dehydrogenase
53	BTCD_v1_2137	*tiaE*	1.1.1.79, 1.1.1.81	2-oxo-carboxylic acid reductase	2,03	2,21	glyoxylate <=glycolate / methylglyoxal <=> lactaldehyde
54	BTCD_v1_2296	*levD*		mannose-specific PTS enzymes: IIA component	5,05	3,87	EIIABCD mannose fructose PTS dependent uptake
55	BTCD_v1_2297	*levE*		mannose-specific PTS enzymes: IIB component	4,66	3,37	
56	BTCD_v1_2298	*levF*		mannose-specific PTS enzyme IIC component	4,41	3,62	
57	BTCD_v1_2299	*levG*		mannose-specific PTS enzyme IID component	3,16	2,82	

58	BTCD_v1_2369	*bglH*	3.2.1.86	aryl-phospho-beta-d-glucosidase	2,36	2,53	carbohydrate metabolism
59	BTCD_v1_2370	*bglH*	3.2.1.86	aryl-phospho-beta-d-glucosidase	2,05	2,38	
60	BTCD_v1_2378	*bglP*		PTS system, beta-glucoside-specific IIABC component	3,05	3,11	Sugar transporter
61	BTCD_v1_2426	-		conserved protein of unknown function	2,26	2,24	unknown
62	BTCD_v1_2603	-		putative transcriptional regulator MtlR with PTS EIIAB components	2,15	2,75	carbohydrate metabolism
63	BTCD_v1_2604	*mtlA*		mannitol-specific PTS enzyme IIABC component	3,5	3,45	carbohydrate metabolism
64	BTCD_v1_2621	-		protein of unknown function	2,4	2,46	unknown
65	BTCD_v1_misc_RNA_13	-		RNaseP_bact_b	2,75	3,96	RNA
66	BTCD_v1_misc_RNA_2	-		6S	2,77	2,59	6S RNA*
67	BTCD_v1_misc_RNA_27	-		TPP	2,06	2,45	RNA
68	BTCD_v1_misc_RNA_28	-		TPP	2.17	2,16	
79	BTCD_v1_tRNA24	-		Trp tRNA	2,39	2,89	tRNA
70	BTCD_v1_tRNA29	-		Leu tRNA	2,1	2,43	tRNA
71	BTCD_v1_tRNA77	-		Lys tRNA	3,42	-2,05	tRNA
72	BTCD_v1_tRNA83	-		Gly tRNA	3,02	3,5	tRNA
73	BTCD_v1_tRNA85	-		Arg tRNA	3,49	3,7	tRNA

4.3. Ce qu'il faut retenir de ce chapitre

L'objectif de ce chapitre était de comprendre l'effet souche et l'effet matrice sur le potentiel altérant de *B. thermosphacta*. Pour cela, deux souches représentatives de la diversité génotypique et phénotypique et dont la séquence génomique a été déterminée ont été inoculées sur deux milieux modèles.
Le suivi de la croissance bactérienne de l'inoculation jusqu'à 7 jours de stockage à 8 °C a montré que les deux souches ont été capables de se développer sur les deux matrices.

L'analyse du volatilome a montré que la production des VOCs par *B. thermosphacta* était variable en fonction des jus. Sur le jus de crevette, les souches de *B. thermosphacta* ont produit six VOCs incluant (isobutyraldehyde, 2-méthylbutyraldehyde, méthylbutyraldéhyde, 3-méthylbutyraldehyde, acétoïne et 3-méthyl-1-butanol). Tandis que sur le jus de viande, les deux souches ont produit seulement deux molécules (éthanol et acétoïne).

L'analyse du transcriptome a révélé de nombreuses fonctions différentiellement exprimées suivant les jus de matrices. Nous avons spécifiquement recherché les fonctions surexprimées par les deux souches CD 337 et TAP 175 sur le jus de viande ou sur le jus de crevette. Cela nous a permis de mettre en évidence un effet matrice évident sur le métabolisme de *B. thermosphacta*. En effet, les deux souches utilisent les substrats spécifiques à chaque matrice par exemple le *myo*-inositol et l'éthanolamine sur le jus de viande, et le mannose, le mannitol et les beta-glucosides sur le jus de crevette, aboutissant donc à des métabolismes différents en fonction des matrices et donc à une production de molécules volatiles différente.

Les souches *B. thermosphacta* ont produit plus de VOCs sur le jus de crevette par rapport au jus de viande. Ceci laisse penser que *B. thermosphacta* seul altérerait peu la viande et que très probablement une interaction avec le reste du microbiote de la viande permettrait d'accentuer son potentiel altérant. Sur le jus de crevette, *B. thermosphacta* seul produit diverses molécules altérantes. Néanmoins, cela n'exclut pas que l'interaction avec le microbiote de la crevette pourrait intensifier ces capacités altérantes.

Ces résultats permettent de mieux comprendre l'effet de la matrice alimentaire sur le métabolisme de *B. thermosphacta*. Il serait également intéressant d'analyser les fonctions différentiellement exprimées en fonction des souches.

Discussion générale et conclusion

Nassima Illikoud
2018

Discussion générale et perspectives

La réglementation européenne liée à la qualité et la sécurité des produits carnés et de la mer ne prend pas compte les bactéries d'altération dans les critères microbiologiques d'hygiène permettant d'assurer la qualité de ces produits (Règlement (CE) n° 1441/2007). De ce fait, ces bactéries ont reçu peu d'attention de la part des industriels et de la communauté scientifique. Cependant, leur développement sur les produits alimentaires entraine une diminution de la durée de conservation à cause de modifications organoleptiques indésirables qui peuvent aussi entraîner des pertes. Les pertes économiques conséquentes ont fait réagir les producteurs du secteur agro-alimentaire, les amenant à investir dans la recherche dédiée à la compréhension et la maîtrise de ces phénomènes d'altération.

L'altération des aliments est principalement causée par le développement de micro-organismes qui provoquent la formation de molécules responsables de l'apparition d'odeurs, de saveurs, de textures ou de couleurs non souhaitées. Les genres et espèces de bactéries liés à l'altération des viandes et des produits de la mer ont été mis en évidence dans de nombreuses études. Celles-ci incluent principalement des *Pseudomonas*., des *Enterobacteriaceae*, différentes espèces de bactéries lactiques et *B. thermosphacta*.

B. thermosphacta, est une bactérie spécifique de l'altération de nombreux produits carnés. Son potentiel d'altération dans de nombreuses matrices alimentaires a été largement rapporté (Casaburi et al., 2014; Dainty et al., 1989; Jaffrès et al., 2011; Joffraud et al., 2006). Cette espèce bactérienne provoque l'altération principalement par la production de métabolites responsables d'odeurs indésirables tels que des aldéhydes, des cétones et des acides (Illikoud et al., 2018). La diversité des composés produits par *B. thermosphacta* peut dépendre des spécificités génétiques des souches, mais aussi des caractéristiques intrinsèques de la matrice alimentaire, de paramètres extrinsèques tels que les étapes liées au procédé de fabrication ou encore de l'environnement de stockage. Tous ces paramètres peuvent influer considérablement sur le métabolisme et donc sur le potentiel altérant de *B. thermosphacta* (Petruzzi et al., 2017). De plus, l'évolution des bactéries pour s'adapter à ces facteurs environnementaux peut conduite à une diversité génétique et phénotypique entre souches appartenant à une même espèce. A titre d'exemple, il a été montré que la taille du génome

de souches de *L. sakei* isolées de divers produits était très variable en fonction des souches, et pouvait atteindre jusqu'à 25 % de différence par rapport à la taille moyenne du génome de l'espèce (2020 kb). En effet, celle-ci était comprise entre 1815 kb et 2310 kb suivant les souches (Chaillou et al., 2009)

Un autre exemple intéressant concerne l'espèce *L. piscium*, qui comprend à la fois des souches rapportées comme altérantes des viandes (Pothakos et al., 2014; Rahkila et al., 2012) mais également une souche décrite pour ces capacités biopréservatrices sur les produits de la mer (Fall et al., 2012; Saraoui et al., 2016). De plus, une étude conduite par Stohr et al. (2001) sur le saumon fumé a montré que des souches, bien qu'appartenant à une même espèce bactérienne, pouvaient avoir des capacités altérantes différentes et donc des caractéristiques métaboliques différentes.

En ce qui concerne *B. thermosphacta*, très peu de données existent à l'heure actuelle quant à la diversité au sein de cette espèce et aux mécanismes spécifiques mis en place lors du processus d'altération. Ce travail de thèse visait à répondre à ces préoccupations en apportant des connaissances nouvelles notamment par des approches de génomique et transcriptomique, sur cette bactérie emblématique de l'altération d'une large gamme de produits carnés et de produits de la mer.

Les données disponibles dans la littérature sur le potentiel d'altération de *B. thermosphacta* montrent que cette bactérie a des capacités d'altération différentes en fonction des conditions d'emballage mais aussi en fonction des matrices alimentaires. En effet, cette bactérie produit des molécules d'altération différentes selon qu'elle se retrouve sur de la viande ou sur des produits de la mer. Cela laisse penser qu'il existe des différences de métabolisme chez cette espèce en fonction des matrices alimentaires. Cependant, les quelques données disponibles sur la diversité intra-espèce ont porté sur des souches isolées d'une même matrice alimentaire (Papadopoulou et al., 2012b; Stanborough et al., 2017). Ainsi, la question de savoir si les différences entre le type de molécules altérantes produites suivant les matrices résultaient de la capacité des souches et/ou de la nature du substrat sur lequel elles se développaient restait ouverte.

La première partie de ce travail a consisté à étudier la diversité de *B. thermosphacta* au niveau génotypique et phénotypique. Les objectifs étaient de déterminer si des écotypes pouvaient être différentiés, mais aussi s'il existait une corrélation entre l'origine écologique des souches et leur capacité à altérer. Cette partie a permis de montrer qu'il existait bien une diversité importante au sein de l'espèce *B. thermosphacta*. Cependant, aucune corrélation n'a été établie entre le génotype, l'origine écologique, et la capacité des souches à produire des molécules d'altération comme l'acétoïne et le diacétyle.

Les hypothèses permettant d'expliquer la variabilité intra-spécifique concernent l'impact des facteurs environnementaux sur l'évolution des souches et leur adaptation à une niche écologique donnée, à travers l'acquisition de nouveaux gènes par des transferts horizontaux ou l'accumulation de mutations ponctuelles dans leur génome (Santiago and Richard, 2003; Wiedenbeck and Cohan, 2011). Afin d'expliquer les différences génotypiques et phénotypiques que nous avions observées, le génome de quatre souches, représentatives de cette diversité et issues de différentes niches écologiques, a été analysé. La comparaison du répertoire génétique entre ces 4 souches, et avec d'autres dont les génomes étaient disponibles dans les bases de données publiques, a montré des différences essentiellement dans le contenu phagique et plasmidique, et sur la présence de gènes codant pour des composants de surface ou des adhésines. Ces variations pourraient contribuer à des capacités d'adaptation différentes, leur conférant des capacités à coloniser et à persister dans une niche écologique spécifique. Le répertoire de gènes liés à la production de molécules altérantes par *B. thermosphacta* est similaire suivant les souches. Cependant, nous avons observé des mutations ponctuelles au niveau des gènes codant des enzymes impliquées dans la production de certaines molécules altérantes comme le 3-méthylbutanal et le 3-méthylbutanol. En revanche, nous n'avons pas noté de différences pouvant expliquer les capacités différentes des souches à produire de l'acétoïne et du diacétyle.

