Université du Québec
à Rimouski

IMPACT DE LA MARENNINE SUR LE MICROBIOTE DE LARVES DE *MYTILUS EDULIS* EXPOSÉES AU PATHOGÈNE *VIBRIO SPLENDIDUS*

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RÉSUMÉ

Dans les écloseries de bivalves, les pathogènes opportunistes sont associés à de grandes mortalités masse causant des pertes économiques importantes aux producteurs. Afin de contrôler ces évènements et stabiliser la production larvaire, des alternatives aux antibiotiques sont proposées. Les probiotiques ou encore leurs molécules bioactives sont associés à des effets bénéfiques pour les larves à différents niveaux, particulièrement dans l'amélioration de leur résistance aux stresseurs dont les pathogènes bactériens. De nos jours, il est reconnu que la composition du microbiote influence l'état de santé de l'hôte et qu'elle pourrait être une des cibles des probiotiques. Le but de cette étude est de mettre en évidence l'effet protecteur d'une nouvelle molécule bioactive, la marennine, sur les larves de *Mytilus edulis* durant des tests de provocations bactériennes avec le pathogène bactérien opportuniste *Vibrio splendidus* en relation à une modification du microbiote larvaire des individus traités avec la marennine. L'hypothèse de ce mémoire est que l'utilisation de la marennine durant l'élevage larvaire pourrait modifier les conditions prévalant dans le milieu de culture et ainsi modifier la composition du microbiote larvaire menant à une meilleure résistance aux infections bactériennes. La marennine est un pigment bleu produit par la diatomée *Haslea ostrearia* et a déjà démontré un effet bénéfique sur la survie larvaire lorsqu'utilisée à une concentration de 500 $\mu g\, L^{-1}$. Des larves-D et des post-larves ont été exposées à *V. splendidus* en présence et en absence de marennine pendant 96 h. Nos résultats démontrent qu'à cette concentration, la marennine n'a pas d'effet antibactérien direct sur la croissance de *V. splendidus*. De plus, la présence de marennine n'a pas modifié l'abondance de bactéries dans le milieu de culture, indiquant que la marennine n'a pas eu d'effet antibactérien sur la charge bactérienne à laquelle les larves ont été exposées durant les expériences. Toutefois, la présence de marennine a fait augmenter le taux de survie des larves-D, mais pas celui des post-larves. Les analyses moléculaires du microbiote larvaire ont permis de démontrer que l'augmentation du taux de survie larvaire était accompagnée d'une modification de la richesse spécifique du microbiote. Ultimement, nos travaux permettront de mettre en évidence l'importance du microbiote larvaire dans la résistance aux pathogènes durant l'élevage des bivalves.

Mots clés : Microbiote, *Vibrio splendidus*, Pathogène opportuniste, Bivalve, Larve, Écloserie

ABSTRACT

In bivalve hatcheries, opportunistic pathogens have been associated with important mass mortality events of larvae and important economic loss for producers. Alternatives to the use of antibiotics, such as probiotics, have been proposed to limit the occurrence of such events in bivalve hatcheries and thus to stabilize bivalve production. Probiotics and their natural molecules are associated with beneficial effects for larvae at different levels, especially to enhance their resistance to external stressors such as bacterial pathogens. It is now recognized that the composition of the host microbiota influences the host health status and could be a target of probiotics. The aim of this master's thesis is to highlight the protective effect of a new bioactive molecule, marennine, on *Mytilus edulis* larvae during bacterial challenges in relation to a potential modification of the marennine-treated larvae microbiota. The main hypothesis is that the addition of marennine during larvae rearing could modify the conditions prevailing in the rearing medium and, as a consequence, the composition of the larvae microbiota leading to a better resistance to bacterial infections. Marennine is a blue pigment, originating from the diatom *Haslea ostrearia*, which has demonstrated a positive effect on larvae survival at a concentration of $500\,\mu g\,L^{-1}$. D-larvae and post-larvae were exposed for 96 h to *Vibrio splendidus* with and without mareninne. Our results demonstrated that, at this concentration, marennine has no direct antimicrobial effect on *V. splendidus* growth kinetics. In addition, the presence of marennine did not modify the abundance of bacteria in the rearing medium, suggesting no direct antimicrobial effect of marennine on the bacterial load to which larvae were exposed during the experiments. Nevertheless, the presence of marennine increased the survival of D-larvae exposed to the pathogen but have no effect on post-larvae survival. The molecular analysis of the larvae microbiota diversity allowed us to demonstrate that a modification in the larval microbiota's richness occurs while the survival rates increase. Ultimately, our work will enable us to shed light on the importance of the larval microbiota in pathogen-resistance during bivalves rearing process.

Keywords : Microbiota, *Vibrio splendidus*, Opportunistic pathogen, Bivalve, Larvae, Hatchery

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INTRODUCTION GÉNÉRALE

L'aquaculture est un secteur d'activité en fort développement à l'échelle mondiale en en raison de la hausse de la demande en produits aquatiques, mais aussi en raison et de la diminution des ressources naturelles marines disponibles (Sapkota et al., 2008). Au Canada, l'aquaculture s'est principalement développée autour des salmonidés et des bivalves, dont la moule bleue (*Mytilus edulis*) est le bivalve avec la production la plus importante représentant un volume total de 25 800 tonnes produit entre 2011 et 2015 (Fisheries and Oceans Canada, 2017-03-15 2018). Au Québec, la tendance est similaire et la moule bleue est le bivalve le plus cultivé et cette culture représentait une valeur à la ferme à 49,5 millions de dollars en 2013, soit une augmentation de 68% depuis 2009 (Fisheries and Oceans Canada, 2015-03-03 2015, 2017-03-15 2018). Pour répondre à la demande grandissante de fruits de mer, les producteurs de bivalves marins doivent avoir accès à un approvisionnement stable en juvéniles (Helm et al., 2004). La technique de production de juvéniles de moule utilise le cycle naturel couplant un stade larvaire pélagique à une phase de fixation sur un substrat pour capter les jeunes juvéniles sur des collecteurs artificiels.

Comme le succès de l'approvisionnement en juvéniles provenant du milieu naturel via le captage sur des collecteurs artificiels est très variable, les juvéniles produits en écloseries sont susceptibles de devenir de plus en plus populaires auprès des conchyliculteurs (Helm et al., 2004). Or, cette méthode d'élevage larvaire pratiquée pour les bivalves comme la moule bleue concentre un grand nombre d'individus dans un volume d'eau restreint et peut causer un stress important chez les larves. De plus, ces conditions d'élevage peuvent favoriser le développement de bactéries pathogènes entraînant des évènements de mortalité de masse dans les écloseries (Vadstein et al., 2004). Ces mortalités massives sont un enjeu représentant un défi majeur pour la production aquacole et le contrôle de leur occurrence est un enjeu primordial afin d'assurer une production stable de juvéniles. Ces évènements ont été reliés à la présence de plusieurs pathogènes opportunistes appartenant aux genres *Pseu-*

domonas, *Roseovarius*, *Aeromonas* et *Vibrio* (Tubiash et al., 1970; Beaz-Hidalgo et al., 2010). Les pathogènes du genre *Vibrio* sont les agents infectieux les plus souvent identifiés dans les élevages larvaires (Paillard et al., 2004). Afin de contrôler les pertes liées aux infections bactériennes, l'antibiothérapie a longtemps été le moyen privilégié dans l'industrie aquacole (Nicolas et al., 2007). Toutefois, le risque de développement de souches bactériennes pouvant résister à ces antibiotiques est considérable et doit absolument être pris en compte lors du développement de nouveaux traitements (Defoirdt et al., 2011; Falaise et al., 2016). Par ailleurs, l'utilisation des antibiotiques en aquaculture est fortement réglementée autour du globe (Perez-Sanchez et al., 2018). Il est donc primordial de développer des techniques de contrôle alternatives contre les infections bactériennes dans les élevages afin de limiter l'impact des évènements de mortalité de masse causés par des pathogènes opportunistes et assurer le succès de développement des écloseries.