Une étude plus approfondie, basée sur l'analyse combinée de l'expression des gènes et de la production de molécules volatiles a été réalisée. Au cours de cette étude, l'accent a été mis sur l'effet de la matrice alimentaire et/ou l'effet souche, sur la diversité des capacités altérantes de *B. thermosphacta*. Deux souches, représentatives de la diversité de *B. thermosphacta*, ont été inoculées dans deux matrices modèles (jus de viande et jus de

crevette). Les résultats ont montré un réel effet matrice sur la production de molécules volatiles mais aussi sur l'expression des gènes. L'analyse des gènes différentiellement exprimées en fonction des matrices alimentaires, montre que les souches de *B. thermosphacta* adaptent leur métabolisme en fonction des substrats disponibles dans leur environnement. En effet, sur le jus de viande les deux souches utiliseraient préférentiellement le *myo*-inositol et l'éthanolamine alors que sur le jus de crevette, elles consommeraient plutôt le mannose, le mannitol et les beta-glucosides. Ces différents métabolismes pourraient conduire à des flux de carbone différents, et donc expliquer les différences de production de molécules volatiles en fonction des matrices. En effet, nous n'avons pas observé de différence d'expression des gènes impliqués directement dans la synthèse d'acétoïne et de diacétyle. Cependant, le flux métabolique vers des précurseurs de ces molécules comme le pyruvate (dont le transport semble varier suivant la matrice), pourrait lui varier suivant le substrat. Concernant la production de 3-méthylbutanol, nous n'avons pas non plus noté de différence d'expression des gènes entre les deux matrices alors que sa production variait. Ceci pourrait conforter notre hypothèse selon laquelle les mutations ponctuelles que nous avons notées pourraient bien influencer l'activité des enzymes. Enfin, pour d'autres molécules altérantes, (2-methylbutyraldehyde, 3-methylbutyraldehyde, isobutyraldehyde) nous n'avons pas pu corréler leur production à l'expression de gènes en particulier, les voies métaboliques responsables de leur synthèse n'étant pas clairement établies. Cette étude donne un aperçu global sur les mécanismes mis en place par *B. thermosphacta* pour survivre sur différentes matrices alimentaires. Par ailleurs, un effet souche a également été noté sur l'expression différentielle des gènes. En effet, chaque souche montrait aussi un ensemble de gènes dont l'expression variait suivant la matrice, mais qui ne variait pas dans l'autre souche. L'analyse des fonctions codées par ces gènes, dont l'expression est souche spécifique, n'a pas pu être terminée lors de ce travail de thèse. Il serait donc intéressant de les explorer pour savoir si elles contribuent à la croissance, la survie et/ou l'altération spécifique de chaque souche dans une matrice donnée.

Au terme de ce travail, plusieurs perspectives pourraient être envisagées pour approfondir la compréhension des mécanismes d'altération par les souches de *B. thermosphacta* :

Des expériences de cultures en milieux de laboratoire supplémentés avec différents composés (*myo*-inositol, éthanolamine, sucres, pyruvate, cuivre, fer) pourraient être réalisées afin de valider l'hypothèse de l'effet de l'utilisation de différents substrats, disponibles dans la matrice alimentaire, sur la croissance et le métabolisme de *B. thermosphacta*, sur la production des molécules altérantes, et sur l'expression des gènes impliquées dans l'altération.

De plus, des gènes d'intérêt (potentiellement mutés ou différentiellement exprimés en fonction des matrices et/ou des souches) pourraient être sélectionnés afin de valider leur implication dans l'altération par génomique fonctionnelle. Un criblage de quelques gènes cibles pourrait également être envisagé mais sur un plus large éventail de souches et/ou de matrices.

Des travaux pourraient être envisagés pour comprendre comment cette bactérie se comporte en présence d'autres bactéries présentes sur l'aliment, notamment sur la viande, puisque nous avons montré que les souches de *B. thermosphacta* seules semblaient présenter un effet plus modéré sur l'altération de la viande, en produisant uniquement de l'acétoïne et de l'éthanol. Cette interaction entre *B. thermosphacta* et d'autres espèces bactériennes a déjà été suggérée dans une étude précédente conduite par Laursen et al. (2006), qui a montré que *B. thermosphacta* et *C. maltaromaticum* en co-culture entraînaient l'apparition de certaines mauvaises odeurs, qui n'étaient pas produites lorsque ces espèces se développaient séparément l'une de l'autre.

Enfin, une autre perspective serait d'identifier les gènes clés activement impliqués dans l'altération des aliments, afin de les utiliser comme marqueurs génétiques, spécifiques de l'altération par *B. thermosphacta.*

Valorisation des travaux de thèse

Nassima Illikoud

2018

Valorisation des travaux de thèse

Articles dans des revues scientifiques internationales à comité de lecture

- **Illikoud N.**, Jaffrès E., Zagorec M., *Brochothrix thermosphacta*. Elsevier's Reference Module in Life Sciences. https://doi.org/10.1016/B978-0-12-809633-8.12106-5
- **Illikoud N.**, Rossero A., Chauvet R., Pilet M-F., Courcoux P., Charrier T., Jaffrès E., Zagorec M., Genotypic and phenotypic diversity of the food spoilage bacterium *Brochothrix thermosphacta*. *Food Microbiology*. https://doi.org/10.1016/j.fm.2018.01.015
- **Illikoud N.**, Klopp C., Roulet A., Bouchez O, Marsaud N., Jaffrès E., and Zagorec M., One complete and three draft genome sequences of four *Brochothrix thermosphacta* strains, CD 337, TAP 175, BSAS1 3 and EBP 3070 - *Extended Genome Report.* Soumis dans *Standards in Genomic Sciences* (Référence SIGS-D-18-00045).
- **Illikoud N.,** Gohier R., Werner D., Barrachina C., Roche D., Jaffrès E., and Zagorec M., Transcriptome and volatilome analysis of food-spoilage associated bacterium *Brochothrix thermosphacta*. En preparation

Communications en congrès nationaux et internationaux

- Communications orales

  - **Illikoud N.**, Rossero A., Pilet M-F., Courcoux P., Chauvet R., Charrier T., Jaffrès E. et Zagorec M., Genotypic and phenotypic characterization of the food spoilage *bacterium Brochothrix thermosphacta*. *Microbial Spoilers of Food,* 2017, Quimper (France) Congrès international

  - **Illikoud N.**, Jaffrès E., Pilet M-F., Chauvet R., Charrier T., Zagorec M., Diversité de *Brochothrix thermosphacta* et capacité altérante. *5e Edition des Journées Scientifiques de l'ED-VENAM*, 2016, Nantes (France)

  - **Illikoud N.**, Chauvet R., Charrier T., Jaffrès E. et Zagorec M., Genetic diversity of *Brochothrix thermosphacta* and food spoilage. *3rd International Conference on Microbial Diversity: The challenge of Complexity*, 2015, Perugia (Italie). Congrès international

- Posters

  - **Illikoud N.**, Jaffrès E., Pilet M-F., Chauvet R., Courcoux P., Charrier T., Zagorec M., Diversité de *Brochothrix thermosphacta* et capacité altérante. *13e Congrès de la Société Française de Microbiologie*, 2017, Paris (France). Congrès national

  - **Illikoud N.**, Chauvet R., Pilet M-F., Courcoux P., Charrier T., Jaffrès E. et Zagorec M., Diversity of *Brochothrix thermosphacta* and food spoilage. *25th International ICFMH Conference - FoodMicro.* 2016, Dublin (Irlande). Congrès international

  - **Illikoud N.**, Jaffrès E., Pilet M-F., Zagorec M., Diversité génétique de *Brochothrix thermosphacta* et altération des aliments. *11e Congrès de la Société Française de Microbiologie*, 2015, Paris (France). Congrès national

  - **Illikoud N.**, Jaffrès E., Pilet M-F., Zagorec M., Diversité génétique de *Brochothrix thermosphacta* et capacité altérante. *20e colloque du Club des Bactéries Lactiques : Nouveaux défis pour la recherche et l'industrie*, 2015, Lille (France). Congrès national

  - **Illikoud N.**, Jaffrès E., Pilet M-F., Zagorec M., Caractérisation des gènes impliqués dans l'expression du potentiel d'altération des aliments chez *Brochothrix thermosphacta*. *4e Edition des Journées Scientifiques de l'ED-VENAM*, 2014, Angers (France)

Références bibliographiques

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References bibliographiques

Adams, W.J., DeForest, D.K., Tear, L.M., Payne, K., Brix, K.V., 2015. Long-term monitoring of arsenic, copper, selenium, and other elements in Great Salt Lake (Utah, USA) surface water, brine shrimp, and brine flies. Environmental Monitoring and Assessment 187, 118.

Adékambi, T., Drancourt, M., Raoult, D., 2009. The *rpoB* gene as a tool for clinical microbiologists. Trends in Microbiology 17, 37-45.

Afzal, M.I., Ariceaga, C.C.G., Boulahya, K.-A., Jacquot, M., Delaunay, S., Cailliez-Grimal, C., 2017. Biosynthesis and role of 3-methylbutanal in cheese by lactic acid bacteria: Major metabolic pathways, enzymes involved, and strategies for control. Critical Reviews in Food Science and Nutrition 57, 399-406.

Almeida, M., Hébert, A., Abraham, A.-L., Rasmussen, S., Monnet, C., Pons, N., et al., 2014. Construction of a dairy microbial genome catalog opens new perspectives for the metagenomic analysis of dairy fermented products. BMC Genomics 15, 1101.

Anders, S., Huber, W., 2010. Differential expression analysis for sequence count data. Genome Biology 11, R106.

Andreani, N.A., Carraro, L., Martino, M.E., Fondi, M., Fasolato, L., Miotto, G., et al., 2015. A genomic and transcriptomic approach to investigate the blue pigment phenotype in *Pseudomonas fluorescens*. International Journal of Food Microbiology 213, 88-98.

Andreani, N.A., Fasolato, L., 2017. *Pseudomonas* and related genera, The Microbiological Quality of Food. Elsevier, pp. 25-59.

Andreani, N.A., Martino, M.E., Fasolato, L., Carraro, L., Montemurro, F., Mioni, R., et al., 2014. Tracking the blue: A MLST approach to characterise the Pseudomonas fluorescens group. Food Microbiology 39, 116-26.

Andreevskaya, M., Hultman, J., Johansson, P., Laine, P., Paulin, L., Auvinen, P., Björkroth, J., 2016. Complete genome sequence of *Leuconostoc gelidum* subsp. *gasicomitatum* KG16-1, isolated from vacuum-packaged vegetable sausages. Standards in Genomic Sciences 11, 40.

Andreevskaya, M., Johansson, P., Laine, P., Smolander, O.-P., Sonck, M., Rahkila, R., et al., 2015. Genome sequence and transcriptome analysis of meat spoilage lactic acid bacterium *Lactococcus piscium* MKFS47. Applied and Environmental Microbiology.

Andrews, S.C., Robinson, A.K., Rodríguez-Quiñones, F., 2003. Bacterial iron homeostasis. FEMS Microbiology Reviews 27, 215-37.

Argyri, A.A., Doulgeraki, A.I., Blana, V.A., Panagou, E.Z., Nychas, G.-J.E., 2011. Potential of a simple HPLC-based approach for the identification of the spoilage status of minced beef stored at various temperatures and packaging systems. International Journal of Food Microbiology 150, 25-33.

Aru, V., Pisano, M.B., Savorani, F., Engelsen, S.B., Cosentino, S., Cesare Marincola, F., 2016. Metabolomics analysis of shucked mussels' freshness. Food Chemistry 205, 58-65.

Asensio, M.A., Ordoñez, J.A., Sanz, B., 1988. Effect of carbon dioxide and oxygen enriched atmospheres on the shelf-life of refrigerated pork packed in plastic bags. Journal of Food Protection 51, 356-60.

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., et al., 2000. Gene ontology: Tool for the unification of biology. Nature Genetics 25, 25.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., et al., 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology 19, 455-77.

Barbuddhe, S.B., Maier, T., Schwarz, G., Kostrzewa, M., Hof, H., Domann, E., et al., 2008. Rapid identification and typing of *Listeria* Species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Applied and Environmental Microbiology 74, 5402-07.

Binnewies, T.T., Motro, Y., Hallin, P.F., Lund, O., Dunn, D., La, T., et al., 2006. Ten years of bacterial genome sequencing: comparative-genomics-based discoveries. Functional & Integrative Genomics 6, 165-85.