1.1 Les *Vibrionaceae*

Les *Vibrionaceae* sont des pathogènes fréquemment identifiés lors d'évènement de mortalité de masse chez les bivalves. Ces bacilles à Gram-négatif, arborant un flagelle polaire et ayant besoin de sodium (Na^+) pour leur croissance sont des microorganismes ubiquitaires dans les milieux aquatiques ont été décrits pour la première fois par Véron en 1965 (Véron, 1965). En 2013, 142 espèces de *Vibrionaceae* étaient répertoriées (Sawabe et al., 2013). Cette famille comprend notamment l'espèce bien connue *Vibrio cholerae*, l'agent infectieux du choléra qui représente actuellement un problème de santé publique majeur pour près du tiers des pays du monde (Ali et al., 2015).

Le nombre important d'espèces classées dans la famille des *Vibrionaceae* en fait un groupe particulièrement diversifié au sein duquel le pouvoir pathogène des espèces est très variable. À l'opposé de *V. cholerae*, on retrouve l'espèce bioluminescente *V. fischeri* qui s'associe avec la seiche *Euprymna scolopes*. Cette association symbiotique permet à *V. fischeri* de coloniser un organe spécialisé de la seiche et d'y perdurer tout en permettant à son hôte

d'utiliser la lumière que la bactérie peut produire (Ruby, 1996). Les 2 espèces précédemment mentionnées représentent les deux extrêmes du spectre des phénotypes (pathogène stricte et non-pathogène) que l'on peut retrouver pour les espèces de la famille des *Vibrionaceae*. Certaines espèces, quant à elles, sont décrites comme des pathogènes opportunistes. Ces bactéries infectent leur hôte seulement lorsque ce dernier présente une ou plusieurs conditions qui ne sont habituellement pas rencontrées chez les individus sains. Par exemple, une plaie ouverte peut servir de voie d'entrée pour ces pathogènes ou encore un système immunitaire compromis peut permettre à une bactérie normalement contrôlée par le système immunitaire de développer son potentiel infectieux. À titre d'exemple, l'espèce *V. vulnificus* est un pathogène opportuniste pouvant infecter les humains via l'ingestion de fruits de mer contaminés ou encore via l'exposition à de l'eau de mer contenant le pathogène. Il est reporté que les cas d'infections de *V. vulnificus* s'aggravent rapidement si les hôtes ont déjà des conditions médicales particulières comme le diabète, l'alcoolisme ou encore la leucémie (Oliver, 2005). Les travaux de recherche sur cette infection se sont principalement concentrés sur les humains (Strom and Paranjpye, 2000; Phillips and Satchell, 2017; Baker-Austin and Oliver, 2018) et sur la sécurité alimentaire concernant la production et la manutention des fruits de mer (Gopal et al., 2005). Or, la présence de pathogènes opportunistes dans les eaux naturelles (Mansergh and Zehr, 2014), les systèmes aquacoles (Vandenberghe et al., 2003) et même dans les communautés microbiennes naturellement associées aux tissus des animaux sains (Vandenberghe et al., 2003; Asmani et al., 2016; Vezzulli et al., 2018) soulève l'importance de l'identification des facteurs biotiques et abiotiques déclenchant leur pathogénie. Ce groupe de bactéries est notamment responsable de maladies appelées couramment vibrioses qui affectent plusieurs élevages aquacoles entraînant des pertes économiques importantes (Beaz-Hidalgo et al., 2010). Dans les conditions particulières associées aux systèmes aquacoles (forte densité d'élevage, stress de manipulation), les *Vibrionaceae* peuvent échapper à la résistance du système immunitaire de nombreuses espèces aquacoles comme les poissons, les crustacés et les bivalves et déclencher des infections pouvant conduire à des évènements de mortalité de masse (Velji et al., 1992; Beaz-Hidalgo et al., 2010; Liu et al., 2013). Les larves

produites en écloseries, qui est le stade ontogénique le plus sensible (Rayssac et al., 2010), sont particulièrement vulnérables face aux pathogènes du genre *Vibrio* (Elston and Leibovitz, 1980; Genard et al., 2013, 2014). Une meilleure compréhension des agents infectieux des vibrioses et de leurs interactions avec les larves et avec les autres bactéries du milieu est donc nécessaire afin de développer de nouvelles stratégies afin de contrer les effets néfastes de ces infections bactériennes.

1.2 Microbiote et immunité

Récemment, beaucoup d'attention s'est portée sur la compréhension du rôle du microbiote (communauté microbienne naturelle associée aux tissus d'un organisme) sur l'état physiologique de l'hôte. Il est aujourd'hui reconnu que le microbiote contribue significativement au développement et à la condition physiologique de l'hôte (Zilber-Rosenberg and Rosenberg, 2008; Rosenberg and Zilber-Rosenberg, 2016). Selon le concept de l'hologénome, le «super-organisme» qui est composé de l'hôte animal et du microbiote, la partie microbienne de l'holobionte représente l'élément de l'hologénome le plus variable. En effet, le microbiote peut réagir plus rapidement à une variation dans les conditions environnementales que l'hôte. Considérant que le microbiote régule en partie les réponses physiologiques de l'hôte face à un stress externe, de nombreux travaux se sont penchés sur le rôle du microbiote dans la colonisation des tissus par des agents infectieux (Stecher and Hardt, 2008; Ribet and Cossart, 2015). L'effet antagoniste des bactéries intestinales contre la colonisation des pathogènes bactériens dans les tissus animaux est bien connu et a déjà été démontré par des expériences utilisant des techniques de culture classiques (Freter, 1962; Bartlett et al., 1977). Des travaux plus récents ont démontré l'implication du microbiote dans la virulence des pathogènes, ce qui défi la vision classique du «un pathogène, une maladie» provenant des postulats de Koch classiques. Par exemple, Duran-Pinedo et al. (2014) ont démontré que les bactéries commensales du microbiote oral contribuaient significativement à l'expression des facteurs de virulences des pathogènes reconnus causant la périodontite. Les auteurs ont

noté qu'un débalancement du microbiote, ou dysbiose, semblait être nécessaire pour l'expression de la pathogénie de ces bactéries. Les mécanismes de la transition du microbiote d'un état commensal à un état dysbiotique et l'implication de cette transition sur la susceptibilité aux pathogènes demeurent difficiles à étudier dû au grand nombre de facteurs pouvant influencer l'homéostasie à l'interface hôte-microbiote. Deux hypothèses non exclusives sont actuellement proposées : (1) l'hypothèse des nutriments (*food hypothesis*) et (2) l'hypothèse de la défense (*killing hypothesis*) (Stecher and Hardt, 2008). L'hypothèse des nutriments suppose que lorsque l'hôte fait face à un stress externe, son état physiologique entraîne une modification des nutriments disponibles pour les bactéries de son microbiote, modifiant ainsi la capacité des tissus à résister à la colonisation par une bactérie pathogène. L'augmentation de la quantité de nutriments pourrait favoriser la croissance de bactéries pathogènes (reconnues pour croître rapidement dans un environnement enrichi en nutriments) et entraîner en parallèle un changement de diversité du microbiote (Ribet and Cossart, 2015). L'hypothèse de la défense stipule qu'une modification du microbiote peut être induite par la production importante de molécules antimicrobiennes telles que la défensine, un peptide antimicrobien, ou encore la phospholipase par l'hôte (Stecher and Hardt, 2008). Ces molécules sont peu spécifiques et une production accrue de ces dernières dans l'environnement du microbiote peut accidentellement affecter grandement les bactéries commensales du microbiote (dommages collatéraux). La réduction de la diversité spécifique et de la densité totale de bactéries du microbiote pourrait favoriser la colonisation de cet environnement par une bactérie pathogène présentant une résistance supérieure vis-à-vis des molécules antimicrobiennes. Afin de mieux comprendre cette dynamique complexe entre l'état physiologique de l'hôte, son microbiote et un pathogène bactérien opportuniste, une caractérisation de la diversité spécifique et fonctionnelle du microbiote des organismes hôtes est nécessaire.

1.3 Le microbiote des bivalves marins

L'étude du microbiote des bivalves marins se nourrissant en filtrant l'eau environnante est particulièrement intéressante parce que ces organismes maintiennent un lien étroit avec leur environnement, mais aussi parce qu'une partie de leur alimentation est constituée de bactéries planctoniques potentiellement pathogènes. Les travaux de Beeson and Johnson (1967) sur la microflore normale du haricot de mer (*Donax gouldii*) en milieu naturel ont pu démontrer que ces bivalves maintenaient une communauté bactérienne différente de celle retrouvée dans leur environnement de croissance. Ces travaux font partie des premiers à avoir suggéré que les conditions physiologiques des organismes créaient un environnement interne distinctif de celui de l'eau de mer environnante et que, contrairement à la pensée populaire, la communauté bactérienne dans l'eau environnante n'était pas le reflet de la communauté du microbiote. Parmi les bactéries isolées des tissus des organismes hôtes durant cette étude, plusieurs étaient de la famille des *Vibrionaceae*. Récemment, Chauhan et al. (2018) ont décrit le microbiote d'huîtres américaines (*Crassostrea virginica*) provenant du milieu naturel. Le microbiote des huîtres était dominé par quelques taxons comme *Cyanobacteria* sp. et *Pelagibacter* sp., mais aussi par *Photobacterium* sp., un pathogène opportuniste de la famille des *Vibrionaceae* qui peut également former une symbiose avec l'hôte. La présence de pathogènes opportunistes dans le microbiote de bivalves sains n'est pas surprenante considérant que ces derniers sont retrouvés naturellement dans les eaux naturelles (Mansergh and Zehr, 2014; Takemura et al., 2014). Toutefois, le rôle des pathogènes opportunistes de la famille des *Vibrionaceae* dans le maintien de l'homéostasie des bivalves sains et les facteurs déclenchant leur pathogénie demeurent à élucider (Pruzzo et al., 2005).

Les travaux de caractérisation de la diversité des *Vibrionaceae* du microbiote des bivalves se sont principalement intéressés aux stades adultes (Romalde et al., 2014). Or, les bivalves ont la particularité d'avoir à se métamorphoser pour passer du stade larvaire au stade juvénile. Pendant cette métamorphose, le système de la prophenoloxidase (proPO) s'active (Smith and Söderhäll, 1991). Ce système agit comme un système immunitaire inné rudimen-

taire qui permet aux bivalves de se défendre contre les pathogènes par l'opsonisation des cellules étrangères. L'activation de ce système peut être enclenchée par certaines molécules comme le ß-1,3-glucane, le peptidoglycane et certains lipopolysaccharides (Söderhäll and Cerenius, 1998). Ce système est partiellement décrit chez les bivalves adultes et peu de données sont disponibles pour ce qui est des stades larvaires (Bassim et al., 2015b). Une meilleure compréhension des capacités immunitaires des larves de bivalves est nécessaire afin de mieux comprendre les facteurs biotiques et abiotiques régulant ces dernières et plus particulièrement le rôle possible du microbiote larvaire des bivalves dans leur immunité.

Considérant l'importance du microbiote dans la colonisation des tissus animaux par les pathogènes et de son implication dans la physiologie de son hôte, la caractérisation de la diversité spécifique du microbiote larvaire de bivalves sous différentes conditions s'avère nécessaire. L'utilisation des espèces élevées en aquaculture comme modèle d'étude est une avenue très intéressante pour étudier les relations entre les larves de bivalves, leur microbiote et les pathogènes bactériens opportunistes couramment retrouvés dans les systèmes d'élevage (Vandenberghe et al., 2003). Plusieurs de ces pathogènes bactériens opportunistes ont notamment été retrouvés dans le microbiote d'individus sains, dont des membres de la famille des *Vibrionaceae* (Vandenberghe et al., 2003; Asmani et al., 2016; Vezzulli et al., 2018).

1.4 Contrôle des évènements de mortalité de masse en écloseries

L'occurrence d'évènements de mortalité de masse dans les écloseries représente une des limitations majeures à la production de juvéniles en écloserie. Les pathogènes du genre *Vibrio* sont les agents infectieux les plus souvent reportés dans le cas d'infections bactériennes dans les systèmes d'élevage de larves (Paillard et al., 2004). Les travaux d'Elston and Leibovitz (1980) sur la pathogenèse de la vibriose chez les huîtres américaines ont démontré que les infections progressaient différemment selon l'isolat bactérien utilisé. Les observations faites durant cette étude ont permis de déterminer quels signes cliniques observer afin de diagnostiquer la vibriose lors des premières étapes de l'infection des huîtres. Par exemple, il

est possible d'observer le détachement des cellules du manteau ou encore une atrophie des viscères (Elston and Leibovitz, 1980).

Depuis les travaux de Davis and Chanley (1956), l'utilisation d'antibiotiques a gagné en popularité auprès des aquaculteurs afin de limiter l'impact des infections dans les élevages. La streptomycine, la pénicilline et le chloramphénicol ont longtemps été les antibiotiques de choix pour réduire l'impact des infections bactériennes dans les élevages larvaires. Toutefois, l'utilisation systématique et intensive d'antibiotiques dans les milieux d'élevage accroît les risques liés au développement de résistance aux antibiotiques (Defoirdt et al., 2011; Falaise et al., 2016) et au transfert de cette capacité dans les réseaux trophiques (Sapkota et al., 2008). Les traitements à l'aide d'antibiotiques sont devenus un enjeu majeur pour la production aquacole (Watts et al., 2017). En effet, lorsqu'une population de bactéries devient résistante à l'utilisation d'antibiotiques courants, cela peut mener à la persistance des maladies difficilement traitables dans les systèmes d'élevage.

L'utilisation de techniques de contrôle biologique en aquaculture afin de limiter l'impact des pathogènes bactériens est une voie intéressante, car la diversité de leur mode d'action réduit significativement la probabilité de l'apparition de résistances spécifiques (Nicolas et al., 2007). Les probiotiques vivants, généralement composés de bactéries vivantes ayant un effet bénéfique sur la santé sur l'hôte (Joint FAO/WHO Expert Consultation, 2001), sont une de ces alternatives. Dès 1907, les observations de Metchnikoff en lien avec la consommation de produits laitiers fermentés lui ont permis de stipuler qu'il serait possible de moduler la flore (microbiote) humaine afin de remplacer les bactéries néfastes par des bactéries bénéfiques. Plus d'un siècle après ces observations, les effets bénéfiques de plusieurs bactéries sur la santé de l'hôte non seulement chez l'humain, mais également chez plusieurs espèces élevées en aquaculture furent démontrés (Kesarcodi-Watson et al., 2016; Sohn et al., 2016a,b; Zorriehzahra et al., 2016; Sharifuzzaman and Austin, 2017). Les effets immunostimulants de certaines espèces bactériennes utilisées en aquaculture permettent de contrer l'implantation d'un pathogène spécifique ou encore d'accroître la résistance aux ma-

ladies de manière non spécifique (Akhter et al., 2015). Par exemple, la bactérie *Pseudoalteromonas* sp. X153, isolée de la surface d'une roche à Saint-Anne-du-Portzic (Bretagne, France) a démontré un effet protecteur sur les palourdes (*Ruditapes philippinarum*) au stade larvaire (Longeon et al., 2004) . Les auteurs de cette étude ont démontré que non seulement *Pseudoalteromonas* sp. X153 n'était pas toxique pour les larves de palourdes, mais également qu'elle avait un effet protecteur pour des larves de coquilles Saint-Jacques (*Pecten maximus*). Une molécule bioactive, P-153, se retrouvant à la fois dans les cellules du probiotique et dans l'eau d'élevage a été identifiée comme étant un des possibles acteurs principaux dans le mode d'action de *Pseudoalteromonas* sp. X153. Les probiotiques, ou encore leurs biomolécules actives sont donc une avenue intéressante pour réduire l'utilisation systématique des antibiotiques, mais également pour rendre les pratiques aquacoles plus durables et plus sécuritaires (Balcazar et al., 2006; Defoirdt et al., 2011; Beaz-Hidalgo et al., 2010; Gastineau et al., 2012).