Blakely, G.W., 2015. Chapter 15 - Mechanisms of Horizontal Gene Transfer and DNA Recombination A2 - Tang, Yi-Wei, in: Sussman, M., Liu, D., Poxton, I., Schwartzman, J. (Eds.), Molecular Medical Microbiology (Second Edition), 2nd ed. Academic Press, Boston, pp. 291-302.

Blickstad, E., 1983. Growth and end product formation of two psychrotrophic *Lactobacillus* spp. and *Brochothrix thermosphacta* ATCC 11509T at different pH values and temperatures. Applied and Environmental Microbiology 46, 1345-50.

Bohaychuk, V.M., Greer, G.G., 2003. Bacteriology and storage life of moisture-enhanced pork. Journal of Food Protection 66, 293-99.

Böhme, K., Cremonesi, P., Severgnini, M., Villa, T.G., Fernandez-No, I.C., Barros-Velazquez, J., et al., 2014. Detection of food spoilage and pathogenic bacteria based on ligation detection reaction coupled to flow-through hybridization on membranes. BioMed Research International 2014, 1-11.

Böhme, K., Fernández-No, I., Gallardo, J., Cañas, B., Calo-Mata, P., 2011. Safety assessment of fresh and processed seafood products by MALDI-TOF mass fingerprinting. Food Bioprocess Technology 4, 907-18.

Borch, E., Kant-Muermans, M.-L., Blixt, Y., 1996. Bacterial spoilage of meat and cured meat products. International Journal of Food Microbiology 33, 103-20.

Borch, E., Molin, G., 1989. The aerobic growth and product formation of *Lactobacillus*, *Leuconostoc*, *Brochothrix*, and *Carnobacterium* in batch cultures. Applied Microbiology and Biotechnology 30, 81-88.

Borg, I., Groenen, P.J., 2005. Modern multidimensional scaling: Theory and applications. Springer-Verlag, New York, USA.

Bosshard, P.P., Zbinden, R., Abels, S., Böddinghaus, B., Altwegg, M., Böttger, E.C., 2006. 16S rRNA gene sequencing versus the API 20 NE system and the VITEK 2 ID-GNB card for identification of

nonfermenting Gram-negative bacteria in the clinical laboratory. Journal of Clinical Microbiology 44, 1359-66.

Braun, P., Sutherland, J.P., 2004. Predictive modelling of growth and measurement of enzymatic synthesis and activity by a cocktail of *Brochothrix thermosphacta*. International Journal of Food Microbiology 95, 169-75.

Büchner, L.M., 2012. Corporate social responsibility and sustainability from a global, European and corporate perspective. Corporate social responsibility and sustainable governance. Eurolimes, 41-55.

Cabrera, M.C., Ramos, A., Saadoun, A., Brito, G., 2010. Selenium, copper, zinc, iron and manganese content of seven meat cuts from Hereford and Braford steers fed pasture in Uruguay. Meat Science 84, 518-28.

Carbonnelle, E., Mesquita, C., Bille, E., Day, N., Dauphin, B., Beretti, J.L., et al., 2011. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. Clinical Biochemistry 44, 104-9.

Carlson, M., Pages, H., Aboyoun, P., Falcon, S., Morgan, M., Sarkar, D., Lawrence, M., 2011. Genomic features: Tools for making and manipulating transcript centric annotations. R package version 1.4.3.

Carpenter, C.E., Clark, E., 1995. Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. Journal of Agricultural and Food Chemistry 43, 1824-27.

Casaburi, A., De Filippis, F., Villani, F., Ercolini, D., 2014. Activities of strains of *Brochothrix thermosphacta in vitro* and in meat. Food Research International (Ottawa, Ont.) 62, 366-74.

Casaburi, A., Di Martino, V., Ercolini, D., Parente, E., Villani, F., 2015a. Antimicrobial activity of *Myrtus communis L.* water-ethanol extract against meat spoilage strains of *Brochothrix thermosphacta* and *Pseudomonas fragi in vitro* and in meat. Annals of Microbiology 65, 841-50.

Casaburi, A., Piombino, P., Nychas, G.-J., Villani, F., Ercolini, D., 2015b. Bacterial populations and the volatilome associated to meat spoilage. Food Microbiology 45, 83-102.

Case, R.J., Boucher, Y., Dahllöf, I., Holmström, C., Doolittle, W.F., Kjelleberg, S., 2007. Use of 16S rRNA and *rpoB* genes as molecular markers for microbial ecology studies. Applied and Environmental Microbiology 73, 278-88.

Castellano, P., Vignolo, G., 2006. Inhibition of *Listeria innocua* and *Brochothrix thermosphacta* in vacuum-packaged meat by addition of bacteriocinogenic *Lactobacillus curvatus* CRL705 and its bacteriocins. Letters in Applied Microbiology 43, 194-99.

Cavanagh, A.T., Sperger, J.M., Wassarman, K.M., 2012. Regulation of 6S RNA by pRNA synthesis is required for efficient recovery from stationary phase in *E. coli* and *B. subtilis*. Nucleic Acids Research 40, 2234-46.

Cavanagh, A.T., Wassarman, K.M., 2014. 6S RNA, a global regulator of transcription in *Escherichia coli*, *Bacillus subtilis*, and beyond. Annual Review of Microbiology 68, 45-60.

Ceuppens, S., Li, D., Uyttendaele, M., Renault, P., Ross, P., Ranst, M.V., et al., 2014. Molecular methods in food safety microbiology: Interpretation and implications of nucleic acid detection. Comprehensive Reviews in Food Science and Food Safety 13, 551-77.

Chaillou, S., Chaulot-Talmon, A., Caekebeke, H., Cardinal, M., Christieans, S., Denis, C., et al., 2015. Origin and ecological selection of core and food-specific bacterial communities associated with meat and seafood spoilage. The Isme Journal 9, 1105-18.

Chaillou, S., Daty, M., Baraige, F., Dudez, A.-M., Anglade, P., Jones, R., et al., 2009. Intraspecies genomic diversity and natural population structure of the meat-borne lactic acid bacterium *Lactobacillus sakei*. Applied and Environmental Microbiology 75, 970-80.

Chaillou, S., Lucquin, I., Najjari, A., Zagorec, M., Champomier-Vergès, M.-C., 2013. Population genetics of *Lactobacillus sakei* reveals three lineages with distinct evolutionary histories. PloS One 8, e73253.

Cheng, J., Guffanti, A.A., Krulwich, T.A., 1994. The chromosomal tetracycline resistance locus of *Bacillus subtilis* encodes a Na+/H+ antiporter that is physiologically important at elevated pH. Journal of Biological Chemistry 269, 27365-71.

Chouliara, E., Karatapanis, A., Savvaidis, I.N., Kontominas, M.G., 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C. Food Microbiology 24, 607-17.

Chu, Y., Corey, D.R., 2012. RNA sequencing: Platform selection, experimental design, and data interpretation. Nucleic Acid Therapeutics 22, 271-74.

Clements, J.R.S., Darnell, B., 1980. *Myo*-inositol content of common foods: development of a high-*myo*-inositol diet. The American Journal of Clinical Nutrition 33, 1954-67.

Collins-Thompson, D.L., Rodriguez-Lopez, G., 1980. Influence of sodium nitrite, temperature, and lactic acid bacteria on the growth of *Brochothrix thermosphacta* under anaerobic conditions. Canadian Journal of Microbiology 26, 1416-21.

Coppée, J.-Y., Auger, S., Turlin, E., Sekowska, A., Le Caer, J.-P., Labas, V., et al., 2001. Sulfur-limitation-regulated proteins in *Bacillus subtilis*: a two-dimensional gel electrophoresis study. Microbiology 147, 1631-40.

Corry, J.E.L., 2007. Spoilage organisms of red meat and poultry, in: Mead, G.C. (Ed.), Microbiological analysis of red meat, poultry and eggs. Woodhead Publishing Limited, Cambridge, England, pp. 101-22.

Coton, E., Coton, M., 2009. Evidence of horizontal transfer as origin of strain to strain variation of the tyramine production trait in *Lactobacillus brevis*. Food Microbiology 26, 52-57.

Cutter-Nettles, C., Siragusa, G.R., 1994. Decontamination of beef carcass tissue with nisin using a pilot scale model carcass washer. Food Microbiology 11, 481-89.

Cutter-Nettles, C., Siragusa, G.R., 1996a. Reduction of *Brochothrix thermosphacta* on beef surfaces following immobilization of nisin in calcium alginate gels. Letters in Applied Microbiology 23, 9-12.

Cutter-Nettles, C., Siragusa, G.R., 1996b. Reductions of *Listeria innocua* and *Brochothrix thermosphacta* on beef following nisin spray treatments and vacuum packaging. Food Microbiology 13, 23-33.

Cutter-Nettles, C., Siragusa, G.R., 1997. Growth of *Brochothrix thermosphacta* in ground beef following treatments with nisin in calcium alginate gels. Food Microbiology 14, 425-30.

D'Amato, S., Mazzarrino, G., Rossi, C., Serio, A., López, C.C., Celano, G.V., Paparella, A., 2016. *Thymus Vulgaris* (red thyme) and *Caryophyllus aromaticus* (Clove) essential oils to control spoilage microorganisms in pork under modified atmosphere. Italian Journal of Food Safety 5, 127-30.

Dainty, R., Edwards, R., Hibbard, C., 1984. Volatile compounds associated with the aerobic growth of some *Pseudomonas* species on beef. Journal of Applied Microbiology 57, 75-81.

Dainty, R.H., 1996. Chemical/biochemical detection of spoilage. International Journal of Food Microbiology 33, 19-33.

Dainty, R.H., Edwards, R.A., Hibbard, C.M., 1985. Time course of volatile compound formation during refrigerated storage of naturally contaminated beef in air. Journal of Applied Bacteriology 59, 303-09.

Dainty, R.H., Edwards, R.A., Hibbard, C.M., Marnewick, J.J., 1989. Volatile compounds associated with microbial growth on normal and high pH beef stored at chill temperatures. Journal of Applied Bacteriology 66, 281-89.

Dainty, R.H., Hibbard, C.M., 1980. Aerobic metabolism of *Brochothrix thermosphacta* growing on meat surfaces and in laboratory media. Journal of Applied Bacteriology 48, 387-96.

Dainty, R.H., Hibbard, C.M., 1983. Precursors of the major end products of aerobic metabolism of *Brochothrix thermosphacta*. Journal of Applied Bacteriology 55, 127-33.

Dainty, R.H., Mackey, B.M., 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. Journal of Applied Bacteriology 73, 103s-14s.

Dalgaard, P., 1995. Qualitative and quantitative characterization of spoilage bacteria from packed fish. International Journal of Food Microbiology 26, 319-33.

Dalgaard, P., 2000. Fresh and lightly preserved seafood, in: Man, C.D., Jones, A.A. (Eds.), Shelf-life evaluation of foods, 2nd ed. Aspen Publishing Inc, Maryland, USA, pp. 110-39.

Dalgaard, P., 2006. Microbiology of marine muscle foods, Handbook of Food Science: Technology and Engineering. CRC, pp. 1-20.

Dalgaard, P., Gram, L., Huss, H.H., 1993. Spoilage and shelf-life of cod fillets packed in vacuum or modified atmospheres. International Journal of Food Microbiology 19, 283-94.

Dalgaard, P., Mejlholm, O., Christiansen, T.J., Huss, H.H., 1997. Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. Letters in Applied Microbiology 24, 373-78.

Daubin, V., Ochman, H., 2004. Bacterial genomes as new gene homes: The genealogy of ORFans in *E. coli*. Genome Research 14, 1036-42.

De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., et al., 2009. Bergey's manual of systematic bacteriology: The firmicutes. Springer, New York, NY.

Del Papa, M.F., Perego, M., 2008. Ethanolamine activates a sensor histidine kinase regulating its utilization in *Enterococcus faecalis*. Journal of Bacteriology 190, 7147-56.

Delhalle, L., Korsak, N., Taminiau, B., Nezer, C., Burteau, S., Delcenserie, V., et al., 2016. Exploring the bacterial diversity of Belgian steak tartare using metagenetics and quantitative real-time PCR analysis. Journal of Food Protection 79, 220-29.