1.5 La marennine et son mode d'action potentiel

La marennine, une biomolécule provenant de la diatomée *Haslea ostrearia*, a été proposée comme traitement alternatif aux antibiotiques en aquaculture (Pouvreau et al., 2006, 2008). Ce pigment a démontré un effet inhibiteur de la croissance de différentes bactéries pathogènes en culture pure dont plusieurs de la famille des *Vibrionaceae* comme *V. tasmaniensis*, *V. aesturianus* et *V. splendidus* (Defoirdt et al., 2011; Gastineau et al., 2012, 2014) à des concentrations variant entre $19.14\,\mathrm{mg\,L^{-1}}$ pour *V. tasmaniensis* (Falaise et al., 2016) et $2.89\,\mathrm{mg\,L^{-1}}$ pour *V. splendidus* (Gastineau et al., 2014). Toutefois, Turcotte et al. (2016) ont mis en lumière un effet cytotoxique de la marennine sur les larves de bivalves à ces concentrations. C'est pourquoi la marennine a été utilisée à une concentration de $500\,\mathrm{\mu g\,L^{-1}}$ dans les élevages larvaires durant leurs travaux. Leurs résultats ont pu démontrer le fort potentiel protecteur de cette biomolécule pour l'élevage de bivalves marins. Les auteurs ont démontré que la marennine avait un effet bénéfique sur le taux de survie des larves-D de *M. edulis* lors

d'expositions à *V. splendidus* sans que l'abondance bactérienne dans les milieux de culture des larves ne soit affectée. Le mode d'action de la marennine à cette concentration ne semble donc pas venir d'un quelconque effet antimicrobien et demeure donc à élucider.

1.6 Objectifs et hypothèses

Dans ce mémoire nous validerons l'hypothèse qu'un des modes d'action possible de la marennine, lorsqu'utilisée à des concentrations de l'ordre de celle utilisée par Turcotte et al. (2016), pourrait être l'interaction avec le microbiote larvaire de *M. edulis*. Le premier objectif spécifique de ce projet est de démontrer l'absence d'un effet antimicrobien de la marennine contre *V. splendidus* à une concentration de $500\,\mu g\,L^{-1}$. Le deuxième objectif spécifique est de mettre en évidence l'effet de la marennine à une concentration de $500\,\mu g\,L^{-1}$ sur les communautés bactériennes indigènes, dans l'eau d'élevage et du microbiote larvaire, lors de tests de provocations bactériennes avec *V. splendidus*.

Élucider les mécanismes d'action de la marennine lors de son utilisation en conchyliculture permettrait non seulement d'optimiser son utilisation, mais également d'étendre son potentiel à plus grande échelle dans un contexte de développement durable de l'exploitation des ressources marines.

ARTICLE 1

IMPACT DE LA MARENNINE SUR LE MICROBIOTE DE LARVES DE *MYTILUS EDULIS* EXPOSÉES AU PATHOGÈNE *VIBRIO SPLENDIDUS* (IMPACT OF MARENNINE ON THE MICROBIOTA OF *MYTILUS EDULIS* LARVAE EXPOSED TO THE PATHOGEN *VIBRIO SPLENDIDUS*)

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2.1 Résumé

Dans les écloseries de bivalves, les pathogènes bactériens opportunistes ont été identifiés comme étant responsables des évènements de mortalité de masse causant des pertes économiques importantes pour les conchyliculteurs. De nouvelles techniques de contrôle ont été proposées afin de remplacer l'utilisation d'antibiotiques dans les élevages. En effet, les probiotiques et les molécules bioactives d'origine naturelle ont déjà été suggérés afin de limiter l'occurrence de ces évènements de mortalité de masse. Ces microorganismes et ces molécules sont associés à des effets bénéfiques sur les hôtes, plus particulièrement sur leur résistance face à des stress externes comme un pathogène bactérien. De nos jours, il est bien reconnu que le microbiote joue un rôle important dans le maintien de l'état de santé d'un organisme. Le but de cet article est de mettre en évidence l'effet protecteur d'une nouvelle molécule bioactive naturelle extraite de la diatomée *Haslea ostrearia* en relation avec une potentielle modification du microbiote larvaire de *Mytilus edulis*. L'hypothèse principale est que cette modification du microbiote de larves traitées avec la marennine améliorera la résistance des larves contre les pathogènes bactériens. L'effet protecteur de la marennine a

déjà été démontré chez *M. edulis* à une concentration de 500 µg L^{-1}. Dans cette étude, des larves-D (J_9) et des post-larves (J_{29}) ont été exposées au pathogène opportuniste *Vibrio splendidus* pendant 96 h en présence et en absence de marennine. Nos résultats démontrent qu'à une concentration de 500 µg L^{-1}, la marennine n'a pas d'effet antibactérien sur la croissance du pathogène en culture pure. De plus, la présence de marennine n'a pas démontré d'effet sur l'abondance bactérienne dans le milieu de culture lors de l'exposition des larves indiquant que la présence de marennine n'influence pas la charge bactérienne à laquelle les larves sont exposées durant les expériences. Toutefois, le taux de survie des larves-D a significativement augmenté en présence de marennine lors des expositions aux pathogènes. Les analyses moléculaires ont révélé que l'augmentation du taux de survie larvaire était accompagnée d'une modification significative de la richesse spécifique du microbiote des larves. Ces travaux permettront de mieux comprendre le rôle que le microbiote joue dans la résistance aux pathogènes opportunistes en présence de biomolécules en aquaculture.

2.2 Abstract

In bivalve hatcheries, opportunistic pathogens have been associated with important mass mortality events of larvae and important economic loss for producers. Alternatives to the use of antibiotics, such as probiotics, have been proposed to limit the occurrence of such events in bivalve hatcheries and thus to stabilize bivalve production. Probiotics and their natural molecules are associated with beneficial effects for larvae at different levels, especially to enhance their resistance to external stressors such as bacterial pathogens. It is now recognized that the composition of the host microbiota influences the host health status and could be a target of probiotics. The aim of this master's thesis is to highlight the protective effect of a new bioactive molecule, marennine, on *Mytilus edulis* larvae during bacterial challenges in relation to a potential modification of the marennine-treated larvae microbiota. The main hypothesis is that the addition of marennine during larvae rearing could modify the conditions prevailing in the rearing medium and, as a consequence, the composi-

tion of the larvae microbiota leading to a better resistance to bacterial infections. Marennine is a blue pigment, originating from the diatom *Haslea ostrearia*, which has demonstrated a positive effect on larvae survival at a concentration of 500 µg L^{-1}. D-larvae and post-larvae were exposed for 96 h to *Vibrio splendidus* with and without mareninne. Our results demonstrated that, at this concentration, marennine has no direct antimicrobial effect on *V. splendidus* growth kinetics. In addition, the presence of marennine did not modify the abundance of bacteria in the rearing medium, suggesting no direct antimicrobial effect of marennine on the bacterial load to which larvae were exposed during the experiments. Nevertheless, the presence of marennine increased the survival of D-larvae exposed to the pathogen but have no effect on post-larvae survival. The molecular analysis of the larvae microbiota diversity allowed us to demonstrate that a modification in the larval microbiota's richness occurs while the survival rates increase. Ultimately, our work will enable us to shed light on the importance of the larval microbiota in pathogen-resistance during bivalves rearing process.

2.3 Introduction

During the last few decades, fish and shellfish farming industries have experienced an important increase in their production rates in response to the growing seafood demand worldwide. From 1960 to 2013, the world marine products consumption per capita almost doubled increasing from an average of 9.9 kg to 19.7 kg per year (FAO, 2016). As a large proportion of natural seafood stocks being fully fished (58.1%) or overfished (31.4%), the share of aquaculture products in the global seafood consumption also increased from 7% to 39% between 1974 and 2004 (FAO, 2016). In order to meet the demand, producers need to have access to a large and stable supply of juveniles (Helm et al., 2004). Consequently, hatcheries are most likely to gain popularity, especially among bivalve producers who often rely on hatchery-reared juveniles due to the high spatiotemporal variability of the natural recruitment success (Helm et al., 2004).

The success of hatcheries, and ultimately the bivalve production rates depend on the

larvae survival in the rearing system. Bacterial infections are known to be a major bottleneck to hatchery-reared juvenile bivalve production by causing massive mortality (mass mortality events). Many opportunistic bacterial pathogens from the genera *Vibrio*, *Pseudomonas*, *Aeromonas* and *Roseovarius* were reported to be linked to mass mortality events in bivalve hatcheries (Tubiash et al., 1970; Paillard et al., 2004). These organisms exert their pathogenicity only in specific environmental or larvae physiological conditions that are still poorly understood. The seemingly random nature of mass mortality events is a major obstacle to achieve steady production rates. Understanding and therefore controlling disease outbreaks in hatcheries is crucial to assure the stability of the hatchery production.

Prior to the 1980s, antibiotics were regularly used by producers in bivalve hatcheries to reduce the impact of bacterial infection on their production (Asmani et al., 2016). However, the development of antibiotic resistance (Defoirdt et al., 2011; Falaise et al., 2016) and the risk of transmission of these resistant strains in the food web (Sapkota et al., 2008) led to strict regulations in several countries (Hernandez and Serrano, 2005; FDA, 2007). The use of antibiotics is therefore not a sustainable solution for controlling the occurrence of mass mortality events in bivalve hatcheries.

Probiotics and natural bioactive molecules have been proposed as alternatives to antibiotics in animal farming (Balcazar et al., 2006; Defoirdt et al., 2011; Beaz-Hidalgo et al., 2010; Gastineau et al., 2012; Falaise et al., 2016). A wide range of organisms, or their cellular components, was tested as probiotics for their positive effect in aquaculture (Irianto and Austin, 2002). For example, Chilean scallop larvae (*Argopecten purpuratus*) were able to complete the larval pelagic phase without any antibiotic treatment when exposed to inhibitor-producing bacteria strains (*Vibrio* sp. C33, *Pseudomonas* sp. 11 and *Bacillus* sp. B2) (Riquelme et al., 2001). The strains *Pseudoalteromonas* sp. X153 (Longeon et al., 2004) and *Phaeobacter gallaeciensis* X34 (Genard et al., 2014) also demonstrated a positive effect on cultured scallop larvae (*Pecten maximus*) survival. Azadirachtin, an extract from the neem tree (*Azadirachta indica*), also demonstrated a beneficial effect acting as an immunostimulant

on goldfish (*Carassius auratus*) and increased their survival rate when challenged against *Aeromonas hydrophila* (Kumar et al., 2013). Recently, marennine, a blue-green pigment produced by the diatom *Haslea ostrearia*, was suggested as an interesting bioactive molecule for bivalve hatcheries (Turcotte et al., 2016). This natural pigment has shown promising results by reducing the mortality rate of blue mussels larvae (*Mytilus edulis*) challenged with *V. splendidus*. Unfortunately, little is known about the mode of action underlying the beneficial effects of marennine in bivalves farming (Pouvreau et al., 2008; Gastineau et al., 2012), except the study of Tardy-Laporte et al. (2013) demonstrating the interaction of marennine with lipopolysaccharides related to higher rigidity of membrane cells in Gram negative bacteria. Improvement of the use of the feed, immunostimulation, antibacterial activity, alteration of the microbial metabolism and competitive exclusion are some of the proposed, but poorly documented, hypothetical modes of action of probiotics in aquaculture (Irianto and Austin, 2002; Prado et al., 2010). Unravelling the role of marennine as a water-additive in bivalve hatcheries is crucial in order to safely expand its utilization.

In this paper, we test the hypothesis that the interaction of marennine with the larval microbiota could be the mode of action contributing to the observed beneficial effects on bivalve larvae survival. The artificial addition of marennine in the rearing medium might modify the bacterial community within the rearing medium itself and induce a change in the bacterial community recruited by the larvae to form their microbiota. In addition, marennine could interfere with the quorum sensing system within the larvae resulting in a modification of the microbiota formation (Kalia, 2013).

Nowadays, it is well known that the taxonomic diversity, the abundance and the physiological structure of the microbiota affect its host health condition (Lopez et al., 2014; Laterza et al., 2016; Marchesi et al., 2016). In the case of bivalves, it has been suggested that a shift in the structure and the specific diversity of the microbiota in adults might prevent bacterial pathogen to settle within the host (Froelich and Oliver, 2013). These microbiota modifications might lead to a better pathogen resistance in larvae and contribute to the beneficial

effect of marennine addition observed in bivalve hatcheries. Therefore, interaction between probiotics or bioactive molecules with the natural larvae microbiota, the conditions prevailing in the rearing medium and the diversity of bacterial communities might contribute to the observed protective effect of marennine on bivalve larvae in hatcheries.

The aim of this study is to investigate the importance of the larval microbiota in hatchery-reared bivalve larvae when exposed to the opportunistic bacterial pathogen *V. splendidus* in presence or absence of marennine. Blue mussel larvae, the most important shellfish aquaculture production in Canada from 1995 to 2016 (Fisheries and Oceans Canada, 2015-03-03 2015), were exposed to the opportunistic bacterial pathogen *V. splendidus* during bacterial challenge experiments with and without marennine added to the rearing medium. The bacterial community in the rearing medium and the blue mussel larval microbiota were characterized under different bacterial contamination and treatment conditions in order to better understand the importance of the larval microbiota in the prevention of mass mortality events with marennine as a natural water-additive. The bacterial community abundance in the rearing medium was characterized with flow cytometry and the diversity of communities in the rearing medium and larval microbiota were investigated using the denaturing gradient gel electrophoresis (DGGE) technique. Taken together, these analyses have helped to shed light on the host-pathogen-microbiota interplay during the rearing process of marine bivalve's larvae in hatcheries.

2.4 Material and methods

2.4.1 Marennine solution

Marennine was obtained from *H. ostrearia* culture produced as described in Gastineau et al. (2014) and Turcotte et al. (2016) and the pigment was purified by the method of Pouvreau et al. (2006) to obtain a solution in nanopure water (pH 7.2) that was autoclaved. The solution was filtered on 0.2 µm pore-sized cellulose acetate membrane before determining

the solution concentration by spectrophotometry at 656 nm with the specific extinction coefficient of 12 L g^{-1} cm^{-1} according to Turcotte et al. (2016).

2.4.2 Bacterial culture condition

V. splendidus 7SHRW, a strain isolated from the Gulf of St. Lawrence (Qc, Canada) (Mateo et al., 2009), was grown overnight in 10 mL of salty LB medium (25 g L^{-1} NaCl, pH 7.2, autoclaved) at room temperature prior to each experiment (bacterial growth kinetic and bacterial challenges). This strain is recognized for its ability to infect blue mussel larvae (Turcotte et al., 2016). After incubation, cells were centrifuged at 3000 g for 5 min and the cell pellet was washed twice in sterile physiological water (9 g L^{-1} NaCl, pH 7.2, autoclaved). Then, bacterial cells were suspended in sterile physiological water to obtain a stock solution at 10^9 cell mL^{-1}.

2.4.3 *V. splendidus* growth kinetic

The effect of marennine on *V. splendidus* growth was assessed by spectrophotometry. The three final concentrations of marennine used were 100 µg L^{-1}, 500 µg L^{-1}, 1000 µg L^{-1}. Negative controls without bacterial cells were used for each of the concentrations tested. A marennine-free positive control with bacteria was also included. Each condition was replicated 5 times. The experiment was performed in a 96-wells microplate in a final volume of 200 µL. Each well contained 100 µL of salty LB 2X medium (50 g L^{-1} NaCl, pH 7.2, autoclaved), 50 µL of *Vibrio* cells at 10^6 cell mL^{-1} (or physiological water, (9 g L^{-1} NaCl, pH 7.2, autoclaved) and 50 µL of marennine (or nanopure water (pH 7.2) as a control) at desired concentrations. Cell growth was estimated by measuring the suspension's OD at 595 nm every 30 min during 48 h. The OD reads were performed automatically with a SpectraMax 190 (Molecular devices, CA, USA) plate reader running the SoftMax Pro software. Growth curves were obtained by plotting OD values against time. Specific growth rate was es-

timated from the slope of the ln-transformed curves as recommended by Beaulieu et al. (2015). Growth inhibition was calculated by comparing the OD values of the curves from each tested marennine concentration after 24 h to the OD value of the positive control.