Denes, T., Vongkamjan, K., Ackermann, H.-W., Moreno Switt, A.I., Wiedmann, M., den Bakker, H.C., 2014. Comparative genomic and morphological analyses of *Listeria* phages isolated from farm environments. Applied and Environmental Microbiology 80, 4616-25.

Dias, F.S., Ramos, C.L., Schwan, R.F., 2013. Characterization of spoilage bacteria in pork sausage by PCR-DGGE analysis. Food Science and Technology (Campinas) 33, 468-74.

Dieckmann, R., Helmuth, R., Erhard, M., Malorny, B., 2008. Rapid classification and identification of *Salmonellae* at the species and subspecies levels by whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry. Applied and Environmental Microbiology 74, 7767-78.

Dischinger, J., Josten, M., Szekat, C., Sahl, H.-G., Bierbaum, G., 2009. Production of the novel two-peptide lantibiotic lichenicidin by *Bacillus licheniformis* DSM 13. PloS One 4, e6788.

Dorscht, J., Klumpp, J., Bielmann, R., Schmelcher, M., Born, Y., Zimmer, M., et al., 2009. Comparative genome analysis of *Listeria* bacteriophages reveals extensive mosaicism, programmed translational frameshifting, and a novel prophage insertion site. Journal of Bacteriology 191, 7206-15.

Doulgeraki, A.I., Ercolini, D., Villani, F., Nychas, G.-J.E., 2012. Spoilage microbiota associated to the storage of raw meat in different conditions. International Journal of Food Microbiology 157, 130-41.

Doulgeraki, A.I., Paramithiotis, S., Kagkli, D.M., Nychas, G.-J.E., 2010. Lactic acid bacteria population dynamics during minced beef storage under aerobic or modified atmosphere packaging conditions. Food Microbiology 27, 1028-34.

Drosinos, E.H., Lambropoulou, K., Mitre, E., Nychas, G.-J.E., 1997. Attributes of fresh gilt-head seabream (*Sparus aurata*) fillets treated with potassium sorbate, sodium gluconate and stored under a modified atmosphere at 0±1°C. Journal of Applied Microbiology 83, 569-75.

Drosinos, E.H., Nychas, G.-J.E., 1996. *Brochothrix thermosphacta*, a dominant microorganism in Mediterranean fresh fish (*Sparus aurata*) stored under modified atmosphere. Italian Journal of Food Science 8, 323-29.

Drosinos, E.H., Nychas, G.J.E., 1997. Production of acetate and lactate in relation to glucose content during modified atmosphere storage of gilt-head seabream (*Sparus aurata*) at 0±1°C. Food Research International 30, 711-17.

Dunn, W.B., Ellis, D.I., 2005. Metabolomics: Current analytical platforms and methodologies. TrAC Trends in Analytical Chemistry 24, 285-94.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460-61.

Edwards, D.J., Holt, K.E., 2013. Beginner's guide to comparative bacterial genome analysis using next-generation sequence data. Microbial Informatics and Experimentation 3, 2-2.

Ellin Doyle, M., 2011. Microbial food spoilage — losses and control strategies a brief review of the literature.

Ercolini, D., Ferrocino, I., Nasi, A., Ndagijimana, M., Vernocchi, P., La Storia, A., et al., 2011. Monitoring of microbial metabolites and bacterial diversity in beef stored under different packaging conditions. Applied and Environmental Microbiology 77, 7372-81.

Ercolini, D., Russo, F., Nasi, A., Ferranti, P., Villani, F., 2009. Mesophilic and psychrotrophic bacteria from meat and their spoilage potential *in vitro* and in beef. Applied and Environmental Microbiology 75, 1990-2001.

Ercolini, D., Russo, F., Torrieri, E., Masi, P., Villani, F., 2006. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Applied and Environmental Microbiology 72, 4663-71.

Everitt, B.S., Landau, S., Leese, M., 2001. Clustering analysis. Arnold, London, UK.

Ewing, B., Hillier, L., Wendl, M.C., Green, P., 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Research 8, 175-85.

Falentin, H., Deutsch, S.-M., Loux, V., Hammani, A., Buratti, J., Parayre, S., et al., 2016. Permanent draft genome sequence of the probiotic strain *Propionibacterium freudenreichii* CIRM-BIA 129 (ITG P20). Standards in Genomic Sciences 11, 6.

Fall, P.A., Leroi, F., Cardinal, M., Chevalier, F., Pilet, M.F., 2010. Inhibition of *Brochothrix thermosphacta* and sensory improvement of tropical peeled cooked shrimp by *Lactococcus piscium* CNCM I-4031. Letters in Applied Microbiology 50, 357-61.

Fall, P.A., Pilet, M.F., Leduc, F., Cardinal, M., Duflos, G., Guérin, C., et al., 2012. Sensory and physicochemical evolution of tropical cooked peeled shrimp inoculated by *Brochothrix thermosphacta* and *Lactococcus piscium* CNCM I-4031 during storage at 8 °C. International Journal of Food Microbiology 152, 82-90.

Fogerty, A.C., Whitfield, F.B., Svoronos, D., Ford, G.L., 1991. The composition of the fatty acids and aldehydes of the ethanolamine and choline phospholipids of various meats. International Journal of Food Science & Technology 26, 363-71.

Fougy, L., Desmonts, M.-H., Coeuret, G., Fassel, C., Hamon, E., Hézard, B., et al., 2016. Reducing salt in raw pork sausages increases spoilage and correlates with reduced bacterial diversity. Applied and Environmental Microbiology 82, 3928-39.

Franke, C., Beauchamp, J., 2017a. Real-time detection of volatiles released during meat spoilage: A case study of modified atmosphere-packaged chicken breast fillets inoculated with *Br. thermosphacta*. Food Analytical Methods 10, 310-19.

Franke, C., Beauchamp, J., 2017b. Real-time detection of volatiles released during meat spoilage: A case study of modified atmosphere-packaged chicken breast fillets inoculated with *Br. thermosphacta*. Food Anal. Method. 10, 310-19.

Fraser-Liggett, C.M., 2005. Insights on biology and evolution from microbial genome sequencing. Genome Research 15, 1603-10.

Frausto da Silva, J.J.R., Williams, R.J.P., 2001. The biological chemistry of the elements: The inorganic chemistry of life, 2nd ed. Clarenden Press, Oxford.

García-Lopez, M., Prieto, M., Otero, A., 1998. The physiological attributes of Gram-negative bacteria associated with spoilage of meat and meat products, in: Davies, A.R., Board, R.J., Board, R.G. (Eds.), The microbiology of meat and poultry. Blackie Academic and Professional, London, p. 1e34.

García, P., Martínez, B., Obeso, J.M., Rodríguez, A., 2008. Bacteriophages and their application in food safety. Letters in Applied Microbiology 47, 479-85.

Gardner, G.A., 1966. A selective medium for the enumeration of *Microbacterium thermosphactum* in meat and meat products. Journal of Applied Bacteriology 29, 455-60.

Gardner, G.A., 1985. Streptomycin-thallous acetate-actidione (STAA) agar: A medium for the selective enumeration of *Brochothrix thermosphacta*. International Journal of Food Microbiology 2, 69-70.

Garot, G., 2015. Lutte contre le gaspillage alimentaire : propositions pour une politique publique Ministère de l'Ecologie, du Développement durable et de l'Energie/Ministre de l'Agriculture, de l'Agroalimentaire et de la Forêt.

Garsin, D.A., 2010. Ethanolamine utilization in bacterial pathogens: Roles and regulation. Nature Reviews Microbiology 8, 290.

Gerner-Smidt, P., Hise, K., Kincaid, J., Hunter, S., Rolando, S., Hyytiä-Trees, E., et al., 2006. PulseNet USA: a five-year update. Foodbourne Pathogens & Disease 3, 9-19.

Gevrekci, A.Ö., 2017. The roles of polyamines in microorganisms. World Journal of Microbiology and Biotechnology 33, 204.

Ghyselinck, J., Van Hoorde, K., Hoste, B., Heylen, K., De Vos, P., 2011. Evaluation of MALDI-TOF MS as a tool for high-throughput dereplication. Journal of Microbiological Methods 86, 327-36.

Gibbons, N., Murray, R., 1978. Proposals concerning the higher taxa of bacteria. International Journal of Systematic and Evolutionary Microbiology 28, 1-6.

Gill, C.O., 1983. Meat spoilage and evaluation of the potential storage life of fresh meat. Journal of Food Protection 46, 444-52.

Gill, C.O., Newton, K.G., 1977. The development of aerobic spoilage flora on meat stored at chill temperatures. Journal of Applied Bacteriology 43, 189-95.

Gill, S.R., Fouts, D.E., Archer, G.L., Mongodin, E.F., DeBoy, R.T., Ravel, J., et al., 2005. Insights on evolution of virulence and resistance from the complete genome analysis of an early

methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. Journal of Bacteriology 187, 2426-38.

Gilmour, M.W., Graham, M., Van Domselaar, G., Tyler, S., Kent, H., Trout-Yakel, K.M., et al., 2010. High-throughput genome sequencing of two *Listeria monocytogenes* clinical isolates during a large foodborne outbreak. BMC Genomics 11, 120.

Ginson, J., Panda, S.K., Bindu, J., Kamalakanth, C.K., Srinivasa Gopal, T.K., 2015. Effect of high pressure treatment on microbiological quality of Indian white prawn (*Fenneropenaeus indicus*) during chilled storage. Food Microbiology 46, 596-603.

Giroux, M., Ouattara, B., Yefsah, R., Smoragiewicz, W., Saucier, L., Lacroix, M., 2001. Combined effect of ascorbic acid and gamma irradiation on microbial and sensorial characteristics of beef patties during refrigerated storage. Journal of Agricultural and Food Chemistry 49, 919-25.

Gram, L., Dalgaard, P., 2002. Fish spoilage bacteria – problems and solutions. Current Opinion in Biotechnology 13, 262-66.

Gram, L., Huss, H.H., 1996. Microbiological spoilage of fish and fish products. International Journal of Food Microbiology 33, 121-37.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., Givskov, M., 2002. Food spoilage—interactions between food spoilage bacteria. International Journal of Food Microbiology 78, 79-97.

Grau, F.H., 1980. Inhibition of the anaerobic growth of *Brochothrix thermosphacta* by lactic acid. Applied and Environmental Microbiology 40, 433-36.

Graves, L.M., Swaminathan, B., 2001. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. International Journal of Food Microbiology 65, 55-62.

Greer, G.G., Dilts, B.D., 1995. Lactic acid inhibition of the growth of spoilage bacteria and cold tolerant pathogens on pork. International Journal of Food Microbiology 25, 141-51.

Greer, G.G., Dilts, B.D., 2002. Control of *Brochothrix thermosphacta* spoilage of pork adipose tissue using bacteriophages. Journal of Food Protection 65, 861-63.

Greer, G.G., Dilts, B.D., 2006. Control of meatborne *Listeria monocytogenes* and *Brochothrix thermosphacta* by a bacteriocinogenic *Brochothrix campestris* ATCC 43754. Food Microbiology 23, 785-90.

Gribble, A., Brightwell, G., 2013. Spoilage characteristics of *Brochothrix thermosphacta* and *campestris* in chilled vacuum packaged lamb, and their detection and identification by real time PCR. Meat Science 94, 361-68.

Gustavsson, J., Cederberg, C., Sonesson, U., van Otterdijk, R., Meybeck, A., 2011. Global food losses and food waste, Study conducted for the International Congress “Save Food!”. Food and Agriculture Organization of the United Nations.

Gutnick, D., Calvo, J.M., Klopotowski, T., Ames, B.N., 1969. Compounds which serve as the sole source of carbon or nitrogen for *Salmonella typhimurium* LT-2. Journal of Bacteriology 100, 215-19.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95-98.

Han, Y., Gao, S., Muegge, K., Zhang, W., Zhou, B., 2015. Advanced applications of RNA sequencing and challenges. Bioinformatics and Biology Insights 9, 29-46.

Han, Y., Xu, X., Jiang, Y., Zhou, G., Sun, X., Xu, B., 2010. Inactivation of food spoilage bacteria by high pressure processing: Evaluation with conventional media and PCR–DGGE analysis. Food Research International 43, 1719-24.