2.4.4 Bacterial challenges

2.4.4.1 Blue mussel larvae rearing conditions

Spawning adults from a pure population of *M. edulis* (Tremblay et al., 2011) were obtained from St. Peters Bay (Prince Edward Island, Canada; 46.4281° N, 62.6422° W) in June 2017. The latter were used to produce gametes and ultimately larvae following the usual protocol used by R. Tremblay's laboratory (Turcotte et al., 2016). Spawning was induced by thermal shocks from 5°C to 20°C and D-larvae (9 days-old) and metamorphosed post-larvae (29 days-old) were both used for bacterial challenges. All the rearing process was carried out at the *Station aquicole de Pointe-au-Père* of the UQAR/ISMER (Rimouski, Qc, Canada).

2.4.4.2 Treatments

Bacterial challenges were carried out at the UQAR/ISMER's marine microbial ecology laboratory (Rimouski, Qc, Canada) to assess the larvae's microbiota response to the exposure to the opportunistic bacterial pathogen *V. splendidus* and marennine. The experiments were performed in 3 Fernbach flasks (Thermo Fisher Scientific, USA) for each treatment applied at room temperature. A concentration of 10 larvae mL^{-1} was used during the bacterial challenges in a volume of 2.5 L.

Both D-larvae and metamorphosed post-larvae were exposed to four different treatments (three replicates of each): a control without *V. splendidus* or marennine (C), a pathogen treatment with only *V. splendidus* at a final concentration of 10^6cell mL^{-1} (V), a marennine treatment with only marennine at a final concentration of 500 $\mu g\, L^{-1}$ (M) and a treatment

in which larvae were exposed to both *V. splendidus* and marennine at the same concentrations used in V and M treatments (MV). All treatments were performed in UV-treated and filtered (0.2 µm pore-sized filter) sea water from the St. Lawrence estuary (Qc, Canada) having a salinity of 23.09 for the first experiment (D-larvae) and 23.11 for the second experiment (post-larvae). For the purpose of this article, treatments C and M will be referred as unchallenged larvae and treatments V and MV will be referred as challenged larvae.

2.4.4.3 Sampling

Before the challenge experiments, the initial bacterial communities in the seawater inhabiting the rearing medium in the culture tanks (T0) were characterized by sampling 1 L seawater used to fill the tanks in triplicates that were filtered on a 0.2 µm pore-sized Durapore filter (Ø 47 mm) and frozen at -80°C before further analyses. The larval microbiota prior to the challenge experiment was characterized to assess the initial communities (T0). Approximately 10 000 larvae (10 larvae mL^{-1}) were collected on 50 µm mesh, gently washed with sterile seawater (filtered on 0.2 µm and autoclaved) and immediately frozen at -80°C until DNA extraction. During the challenge experiments, two samples of 1 L of each flask were collected after 1 h and 96 h of exposition after gently homogeneous mixing and filtered on 50 µm mesh to collect the larvae. The collected larvae were treated as already described and frozen at -80°C. A sample of 4 mL of the 50 µm mesh filtered water was fixed in the dark for 15 min with 0.2% glutaraldehyde at pH 7 and then frozen at -80°C until further bacterial abundance analyses. Finally, the remaining water was filtered on a 0.2 µm pore-sized Durapore filter (Ø 47 mm) to collect the bacteria present in the rearing medium and frozen at -80°C for DNA extraction.

2.4.4.4 Survival rate

After 96 h of exposition, three samples of 10 mL of rearing medium containing at least 30 individuals were taken to assess the larvae survival rate. The larval survival rate (%) was obtained by calculating the ratio (number of live larvae/total number of larvae) between the control (C) and the treatments (M, V and MV). The control survival rate was set as 100%. In addition, the shell's length of 30 live larvae was measured to monitor the potential effect of marennine on the larval growth during the experiments. Larvae were examined and photographed under 100X with a microscope Olympus BX41 coupled to an Evolution VF camera and use of Image Pro Plus software v5.1 (Media Cybernetics, Silver Spring, MD, USA). The mean of the three counts from each tank was used for statistical analyses.

2.4.4.5 Total bacteria abundance

The total free abundance in the rearing medium was assessed by flow cytometry using a CytoFlex Flow Cytometer (Beckman Coulter Inc., Mississauga, Canada). Frozen samples were thawed at room temperature and then stained in the dark with 0.3 µL of SYBR Green I (10 000X, Invitrogen, Thermo Fisher Scientific, USA). Fluorescent beads (Fluoresbrite YG microsphere 1 µm, Polysciences) were added to each sample prior to their analyses as an internal standard (Lebaron et al., 2002). The sample volume analyzed was determined by weighing the tubes before and after the analyses. Then, the abundance was determined with the latter volume and the number of counted events. Data analyses were performed with the FCSalyzer software (version 0.9.14, Free Software Foundation Inc., Boston, USA). Total bacteria were detected by plotting the green fluorescence recorded at 530 nm (FL1) versus the side angle light scatter (SSC).

2.4.4.6 Bacterial diversity

Bacterial 16S rDNA gene amplification and purification

Total DNA was extracted from larvae and the filters containing the bacteria from the rearing medium using the E.Z.N.A. Mollusc DNA kit (Omega Bio-Tek, Norcross, USA) according to the manufacturer's instructions. Prior to each DNA extraction, larvae were gently washed with physiological water and crushed in a sterile 1.5 mL Eppendorf tube containing 350 µL of ML1 buffer from the extraction kit and the filters were cut into pieces then transferred in a sterile 1.5 mL Eppendorf tube. The extracted DNA from the larvae contained the DNA from the larvae itself, the bacteria within the larvae (microbiota) and the bacteria attached to the shell of the larvae.

The bacterial 16S rDNA gene was amplified by PCR using the universal primers 341F-GC (5'-CGC-CCG-CCG-CGC-CCC-GCG-CCC-GTC-CCG-CCG-CCC-CCG-CCC-GCC-TAC-GGG-AGG-GGA-GAG-3') and 907R (5'-CCG-TCA-ATT-CMT-TTG-AGT-TT -3') from Schäfer and Muyzer (2001). The mix was composed of 5 µL of 10X PCR buffer (QIAGEN, Hilden, DE), 200 µM of DNTPs (VWR, Radnor, USA), 50 pmol of each primers, 1 U of HotStart Taq polymerase (QUIAGEN, Hilden, DE), 200 ng of DNA and sterile water (q.s. water 50 µL). Amplifications were performed in triplicates then pooled to minimize the effect of PCR biases (Perreault et al., 2007). Briefly, a 500 bp fragment from the V4 region coding for the 16S sub-unit of the bacterial ribosome was amplified with the following PCR conditions: an initial denaturation at 94°C for 1 min, followed by 20 cycles consisting of a denaturation step at 94 °C for 1 min, a annealing step at 65°C for 1 min (touchdown of -0.5°C per cycle) and an extension step at 72°C for 3 min. Following these steps, there was 15 cycles consisting of a denaturation step at 94°C for 1 min, an annealing step at 55 °C for 1 min and an extension step at 72°C for 3 min. Finally, a last extension step at 72°C for 3 min. Amplicons were then purified using the MiniElute columns (QIAGEN, Hilden, DE). Purified amplicons were then kept at -20°C until bacterial diversity analyses.

Denaturing gradient gel electrophoresis (DGGE)

The bacterial diversity was assessed using the PCR-DGGE technique described in Schäfer and Muyzer (2001). Analyses were performed with a DGGE-4001-Rev-B (C.B.S. Scientific Company, CA, USA) following Schäfer and Muyzer (2001) recommendations. A denaturing gradient from 30 to 70% was used to allow a good discrimination of operational taxonomic units (OTUs). The migration was performed at 100 V for 16 h and temperature of 60°C. After migration, gels were stained with SYBR Gold at a final concentration of 3X (10000X, Invitrogen, Thermo Fisher Scientific, USA) during 1 h in the dark. A photograph was taken of each gel using UV light (AlphaImager HP, Alpha Innotech, CA, USA).