Hayman, M.M., Baxter, I., O'Riordan, P.J., Stewart, C.M., 2004. Effects of high-pressure processing on the safety, quality, and shelf life of ready-to-eat meats. Journal of Food Protection 67, 1709-18.

Heinz, G., 2007. Preservation and processing technologies to improve availability and safety of meat and meat products in developing countries. FAO, Rome, Italy.

Heinz, G., Hautzinger, P., 2007. Meat processing technology for small to medium scale producers. FAO, Bangkok.

Herbert, R., Hendrie, M.S., Gibson, D., Shewan, J., 1971. Bacteria active in the spoilage of certain sea foods. Journal of Applied Microbiology 34, 41-50.

Hille, F., Richter, H., Wong, S.P., Bratovič, M., Ressel, S., Charpentier, E., 2018. The biology of CRISPR-Cas: Backward and forward. Cell 172, 1239-59.

Hitchener, B.J., Egan, A.F., Rogers, P.J., 1979. Energetics of *Microbacterium thermosphactum* in glucose-limited continuous culture. Applied and Environmental Microbiology 37, 1047-52.

Hoffmann-Ostenhof, O., Pittner, F., 1982. The biosynthesis of *myo*-inositol and its isomers. Canadian Journal of Chemistry 60, 1863-71.

Höll, L., Behr, J., Vogel, R.F., 2016. Identification and growth dynamics of meat spoilage microorganisms in modified atmosphere packaged poultry meat by MALDI-TOF MS. Food Microbiology 60, 84-91.

Holley, R.A., 2014. *Brochothrix*, in: Tortorello, M.L. (Ed.), Encyclopedia of Food Microbiology 2nd ed. Academic Press, Oxford, pp. 331-34.

Hood, D.E., Mead, G.C., 1993. Modified atmosphere storage of fresh meat and poultry, in: Parry, R.T. (Ed.), Principles and applications of modified atmosphere packaging of food. Blackie Academic and Professional, London, pp. 269-98.

Hovda, M.B., Sivertsvik, M., Tore Lunestad, B., Lorentzen, G., Rosnes, J.T., 2007. Characterisation of the dominant bacterial population in modified atmosphere packaged farmed halibut (*Hippoglossus hippoglossus*) based on 16S rDNA-DGGE. Food Microbiology 24, 362-71.

Hunt, M., Silva, N.D., Otto, T.D., Parkhill, J., Keane, J.A., Harris, S.R., 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. Genome Biology 16, 294.

Hwanhlem, N., Ivanova, T., Haertlé, T., Jaffrès, E., Dousset, X., 2017. Inhibition of food-spoilage and foodborne pathogenic bacteria by a nisin Z-producing *Lactococcus lactis* subsp. *lactis* KT2W2L. LWT - Food Science and Technology 82, 170-75.

Hyldig, G., Green-Petersen, D.M.B., 2005. Quality index method—an objective tool for determination of sensory quality. Journal of Aquatic Food Product Technology 13, 71-80.

Igarashi, K., Kashiwagi, K., 1999. Polyamine transport in bacteria and yeast. Biochemical Journal 344, 633-42.

Illikoud, N., Jaffrès, E., Zagorec, M., 2018. *Brochothrix thermosphacta*. Reference Module in Life Sciences, 1-17.

Illikoud, N., Klopp, C., Roulet, A., Bouchez, O., Marsaud, N., Jaffrès, E., Zagorec, M., Submitted. One complete and three draft genome sequences of four *Brochothrix thermosphacta* strains, CD 337, TAP 175, BSAS1 3 and EBP 3070. Standards in Genomic Sciences Reference SIGS-D-18-00045.

Illikoud, N., Rossero, A., Chauvet, R., Courcoux, P., Pilet, M.-F., Charrier, T., et al., *in press* Genotypic and phenotypic characterization of the food spoilage bacterium *Brochothrix thermosphacta*. Food Microbiology.

INCOME consulting - AK2C, 2016. Pertes et gaspillages alimentaires : l'état des lieux et leur gestion par étapes de la chaîne alimentaire ADEME, pp. 1-164.

Ishii, S., Sadowsky, M.J., 2009. Applications of the rep-PCR DNA fingerprinting technique to study microbial diversity, ecology and evolution. Environmental Microbiology 11, 733-40.

Jacxsens, L., Devlieghere, F., Van der Steen, C., Debevere, J., 2001. Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. International Journal of Food Microbiology 71, 197-210.

Jaffrès, E., Lalanne, V., Macé, S., Cornet, J., Cardinal, M., Sérot, T., et al., 2011. Sensory characteristics of spoilage and volatile compounds associated with bacteria isolated from cooked and peeled tropical shrimps using SPME–GC–MS analysis. International Journal of Food Microbiology 147, 195-202.

Jaffrès, E., Sohier, D., Leroi, F., Pilet, M.-F., Prévost, H., Joffraud, J.-J., Dousset, X., 2009. Study of the bacterial ecosystem in tropical cooked and peeled shrimps using a polyphasic approach. International Journal of Food Microbiology 131, 20-29.

Joffraud, J.-J., Cardinal, M., Cornet, J., Chasles, J.-S., Léon, S., Gigout, F., Leroi, F., 2006. Effect of bacterial interactions on the spoilage of cold-smoked salmon. International Journal of Food Microbiology 112, 51-61.

Joffraud, J.-J., Leroi, F., Roy, C., Berdagué, J.L., 2001. Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon. International Journal of Food Microbiology 66, 175-84.

Johansson, P., Paulin, L., Säde, E., Salovuori, N., Alatalo, E.R., Björkroth, K.J., Auvinen, P., 2011. Genome Sequence of a Food Spoilage Lactic Acid Bacterium, Leuconostoc gasicomitatum LMG

18811(T), in Association with Specific Spoilage Reactions. Applied and Environmental Microbiology 77, 4344-51.

Jørgensen, L.V., Huss, H.H., Dalgaard, P., 2000. The effect of biogenic amine production by single bacterial cultures and metabiosis on cold-smoked salmon. Journal of Applied Microbiology 89, 920-34.

Kappock, T.J., Ealick, S.E., Stubbe, J., 2000. Modular evolution of the purine biosynthetic pathway. Current Opinion in Chemical Biology 4, 567-72.

Katoh, K., Misawa, K., Kuma, K.-i., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30, 3059-66.

Kern, C.C., Vogel, R.F., Behr, J., 2014. Differentiation of *Lactobacillus brevis* strains using matrix-assisted-laser-desorption-ionization-time-of-flight mass spectrometry with respect to their beer spoilage potential. Food Microbiology 40, 18-24.

Kilcher, S., Loessner, M.J., Klumpp, J., 2010. *Brochothrix thermosphacta* bacteriophages feature heterogeneous and highly mosaic genomes and utilize unique prophage insertion sites. Journal of Bacteriology 192, 5441-53.

Koren, S., Walenz, B.P., Berlin, K., Miller, J.R., Bergman, N.H., Phillippy, A.M., 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Research 27, 722-36.

Koutsoumanis, K., Nychas, G.-J.E., 1999. Chemical and sensory changes associated with microbial flora of Mediterranean boque (*Boops boops*) stored aerobically at 0, 3, 7, and 10°C. Applied and Environmental Microbiology 65, 698-706.

Koutsoumanis, K., Stamatiou, A., Skandamis, P., Nychas, G.-J.E., 2006. Development of a microbial model for the combined effect of temperature and pH on spoilage of ground meat, and validation of the model under dynamic temperature conditions. Applied and Environmental Microbiology 72, 124-34.

Kröger, C., Fuchs, T.M., 2009. Characterization of the *myo*-inositol utilization island of *Salmonella enterica* serovar Typhimurium. Journal of Bacteriology 191, 545-54.

Krzanowski, W.J., 1990. Principles of multivariate analysis. Oxford University Press, USA.

Kummu, M., de Moel, H., Porkka, M., Siebert, S., Varis, O., Ward, P.J., 2012. Lost food, wasted resources: Global food supply chain losses and their impacts on freshwater, cropland, and fertiliser use. Science of the Total Environment 438, 477-89.

La Storia, A., Ferrocino, I., Torrieri, E., Di Monaco, R., Mauriello, G., Villani, F., Ercolini, D., 2012. A combination of modified atmosphere and antimicrobial packaging to extend the shelf-life of beefsteaks stored at chill temperature. International Journal of Food Microbiology 158, 186-94.

Labadie, J., 1999. Consequences of packaging on bacterial growth. Meat is an ecological niche. Meat Science 52, 299-305.

Lambert, A.D., Smith, J.P., Dodds, K.L., 1991. Shelf life extension and microbiological safety of fresh meat — a review. Food Microbiology 8, 267-97.

Laursen, B.G., Leisner, J.J., Dalgaard, P., 2006. *Carnobacterium* species: Effect of metabolic activity and interaction with *Brochothrix thermosphacta* on sensory characteristics of modified atmosphere packed shrimp. Journal of Agricultural and Food Chemistry 54, 3604-11.

Legakis, N.J., Papavassiliou, J.T., Xilinas, M.E., 1976. Inositol as a selective substrate for the growth of *Klebsiellae* and *Serratiae*. Zentralbl Bakteriol Orig A. 235, 453-58.

Lemay, M.-J., Choquette, J., Delaquis, P.J., Gariépy, C., Rodrigue, N., Saucier, L., 2002. Antimicrobial effect of natural preservatives in a cooked and acidified chicken meat model. International Journal of Food Microbiology 78, 217-26.

Leroi, F., Cornet, J., Chevalier, F., Cardinal, M., Coeuret, G., Chaillou, S., Joffraud, J.-J., 2015. Selection of bioprotective cultures for preventing cold-smoked salmon spoilage. International Journal of Food Microbiology 213, 79-87.

Leroi, F., Fall, P.A., Pilet, M.F., Chevalier, F., Baron, R., 2012. Influence of temperature, pH and NaCl concentration on the maximal growth rate of *Brochothrix thermosphacta* and a bioprotective bacteria *Lactococcus piscium* CNCM I-4031. Food Microbiology 31, 222-28.

Leroi, F., Joffraud, J.-J., Chevalier, F., Cardinal, M., 1998. Study of the microbial ecology of cold-smoked salmon during storage at 8°C. International Journal of Food Microbiology 39, 111-21.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al., 2009a. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078-79.

Li, W., Raoult, D., Fournier, P.-E., 2009b. Bacterial strain typing in the genomic era. FEMS Microbiology Reviews 33, 892-916.

Liang, R., Yu, X., Wang, R., Luo, X., Mao, Y., Zhu, L., Zhang, Y., 2012. Bacterial diversity and spoilage-related microbiota associated with freshly prepared chicken products under aerobic conditions at 4°C. Journal of Food Protection 75, 1057-62.

Lilyblade, A.L., Peterson, D.W., 1962. Inositol and free sugars in chicken muscle post-mortem. Journal of Food Science 27, 245-49.

Liu, S., Graham, J.E., Bigelow, L., Morse, P.D., Wilkinson, B.J., 2002. Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature. Applied and Environmental Microbiology 68, 1697-705.

Lopez-Galvez, D., de la Hoz, L., Ordonez, J.A., 1995. Effect of carbon dioxide and oxygen enriched atmospheres on microbiological and chemical changes in refrigerated tuna (*Thunnus alalunga*) steaks. Journal of Agricultural and Food Chemistry 43, 483-90.

Lucquin, I., Zagorec, M., Champomier-Vergès, M., Chaillou, S., 2012. Fingerprint of lactic acid bacteria population in beef carpaccio is influenced by storage process and seasonal changes. Food Microbiology 29, 187-96.

Ludwig, W., Schleifer, K.-H., Stackebrandt, E., 1984. 16S rRNA analysis of *Listeria monocytogenes* and *Brochothrix thermosphacta*. FEMS Microbiology Letters 25, 199-204.

Ludwig, W., Schleifer, K.-H., Whitman, W., 2009a. Class I. *Bacilli* class nov., in: De Vos, P., Garrity, G., Jones, D., Krieg, N.-R., Ludwig, W., Rainey, F.-A., Schleifer, K.-H., Whitman, W. (Eds.), Bergey's Manual of Systematic Bacteriology., 2nd ed. Spinger, New York, pp. 19-20.

Ludwig, W., Schleifer, K.-H., Whitman, W., 2009b. Order II. *Lactobacillales* ord. nov., in: De Vos, P., Garrity, G., Jones, D., Krieg, N.-R., Ludwig, W., Rainey, F.-A., Schleifer, K.-H., Whitman, W. (Eds.), Bergey's Manual of Systematic Bacteriology., 2nd ed. Springer, New York, p. 464.

Ludwig, W., Schleifer, K.-H., Whitman, W.B., 2009c. "*Listeriaceae*" fam. nov, in: De Vos, P., Garrity, G., Jones, D., Krieg, N.-R., Ludwig, W., Rainey, F.-A., Schleifer, K.-H., Whitman, W. (Eds.), Bergey's Manual of Systematic Bacteriology, 2nd ed. Springer, New York, pp. 244-57.

Lukinmaa, S., Nakari, U.-M., Eklund, M., Siitonen, A., 2004. Application of molecular genetic methods in diagnostics and epidemiology of food-borne bacterial pathogens. APMIS 112, 908-29.

Macaskie, L.E., Sheard, A.G., Dainty, R.H., Henderson, P.J.F., 1984. Glycerol utilization by *Brochothrix thermosphacta*. Journal of Applied Bacteriology 56, 137-43.

Macé, S., Cornet, J., Chevalier, F., Cardinal, M., Pilet, M.-F., Dousset, X., Joffraud, J.-J., 2012. Characterisation of the spoilage microbiota in raw salmon (Salmo salar) steaks stored under vacuum or modified atmosphere packaging combining conventional methods and PCR–TTGE. Food Microbiology 30, 164-72.

Macé, S., Joffraud, J.-J., Cardinal, M., Malcheva, M., Cornet, J., Lalanne, V., et al., 2013. Evaluation of the spoilage potential of bacteria isolated from spoiled raw salmon (Salmo salar) fillets stored under modified atmosphere packaging. International Journal of Food Microbiology 160, 227-38.

Mamlouk, K., Macé, S., Guilbaud, M., Jaffrès, E., Ferchichi, M., Prévost, H., et al., 2012. Quantification of viable *Brochothrix thermosphacta* in cooked shrimp and salmon by real-time PCR. Food Microbiology 30, 173-79.

Marxen, J.C., Pick, C., Kwiatkowski, M., Burmester, T., 2013. Molecular characterization and evolution of haemocyanin from the two freshwater shrimps *Caridina multidentata* (Stimpson, 1860) and *Atyopsis moluccensis* (De Haan, 1849). Journal of Comparative Physiology B 183, 613-24.

Mastromatteo, M., Danza, A., Conte, A., Muratore, G., Del Nobile, M.A., 2010. Shelf life of ready to use peeled shrimps as affected by thymol essential oil and modified atmosphere packaging. International Journal of Food Microbiology 144, 250-56.

McCormick, J.K., Poon, A., Sailer, M., Gao, Y., Roy, K.L., McMullen, L.M., et al., 1998. Genetic characterization and heterologous expression of brochocin-C, an antibotulinal, two-peptide bacteriocin produced by *Brochothrix campestris* ATCC 43754. Applied and Environmental Microbiology 64, 4757-66.

McDonald, K., Sun, D.-W., 1999. Predictive food microbiology for the meat industry: A review. International Journal of Food Microbiology 52, 1-27.

McLean, R.A., Sulzbacher, W.L., 1953. *Microbacterium thermosphactum*, spec nov; a nonheat resistant bacterium from fresh pork sausage. Journal of Bacteriology 65, 428-33.

McMillin, K.W., 2008. Where is MAP Going? A review and future potential of modified atmosphere packaging for meat. Meat Science 80, 43-65.

Médigue, C., Calteau, A., Cruveiller, S., Gachet, M., Gautreau, G., Josso, A., et al., 2017. MicroScope—an integrated resource for community expertise of gene functions and comparative analysis of microbial genomic and metabolic data. Briefings in Bioinformatics, bbx113-bbx13.

Medini, D., Donati, C., Tettelin, H., Masignani, V., Rappuoli, R., 2005. The microbial pan-genome. Current Opinion in Genetics and Development 15, 589-94.

Mejlholm, O., Bøknæs, N., Dalgaard, P., 2005. Shelf life and safety aspects of chilled cooked and peeled shrimps (*Pandalus borealis*) in modified atmosphere packaging. Journal of Applied Microbiology 99, 66-76.

Mignard, S., Flandrois, J.P., 2006. 16S rRNA sequencing in routine bacterial identification: A 30-month experiment. Journal of Microbiological Methods 67, 574-81.

Mikš-Krajnik, M., Yoon, Y.-J., Ukuku, D.O., Yuk, H.-G., 2016. Volatile chemical spoilage indexes of raw Atlantic salmon (*Salmo salar*) stored under aerobic condition in relation to microbiological and sensory shelf lives. Food Microbiology 53, 182-91.

Miller, A., Scanlan, R.A., Lee, J.S., Libbey, L.M., 1973. Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas putrefaciens*, *Pseudomonas fluorescens*, and an *Achromobacter* species. Applied Microbiology 26, 18-21.

Mogk, A., Homuth, G., Scholz, C., Kim, L., Schmid, F.X., Schumann, W., 1997. The GroE chaperonin machine is a major modulator of the CIRCE heat shock regulon of *Bacillus subtilis*. The EMBO Journal 16, 4579-90.

Mollet, C., Drancourt, M., Raoult, D., 1997. *rpoB* sequence analysis as a novel basis for bacterial identification. Molecular Microbiology 26, 1005-11.

Nagorska, K., Ostrowski, A., Hinc, K., Holland, I., Obuchowski, M., 2010. Importance of *eps* genes from *Bacillus subtilis* in biofilm formation and swarming. Journal of applied genetics 51, 369-81.

Nicholson, W.L., 2008. The *Bacillus subtilis ydjL (bdhA)* gene encodes acetoin reductase/2,3-butanediol dehydrogenase. Applied and Environmental Microbiology 74, 6832-38.

Niemand, J.G., Linde, H.J.V.D., Holzapfel, W.H., 1983. Shelf-life extension of minced beef through combined treatments involving radurization. Journal of Food Protection 46, 791-96.

Nieminen, T.T., Koskinen, K., Laine, P., Hultman, J., Säde, E., Paulin, L., et al., 2012. Comparison of microbial communities in marinated and unmarinated broiler meat by metagenomics. International Journal of Food Microbiology 157, 142-49.

Nieminen, T.T., Vihavainen, E., Paloranta, A., Lehto, J., Paulin, L., Auvinen, P., et al., 2011. Characterization of psychrotrophic bacterial communities in modified atmosphere-packed meat with terminal restriction fragment length polymorphism. International Journal of Food Microbiology 144, 360-66.

Ning, Z., Cox, A.J., Mullikin, J.C., 2001. SSAHA: A fast search method for large DNA databases. Genome Research 11, 1725-29.

Noseda, B., Islam, M.T., Eriksson, M., Heyndrickx, M., De Reu, K., Van Langenhove, H., Devlieghere, F., 2012. Microbiological spoilage of vacuum and modified atmosphere packaged Vietnamese *Pangasius hypophthalmus* fillets. Food Microbiology 30, 408-19.

Nowak, A., Czyzowska, A., 2011. *In vitro* synthesis of biogenic amines by *Brochothrix thermosphacta* isolates from meat and meat products and the influence of other microorganisms. Meat Science 88, 571-74.

Nowak, A., Kalemba, D., Krala, L., Piotrowska, M., Czyzowska, A., 2012a. The effects of thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) essential oils on *Brochothrix thermosphacta* and on the shelf life of beef packaged in high-oxygen modified atmosphere. Food Microbiology 32, 212-16.

Nowak, A., Piotrowska, M., 2012. Biochemical activities of *Brochothrix thermosphacta*. Meat Science 90, 410-13.

Nowak, A., Rygala, A., Oltuszak-Walczak, E., Walczak, P., 2012b. The prevalence and some metabolic traits of *Brochothrix thermosphacta* in meat and meat products packaged in different ways. Journal of the Science of Food and Agriculture 92, 1304-10.

Nychas, G.-J.E., Drosinos, E.H., Board, R.G., 1998. Chemecal changes in stored meat, in: Davies, A., Board, R. (Eds.), The microbiology of meat and poultry, 1st ed. Blackie Academic & Professional, London, United Kingdom, pp. 288-326.

Nychas, G.-J.E., Marshall, D.L., Sofos, J.N., 2007. Meat, poultry, and seafood, in: Doyle, M.P., Beuchat, L.R., Montville, T.J. (Eds.), Food Microbiology: Fundamentals and Frontiers, 3rd ed. ASM Press, Whashington, D.C., pp. 105-40.

Nychas, G.-J.E., Skandamis, P.N., 2005. Fresh meat spoilage andmodified atmosphere packaging (MAP), in: Sofos, J.N. (Ed.), Improving the Safety of Fresh Meat. CRC/Woodhead Publishing Limited Cambridge, UK, pp. 461–502.

Nychas, G.-J.E., Skandamis, P.N., Tassou, C.C., Koutsoumanis, K.P., 2008a. Meat spoilage during distribution. Meat Sci. 78, 77-89.

Nychas, G.-J.E., Skandamis, P.N., Tassou, C.C., Koutsoumanis, K.P., 2008b. Meat spoilage during distribution. Meat Science 78, 77-89.

OCDE/FAO, 2017. Perspectives agricoles de l'OCDE et de la FAO 2017-2026, Éditions OCDE, Paris. http://dx.doi.org/10.1787/agr_outlook-2017-fr.

Odeyemi, O.A., Burke, C.M., Bolch, C.C.J., Stanley, R., 2018. Seafood spoilage microbiota and associated volatile organic compounds at different storage temperatures and packaging conditions. International Journal of Food Microbiology.

Ojima-Kato, T., Yamamoto, N., Takahashi, H., Tamura, H., 2016. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) can precisely discriminate the lineages of listeria monocytogenes and species of *Listeria*. PloS One 11, e0159730.

Olaoye, O.A., Onilude, A.A., Ubbor, S.C., 2015. Control of *Brochothrix thermosphacta* in pork meat using *Lactococcus lactis* subsp. *lactis* I23 isolated from beef. Applied Food Biotechnology 2, 7.

Olmedo, P., Hernández, A.F., Pla, A., Femia, P., Navas-Acien, A., Gil, F., 2013. Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples. Risk and nutritional assessment and mercury–selenium balance. Food and Chemical Toxicology 62, 299-307.

Ordonez, J.A., De Pablo, B., Perez de Castro, B., Asensio, M.A., Sanz, B., 1991. Selected chemical and microbiological changes in refrigerated pork stored in carbon dioxide and oxygen enriched atmospheres. Journal of Agricultural and Food Chemistry 39, 668-72.

Ouattara, B., Giroux, M., Smoragiewicz, W., Saucier, L., Lacroix, M., 2002. Combined effect of gamma irradiation, ascorbic acid, and edible coating on the improvement of microbial and biochemical characteristics of ground beef. Journal of Food Protection 65, 981-87.

Ouoba, L.I.I., Parkouda, C., Diawara, B., Scotti, C., Varnam, A.H., 2008. Identification of *Bacillus* spp. from Bikalga, fermented seeds of *Hibiscus sabdariffa*: phenotypic and genotypic characterization. Journal of Applied Microbiology 104, 122-31.

Paleologos, E.K., Savvaidis, I.N., Kontominas, M.G., 2004. Biogenic amines formation and its relation to microbiological and sensory attributes in ice-stored whole, gutted and filleted Mediterranean sea bass (*Dicentrarchus labrax*). Food Microbiology 21, 549-57.