2.4.4.7 Statistical analyses

Larval survival rates were compared using a one-way ANOVA with the treatment (C, M, V and MV) as a factor. The shell's lengths were compared between treatments using a mixed model setting the 30 replications of the measure as the random effect to account for the pseudo-replications, replicates were the three tanks used per treatment and the factor the four treatments. The bacterial abundances in the rearing medium were compared by performing a two-way ANOVA with treatment and sampling time as factors. Where differences were detected, Tukey's HSD test was used as multiple comparison test to determine which means were significantly different. For each model, residuals were screened for normality using the normal probability plot and then tested using the Shapiro-Wilk's statistic. Homogeneity of variances was graphically assessed using residual.

DGGE profiles were analyzed using the Phoretix 1D Software (Non-linear Dynamics, Newcastle upon Tyne, UK) to obtain a presence-absence matrix from the detection of OTUs. Because the triplicates were similar, further analyses were performed with a single sample for each treatment for each larval stage. The latter matrix was then used to calculate distances between sample's profiles from the Jaccard dissimilarity index. Hierarchical analyses

were performed with the UPGMA algorithm to form clusters based on the previously calculated dissimilarity index. The fingerprints of the communities from the rearing medium and the D-larvae microbiota after 96 h of exposition were compared to investigate the resemblance between both communities. The initial communities (T0) were also compared with each treatment. The proportions of matches (%) in terms of the number of shared OTUs between samples were retained.

The variation of the number of conserved, gained and lost OTUs for the D-larvae in the treatments M, V and MV in regard to the control (C) and the initial community (T0) after 96 h of exposition were used to identify variations of OTUs between treatments in regards of the match mismatch analysis presented previously. The total number of OTUs for each treatment, the number of unique OTUs in each treatment and the number of common OTUs between treatments were assessed.

2.5 Results

2.5.1 *V. splendidus* growth kinetic

When exposed to the 4 different marennine concentrations, the lag time of *V. splendidus* ranged from 6.8 h to 7.2 h and the specific growth rate ranged from $0.17\,h^{-1}$ to $0.19\,h^{-1}$ (table 1). No statistical differences were found for the specific growth rate of *V. splendidus* between tested concentrations ($F_{3,16}$=0.27, p=0.84) in regards of the lag time ($F_{3,16}$=2.92, p=0.07).

The exposition to different concentrations of marennine did not result in inhibition of *V. splendidus* growth. All calculated growth inhibitions (%) were under 10%, and therefore marennine concentrations under $1000\,\mu g\,L^{-1}$ were considered as non-effective on the growth of this bacteria (table 1).

Table 1: Effect of marennine on *V. splendidus* 7SHRW lag time, specific growth rate and growth inhibition (standard deviation is shown between parentheses).

Marennine concentration ($\mu g\,L^{-1}$)	Lag time (h)	Specific growth rate (h^{-1})	Growth inhibition (%)
0	7.0 (0.00)	0.19 (0.017)	NA
100	7.0 (0.71)	0.19 (0.013)	-1.70
500	6.8 (0.45)	0.18 (0.006)	4.62
1000	7.2 (0.45)	0.17 (0.013)	6.93

2.5.2 Larval growth and larval survival rate

The mean shell length measured on the D-larvae after 96 h was 183.5 µm. There was no statistical difference of the shell's length between treatments for the D-larvae ($F_{3,8}$=0.78, p=0.54).

In contrast, D-larvae we observed after 96 h of exposition significant survival rate variation between treatments (near 25%; $F_{3,8}$=16.44, p<0.001; figure 1), with a significant lower value for *Vibrio* challenged larvae (p<0.001). No statistical difference was detected among the survival rates of the post-larvae from the different treatments with maximal variation observed lesser than 20% ($F_{3,8}$=1.93, p=0.20).

2.5.3 Bacterial communities

2.5.3.1 Abundance

Cell abundance analyses in the rearing medium showed significant differences between the treatments (C, M, V and MV), the sampling time (1 h and 96 h) and the interaction of these factors (treatment:time) for the both larval stages (table 2).

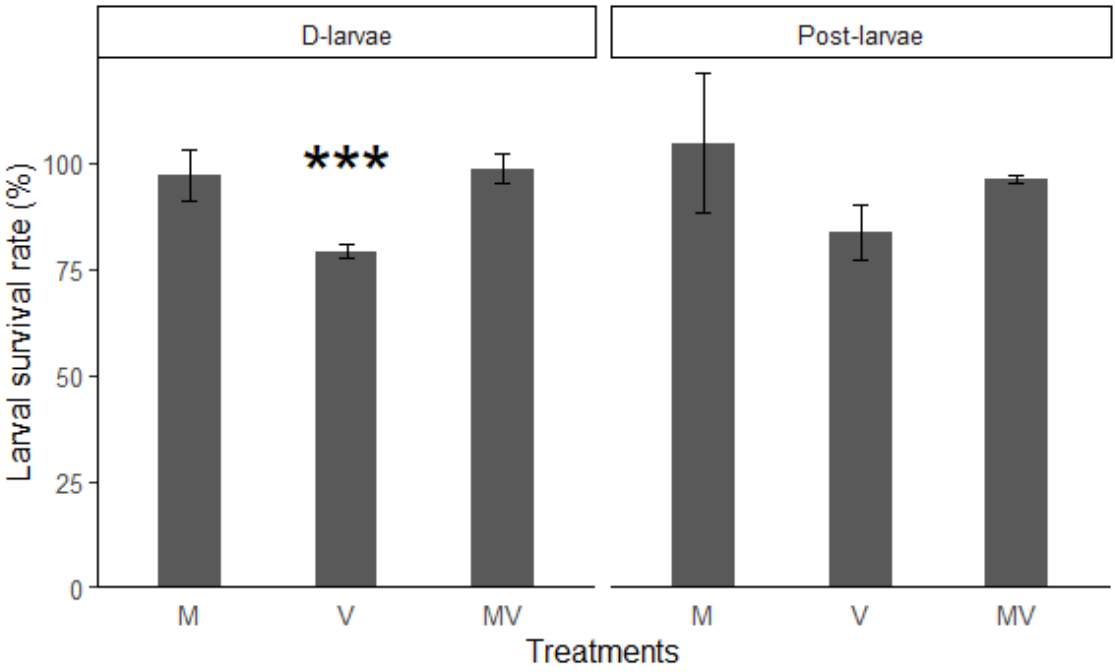

Figure 1: Larval survival rates (%) for the D-larvae and the post-larvae after 96 h of exposition to each treatment (C: control, M: marennine, V: vibrio and MV: marennine + vibrio; error bars: standard deviation; ***: significant difference ($p<0.001$)).

In the flasks of unchallenged larvae (C and M), the abundance of bacteria after 1 h of exposition was under 0.5 x 10^5 cell mL^{-1} and significantly increased by 3 to 5 fold after 96 h for both treatments (figure 2). In the flasks containing challenged larvae (V and MV), bacteria abundances after 1 h of exposition were over 7 × 10^5 cell mL^{-1}, translating the artificial addition of pathogen cells to attain a final concentration of 10^6 cell mL^{-1}, and significantly decreased to less than 2.5 × 10^5 cell mL^{-1} after 96 h (figure 2). The presence of marennine did not significantly affect bacteria abundances measured in the flasks after 96 h (figure 2) of exposition as the values are more similar than the Vibrio treatment (figure 2).

Table 2: Two-way ANOVAs results for the cell abundance analyses for both larval stages (D-larvae and post-larvae) with the treatment (C, M, V and MV) and the sampling time (1 h and 96 h) as factors and the interaction between both factors.