Paoli, G.C., Wijey, C., Nguyen, L.-H., Chen, C.-Y., Yan, X., Irwin, P.L., 2017. Complete genome sequences of two strains of the meat spoilage bacterium *Brochothrix thermosphacta* isolated from ground chicken. Genome Announcements 5.

Papadimitriou, K., Alegría, Á., Bron, P.A., de Angelis, M., Gobbetti, M., Kleerebezem, M., et al., 2016. Stress physiology of lactic acid bacteria. Microbiology and Molecular Biology Reviews 80, 837-90.

Papadopoulou, O.S., Doulgeraki, A.I., Botta, C., Cocolin, L., Nychas, G.-J.E., 2012a. Genotypic characterization of *Brochothrix thermosphacta* isolated during storage of minced pork under aerobic or modified atmosphere packaging conditions. Meat Science 92, 735-38.

Papadopoulou, O.S., Doulgeraki, A.I., Botta, C., Cocolin, L., Nychas, G.-J.E., 2012b. Genotypic characterization of *Brochothrix thermosphacta* isolated during storage of minced pork under aerobic or modified atmosphere packaging conditions. Meat Sci. 92, 735-38.

Papon, M., Talon, R., 1988. Factors affecting growth and lipase production by meat *lactobacilli* strains and *Brochothrix thermosphacta*. Journal of Applied Bacteriology 64, 107-15.

Parlapani, F.F., Mallouchos, A., Haroutounian, S.A., Boziaris, I.S., 2014. Microbiological spoilage and investigation of volatile profile during storage of sea bream fillets under various conditions. International Journal of Food Microbiology 189, 153-63.

Parlapani, F.F., Meziti, A., Kormas, K.A., Boziaris, I.S., 2013. Indigenous and spoilage microbiota of farmed sea bream stored in ice identified by phenotypic and 16S rRNA gene analysis. Food Microbiology 33, 85-89.

Pennacchia, C., Ercolini, D., Villani, F., 2009. Development of a real-time PCR assay for the specific detection of *Brochothrix thermosphacta* in fresh and spoiled raw meat. International Journal of Food Microbiology 134, 230-36.

Pennacchia, C., Ercolini, D., Villani, F., 2011. Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. Food Microbiology 28, 84-93.

Petruzzi, L., Corbo, M.R., Sinigaglia, M., Bevilacqua, A., 2017. Chapter 1 - Microbial spoilage of foods: Fundamentals, in: Bevilacqua, A., Corbo, M.R., Sinigaglia , M. (Eds.), The Microbiological Quality of Food. Woodhead Publishing, pp. 1-21.

Pilet, M.-F., Dousset, X., Barre, R., Novel, G., Desmazeaud, M., Piard, J.-C., 1995. Evidence for two bacteriocins produced by *Carnobacterium piscicola* and *Carnobacterium divergens* isolated from fish and active against *Listeria monocytogenes*. Journal of Food Protection 58, 256-62.

Pin, C., García de Fernando, G.D., Ordóñez, J.A., 2002. Effect of modified atmosphere composition on the metabolism of glucose by *Brochothrix thermosphacta*. Applied and Environmental Microbiology 68, 4441-47.

Piotrowska-Cyplik, A., Myszka, K., Czarny, J., Ratajczak, K., Kowalski, R., Biegańska-Marecik, R., et al., 2017. Characterization of specific spoilage organisms (SSOs) in vacuum-packed ham by culture-plating techniques and MiSeq next-generation sequencing technologies. Journal of the Science of Food and Agriculture 97, 659-68.

Pitts, A.C., Tuck, L.R., Faulds-Pain, A., Lewis, R.J., Marles-Wright, J., 2012. Structural insight into the *Clostridium difficile* ethanolamine utilisation microcompartment. PloS One 7, e48360.

Pothakos, V., Devlieghere, F., Villani, F., Björkroth, J., Ercolini, D., 2015. Lactic acid bacteria and their controversial role in fresh meat spoilage. Meat Science 109, 66-74.

Pothakos, V., Nyambi, C., Zhang, B.-Y., Papastergiadis, A., De Meulenaer, B., Devlieghere, F., 2014. Spoilage potential of psychrotrophic lactic acid bacteria (LAB) species: *Leuconostoc gelidum* subsp. *gasicomitatum* and *Lactococcus piscium*, on sweet bell pepper (SBP) simulation medium under different gas compositions. International Journal of Food Microbiology 178, 120-29.

Radford, D.S., Kihlken, M.A., Borrelly, G.P.M., Harwood, C.R., Brun, N.E., Cavet, J.S., 2003. CopZ from *Bacillus subtilis* interacts *in vivo* with a copper exporting CPx-type ATPase CopA. FEMS Microbiology Letters 220, 105-12.

Rahi, P., Prakash, O., Shouche, Y.S., 2016. Matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI-TOF MS) based microbial identifications: challenges and scopes for microbial ecologists. Frontiers in Microbiology 7.

Rahkila, R., Nieminen, T., Johansson, P., Säde, E., Björkroth, J., 2012. Characterization and evaluation of the spoilage potential of *Lactococcus piscium* isolates from modified atmosphere packaged meat. International Journal of Food Microbiology 156, 50-59.

Rantsiou, K., Greppi, A., Garosi, M., Acquadro, A., Mataragas, M., Cocolin, L., 2012. Strain dependent expression of stress response and virulence genes of *Listeria monocytogenes* in meat juices as determined by microarray. International Journal of Food Microbiology 152, 116-22.

Rattanasomboon, N., Bellara, S.R., Harding, C.L., Fryer, P.J., Thomas, C.R., Al-Rubeai, M., McFarlane, C.M., 1999. Growth and enumeration of the meat spoilage bacterium *Brochothrix thermosphacta*. International Journal of Food Microbiology 51, 145-58.

Refsgaard, H.H.F., Haahr, A.-M., Jensen, B., 1999. Isolation and quantification of volatiles in fish by dynamic headspace sampling and mass spectrometry. Journal of Agricultural and Food Chemistry 47, 1114-18.

Règlement (CE) n° 1441/2007 de la Commission du 5 décembre 2007 modifiant le règlement (CE) n° 2073/2005 concernant les critères microbiologiques applicables aux denrées alimentaires. Journal officiel de l'Union européenne du 07/12/2007

Remenant, B., Jaffrès, E., Dousset, X., Pilet, M.-F., Zagorec, M., 2015. Bacterial spoilers of food: Behavior, fitness and functional properties. Food Microbiology 45, 45-53.

Ritchie, H., Roser, M., 2018. Meat and Seafood Production & Consumption. Published online at OurWorldInData.org. Retrieved from: 'https://ourworldindata.org/meat-and-seafood-production-consumption' [Online Resource].

Rose, N.L., Sporns, P., Stiles, M.E., McMullen, L.M., 1999. Inactivation of nisin by glutathione in fresh meat. Journal of Food Science 64, 759-62.

Rouger, A., Remenant, B., Prévost, H., Zagorec, M., 2017. A method to isolate bacterial communities and characterize ecosystems from food products: Validation and utilization in as a reproducible chicken meat model. International Journal of Food Microbiology 247, 38-47.

Russell, S.M., Fletcher, D.L., Cox, N.A., 1995. Spoilage bacteria of fresh broiler chicken carcasses. Poultry Science 74, 2041-47.

Russo, F., Ercolini, D., Mauriello, G., Villani, F., 2006. Behaviour of *Brochothrix thermosphacta* in presence of other meat spoilage microbial groups. Food Microbiology 23, 797-802.

Sakala, R.M., Hayashidani, H., Kato, Y., Hirata, T., Makino, Y., Fukushima, A., et al., 2002. Change in the composition of the microflora on vacuum-packaged beef during chiller storage. International Journal of Food Microbiology 74, 87-99.

Salzer, R., Kern, T., Joos, F., Averhoff, B., 2016. The *Thermus thermophilus comEA/comEC o*peron is associated with DNA binding and regulation of the DNA translocator and type IV pili. Environmental Microbiology 18, 65-74.

Samelis, J., Kakouri, A., Georgiadou, K.G., Metaxopoulos, J., 1998. Evaluation of the extent and type of bacterial contamination at different stages of processing of cooked ham. Journal of Applied Microbiology 84, 649-60.

Samelis, J., Kakouri, A., Rementzis, J., 2000a. Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4°C. Food Microbiology 17, 329-40.

Samelis, J., Kakouri, A., Rementzis, J., 2000b. The spoilage microflora of cured, cooked turkey breasts prepared commercially with or without smoking. International Journal of Food Microbiology 56, 133-43.

Santiago, E.F., Richard, L.E., 2003. Evolution experiments with microorganisms: The dynamics and genetic bases of adaptation. Nature Reviews Genetics 4, 457.

Saraoui, T., Leroi, F., Björkroth, J., Pilet, M.F., 2016. *Lactococcus piscium*: a psychrotrophic lactic acid bacterium with bioprotective or spoilage activity in food—a review. Journal of Applied Microbiology 121, 907-18.

Scheu, P.M., Berghof, K., Stahl, U., 1998. Detection of pathogenic and spoilage micro-organisms in food with the polymerase chain reaction. Food Microbiology 15, 13-31.

Seiler, N., 1999. A Guide to the Polyamines, in: Cohen, S.S. (Ed.), The Quarterly Review of Biology, pp. 342-43.

Shah, P., Nanduri, B., Swiatlo, E., Ma, Y., Pendarvis, K., 2011. Polyamine biosynthesis and transport mechanisms are crucial for fitness and pathogenesis of *Streptococcus pneumoniae*. Microbiology 157, 504-15.

Shah, P., Swiatlo, E., 2008. A multifaceted role for polyamines in bacterial pathogens. Molecular Microbiology 68, 4-16.

Sharma, C.M., Vogel, J., 2014. Differential RNA-seq: The approach behind and the biological insight gained. Current Opinion in Microbiology 19, 97-105.

Siegrist, T.J., Anderson, P.D., Huen, W.H., Kleinheinz, G.T., McDermott, C.M., Sandrin, T.R., 2007. Discrimination and characterization of environmental strains of *Escherichia coli* by atrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Journal of Microbiological Methods 68, 554-62.

Singhal, N., Kumar, M., Kanaujia, P.K., Virdi, J.S., 2015. MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. Frontiers in Microbiology 6, 791.

Siragusa, G.R., Cutter-Nettles, C., 1993. Brochocin-C, a new bacteriocin produced by *Brochothrix campestris*. Applied and Environmental Microbiology 59, 2326-28.

Skandamis, P.N., Nychas, G.-J.E., 2002. Preservation of fresh meat with active and modified atmosphere packaging conditions. International Journal of Food Microbiology 79, 35-45.

Smit, B.A., Engels, W.J.M., Wouters, J.T.M., Smit, G., 2004. Diversity of l-leucine catabolism in various microorganisms involved in dairy fermentations, and identification of the rate-controlling step in the formation of the potent flavour component 3-methylbutanal. Applied Microbiology and Biotechnology 64, 396-402.

Smith, T.F., Waterman, M.S., 1981. Identification of common molecular subsequences. Journal of Molecular Biology 147, 195-97.

Sneath, P.H.A., 2009. Genus II. *Brochothrix* nov., in: De Vos, P., Garrity, G., Jones, D., Krieg, N.-R., Ludwig, W., Rainey, F.-A., Schleifer, K.-H., Whitman, W. (Eds.), Bergey's Manual of Systematic Bacteriology, 2nd ed. Springer, New York, pp. 257-61.

Sneath, P.H.A., Jones, D., 1976. *Brochothrix*, a new genus tentatively placed in the family *Lactobacillaceae*. International Journal of Systematic and Evolutionary Microbiology 26, 102-04.

Sneath, P.H.A., Jones, D., 1986. Genus *Brochothrix*, in: Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holt, J.G. (Eds.), Bergey's manual of systematic bacteriology, 1st Vol2 ed. The Williams & Wilkins Co., Baltimore, pp. 1249-53.

Snipen, L., Ussery, D.W., 2010. Standard operating procedure for computing pangenome trees. Standards in Genomic Sciences 2, 135-41.

Stackebrandt, E., Jones, D., 2006. The genus *Brochothrix*, in: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (Eds.), The prokaryotes, 3rd ed. Springer US, New York, NY, pp. 477-91.

Stanborough, T., Fegan, N., Powell, S.M., Tamplin, M., Chandry, P.S., 2017. Insight into the genome of *Brochothrix thermosphacta*, a problematic meat spoilage bacterium. Applied and Environmental Microbiology 83, e02786-16.

Stanbridge, L.H., Davis, A.R., 1998. The microbiology of chill-stored meat, in: ed. (Ed.), The microbiology of meat and poultry. Blackie Academic and Professional, London, U.K, pp. 174-219.

Stanley, G., Shaw, K.J., Egan, A.F., 1981. Volatile compounds associated with spoilage of vacuum-packaged sliced luncheon meat by *Brochothrix thermosphacta*. Applied and Environmental Microbiology 41, 816-18.

Stiles, M.E., 1996. Biopreservation by lactic acid bacteria. Antonie van Leeuwenhoek 70, 331-45.

Stohr, V., Joffraud, J.-J., Cardinal, M., Leroi, F., 2001. Spoilage potential and sensory profile associated with bacteria isolated from cold-smoked salmon. Food Research International 34, 797-806.

Stout, E., Klaenhammer, T., Barrangou, R., 2017. CRISPR-Cas technologies and applications in food bacteria. Annual Review of Food Science and Technology 8, 413-37.

Sulzbacher, W.L., McLean, R.A., 1951. The bacterial flora of fresh pork sausage. Food Technology 5, 7-8.

Talon, R., Grimont, P.A.D., Grimont, F., Gasser, F., Boeufgras, J.M., 1988. *Brochothrix campestris* sp. nov. International Journal of Systematic and Evolutionary Microbiology 38, 99-102.

Tanigawa, K., Kawabata, H., Watanabe, K., 2010. Identification and typing of *Lactococcus lactis* by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Applied and Environmental Microbiology 76, 4055-62.

Tettelin, H., Riley, D., Cattuto, C., Medini, D., 2008. Comparative genomics: the bacterial pan-genome. Current Opinion in Microbiology 11, 472-77.

Theron, M.M., Lues, J.F.R., 2007. Organic acids and meat preservation: A review. Food Reviews International 23, 141-58.

Thomson, I.Q., Collins-Thompson, D.L., 1986. Iron requirement of *Brochothrix thermosphacta*. International Journal of Food Microbiology 3, 299-309.

Tiwari, B.K., Valdramidis, V.P., O' Donnell, C.P., Muthukumarappan, K., Bourke, P., Cullen, P.J., 2009. Application of natural antimicrobials for food preservation. Journal of Agricultural and Food Chemistry 57, 5987-6000.

Trevors, J., Cotter, C., 1990. Copper toxicity and uptake in microorganisms. Journal of Industrial Microbiology 6, 77-84.

Tsoy, O., Ravcheev, D., Mushegian, A., 2009. Comparative genomics of ethanolamine utilization. Journal of Bacteriology 191, 7157-64.

Tsugawa, H., Tsujimoto, Y., Arita, M., Bamba, T., Fukusaki, E., 2011. GC/MS based metabolomics: development of a data mining system for metabolite identification by using soft independent modeling of class analogy (SIMCA). BMC Bioinformatics 12, 131.

Tu, L., Mustapha, A., 2002. Reduction of *Brochothrix thermosphacta* and *Salmonella* serotype *Typhimurium* on vacuum-packaged fresh beef treated with nisin and nisin combined with EDTA. Journal of Food Science 67, 302-06.

Vallenet, D., Belda, E., Calteau, A., Cruveiller, S., Engelen, S., Lajus, A., et al., 2013. MicroScope—an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. Nucleic Acids Research 41, D636-D47.

Vallenet, D., Calteau, A., Cruveiller, S., Gachet, M., Lajus, A., Josso, A., et al., 2016. MicroScope in 2017: an expanding and evolving integrated resource for community expertise of microbial genomes. Nucleic Acids Research.

Van Der Vossen, J.M.B.M., Hofstra, H., 1996. DNA based typing, identification and detection systems for food spoilage microorganisms: Development and implementation. International Journal of Food Microbiology 33, 35-49.

Vasilopoulos, C., Ravyts, F., De Maere, H., De Mey, E., Paelinck, H., De Vuyst, L., Leroy, F., 2008. Evaluation of the spoilage lactic acid bacteria in modified-atmosphere-packaged artisan-type cooked ham using culture-dependent and culture-independent approaches. Journal of Applied Microbiology 104, 1341-53.

Vermeiren, L., Devlieghere, F., De Graef, V., Debevere, J., 2005. *In vitro* and *in situ* growth characteristics and behaviour of spoilage organisms associated with anaerobically stored cooked meat products. Journal of Applied Microbiology 98, 33-42.

Vihavainen, E.J., Björkroth, K.J., 2009. Diversity of *Leuconostoc gasicomitatum* associated with meat spoilage. International Journal of Food Microbiology 136, 32-36.

Wang, F., Fu, L., Bao, X., Wang, Y., 2017a. The spoilage microorganisms in seafood with the existed quorum sensing phenomenon. Journal of Food Microbiology 1, 14-19.

Wang, G.-y., Wang, H.-h., Han, Y.-w., Xing, T., Ye, K.-p., Xu, X.-l., Zhou, G.-h., 2017b. Evaluation of the spoilage potential of bacteria isolated from chilled chicken *in vitro* and *in situ*. Food Microbiology 63, 139-46.

Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-seq: A revolutionary tool for transcriptomics. Nature Reviews Genetics 10, 57.

Westerfeld, W.W., 1945. A Colorimetric determination of blood acetoin Journal of Biological Chemistry 161, 495-502.

Wiedenbeck, J., Cohan, F.M., 2011. Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. FEMS Microbiology Reviews 35, 957-76.

Williams, P., 2007. Nutritional composition of red meat. Nutrition & Dietetics 64.

Woese, C.R., Kandler, O., Wheelis, M.L., 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proceedings of the National Academy of Sciences 87, 4576-79.

Wolska, K., Szweda, P., 2012. Genotyping techniques for determining the diversity of microorganisms, Genetic Diversity in Microorganisms. InTech.

Wortham, B.W., Oliveira, M.A., Patel, C.N., 2007. Polyamines in bacteria: Pleiotropic effects yet specific mechanisms, in: Perry, R.D., Fetherston, J.D. (Eds.), The Genus Yersinia: From Genomics to Function. Springer New York, New York, NY, pp. 106-15.

Xu, Y., Anyogu, A., Ouoba, L.I., Sutherland, J.P., 2010. Genotypic characterization of *Brochothrix* spp. isolated from meat, poultry and fish. Letters in Applied Microbiology 51, 245-51.

Yao, X., Lu, C.-D., 2014. Functional characterization of the *potRABCD* operon for spermine and spermidine uptake and regulation in *Staphylococcus aureus*. Current Microbiology 69, 75-81.

Yendrek, C.R., Ainsworth, E.A., Thimmapuram, J., 2012. The bench scientist's guide to statistical analysis of RNA-Seq data. BMC Research Notes 5, 506.

Yoshida, K.-I., Shibayama, T., Aoyama, D., Fujita, Y., 1999. Interaction of a repressor and its binding sites for regulation of the *Bacillus subtilis* iol divergon. Journal of Molecular Biology 285, 917-29.

Yoshida, K.-i., Yamaguchi, M., Ikeda, H., Omae, K., Tsurusaki, K.-i., Fujita, Y., 2004. The fifth gene of the *iol* operon of *Bacillus subtilis*, *iolE*, encodes 2-keto-myo-inositol dehydratase. Microbiology 150, 571-80.

Yoshida, K.-I., Yamamoto, Y., Omae, K., Yamamoto, M., Fujita, Y., 2002. Identification of two *myo*-inositol transporter genes of *Bacillus subtilis*. Journal of Bacteriology 184, 983-91.

Yoshida, K.I., Aoyama, D., Ishio, I., Shibayama, T., Fujita, Y., 1997. Organization and transcription of the *myo*-inositol operon, *iol,* of *Bacillus subtilis*. Journal of Bacteriology 179, 4591-8.

Zhang, H., Lountos, G., Bun Ching, C., Jiang, R., 2010. Engineering of glycerol dehydrogenase for improved activity towards 1, 3-butanediol.

Zhang, Y., Morar, M., Ealick, S.E., 2008. Structural biology of the purine biosynthetic pathway. Cellular and Molecular Life Sciences 65, 3699-724.

Zhou, Y., Liang, Y., Lynch, K.H., Dennis, J.J., Wishart, D.S., 2011. PHAST: A fast phage search tool. Nucleic Acids Research 39, W347-W52.

UNIVERSITE ECOLOGIE
BRETAGNE GEOSCIENCES
LOIRE AGRONOMIE ALIMENTATION

UNIVERSITÉ DE NANTES

Titre : **Caractérisation des mécanismes d'altération des produits carnés et de la mer par *Brochothrix thermosphacta***

Mots clés : *Brochothrix thermosphacta* ; Altération ; Acétoïne ; Diacétyle ; Diversité ; Génomique comparative ; Volatilome ; Transcriptomique.

Résumé : L'altération microbiologique des aliments entraîne des pertes économiques considérables. Elle résulte du développement et du métabolisme de microorganismes produisant des odeurs, flaveurs, textures ou des couleurs indésirables.
Brochothrix thermosphacta, est l'une des principales bactéries d'altération des produits carnés et de la mer. Elle produit des métabolites responsables d'odeurs désagréables, variant suivant les souches, les aliments et les communautés bactériennes qui l'entourent. Nous avons évalué sa diversité génétique et phénotypique au sein d'une collection de souches issues de diverses origines écologiques. Différentes méthodes ont discriminé les souches en groupes distincts, révélant une diversité significative. Cependant, celle-ci n'est pas liée à l'environnement dont les souches ont été isolées.
Quatre souches représentatives de cette diversité ont été analysées par génomique comparative. Le contenu génomique est similaire, les différences résidant dans le contenu en phages et/ou plasmides et en composants de surface qui peuvent contribuer à leur adaptation écologique. Des mutations ponctuelles peuvent être responsables des différentes capacités à produire des molécules d'altération.
Une étude transcriptomique a montré que les gènes différentiellement exprimés lors de la croissance sur une matrice viande ou produit de la mer codent des fonctions importantes pour la croissance et la survie sur ces niches spécifiques. L'analyse du volatilome a montré que *B. thermosphacta* produit différentes molécules responsables d'odeurs désagréables qui varient suivant la matrice. Par conséquent, nous avons montré un effet matrice plutôt qu'un effet souche sur le potentiel d'altération de *B. thermosphacta*.

Title: **Characterization of meat and seafood spoilage mechanisms by *Brochothrix thermosphacta***

Keywords : *Brochothrix thermosphacta*; Spoilage; Acetoin; Diacetyl; Diversity; Comparative genomics; Volatilome; Transcriptomics

Abstract: Microbial spoilage of food causes considerable economic losses worldwide. It results from the development and metabolism of microorganisms which produce undesirable odors, flavors, textures, or colors. *Brochothrix thermosphacta* is one of the main spoilage bacteria of meat and seafood products. It produces various metabolites responsible for off-odors, which seem to depend on strains, food type, and bacterial communities surrounding it. We assessed the genetic and phenotypic diversity of *B. thermosphacta* among a strain collection issued from various origins (seafood, meat, dairy products, and environment). Different methods were applied to discriminate strains into distinct groups, revealing a significant diversity. However, the intra-species diversity is not related to the environment strains were isolated from.
Four strains representative of intra-species diversity were analyzed by comparative genomics. The genomic content is similar and the only differences reside in phage and plasmid content and on surface components that may contribute to their ecological adaptation. Point mutations may be responsible for different abilities to produce spoilage molecules.
A transcriptomic study showed that the genes differentially expressed on seafood or meat matrices encoded functions important for growth and survival on these specific niches. The volatilome analysis showed that *B. thermosphacta* produced different molecules responsible for off-odors that vary depending on the growth matrix. Therefore, we showed a matrix effect rather than a strain effect on the spoilage potential of *B. thermosphacta.*

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