Larval stage		DF	F	p-value
D-larvae	Treatment	3	50.7	<0.001
	Time	1	53.9	<0.001
	Treatment:Time	3	88.1	<0.001
	Residuals	16	-	-
Post-larvae	Treatment	3	188.4	<0.001
	Time	1	356.4	<0.001
	Treatment:Time	3	228.0	<0.001
	Residuals	16	-	-

2.5.3.2 Diversity

Microbial diversity

After 96 h of exposition, the D-larvae microbiota genetic fingerprints were separated in two clusters. A first cluster composed of the fingerprint from the initial larval microbiota (T0), the control (C) and the challenged marennine-treated D-larvae (MV) and a second one composed of the fingerprint from the challenged (V) and marennine-treated (M) D-larvae microbiota (figure 3). For the post-larvae microbiota, the fingerprints of the challenged larvae (V and MV) are clustered with the unchallenged marennine-treated post-larvae microbiota (M) (figure 3).

The total number of OTUs decreased from 26 in T0 to 20 in the MV treatment. The total number of OTUs increased from 20 (T0) to 35 in V and 41 in M in the microbiota fingerprints from the second cluster (figure 3). In the rearing medium, the fingerprint of the challenged marennine-treated rearing medium of the D-larvae (MV) formed a cluster with

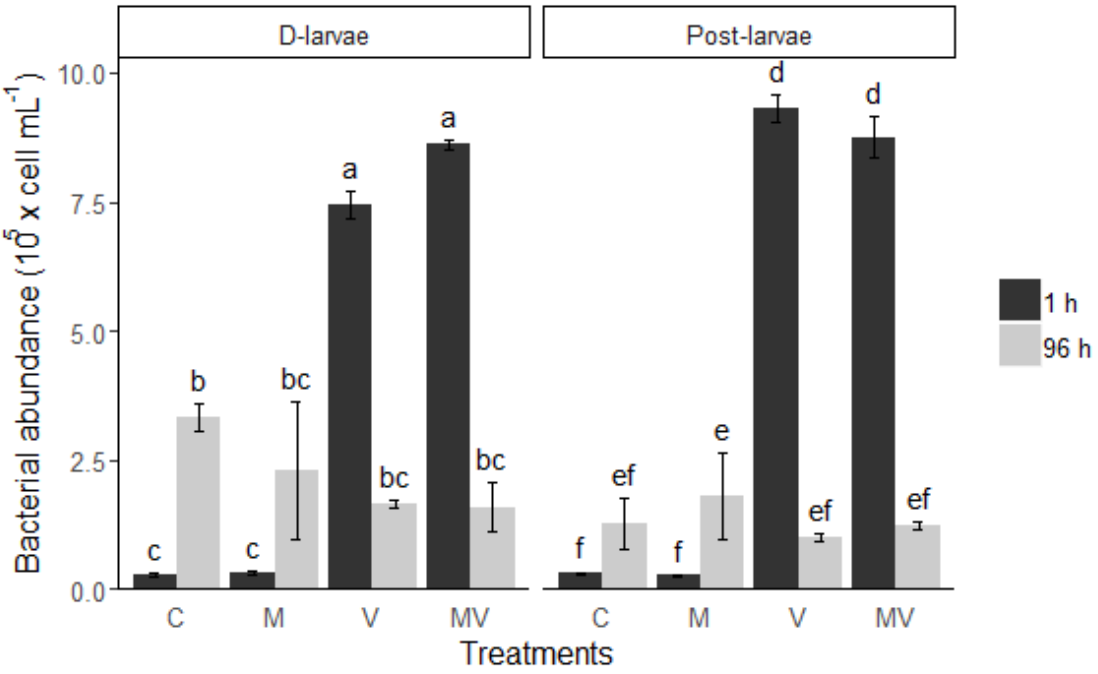

Figure 2: Bacterial abundance in the rearing medium after 1 h and 96 h of exposition to each treatment (C: control, M: marennine, V: vibrio and MV: marennine + vibrio; standard deviation is shown with error bars; letters indicate groups formed by the Tukey's HSD post-hoc analysis and one set of letters (a, b and c) were assigned to the analysis of D-larvae while another set (d, e and f) were assigned to the post-larvae analysis.

the control (C), both with a decreased total number of OTUs representing respectively 20 and 26 compared to the initial community (T0) which had 43 OTUs in total (figure 3).

Notably, both communities from the rearing medium and the microbiota from the challenged D-larvae marennine-treated treatment (MV) has a total of 20 OTUs (figure 3) from which only 11 were common to the rearing medium and the microbiota. For the D-larvae and the post-larvae, the treatment M is less dissimilar from the initial communities (T0) in the rearing medium with 28.57% and 66.68% respectively (figure 3).

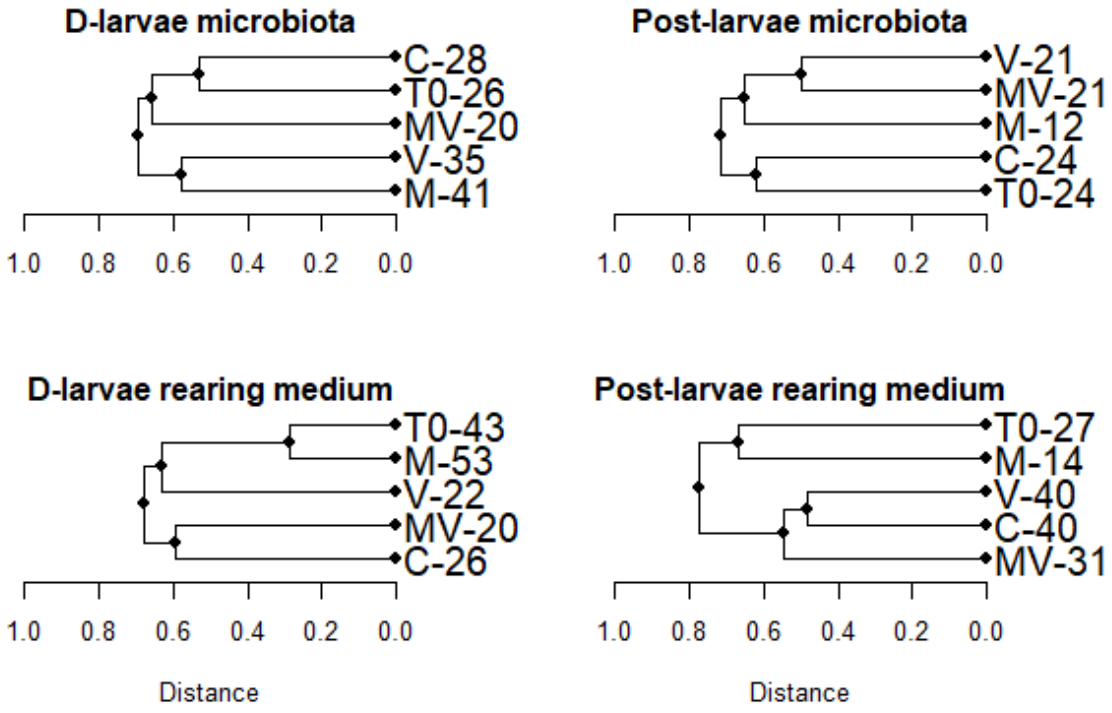

Figure 3: Dendrograms of the genetic fingerprints of the bacterial communities sampled in the microbiota and the rearing medium of blue mussel D-larvae and post-larvae at the beginning of the experiment (T0) and exposed to four different treatments (C: control, M: marennine, V: vibrio and MV: marennine + vibrio) during 96 h. The cluster analysis was based on the Jaccard coefficient similarity indicator and the dendrograms were constructed with the UPGMA algorithm using the vegan package (version 2.5-1) built for R (version 3.5). Numbers are the total number of OTUs recorded in each treatment.

Match-mismatch between the rearing medium and the larval microbiota diversity

The proportion of matches between the community in the rearing medium and the community composing the microbiota of the D-larvae was 34.2% for T0 and 34.4% for the treatment C. This proportion was more important for the treatment M and V being respectively 67.5% and 41.9%. The proportion of matches in the MV treatment was lowest with 26.9% (figure 4).

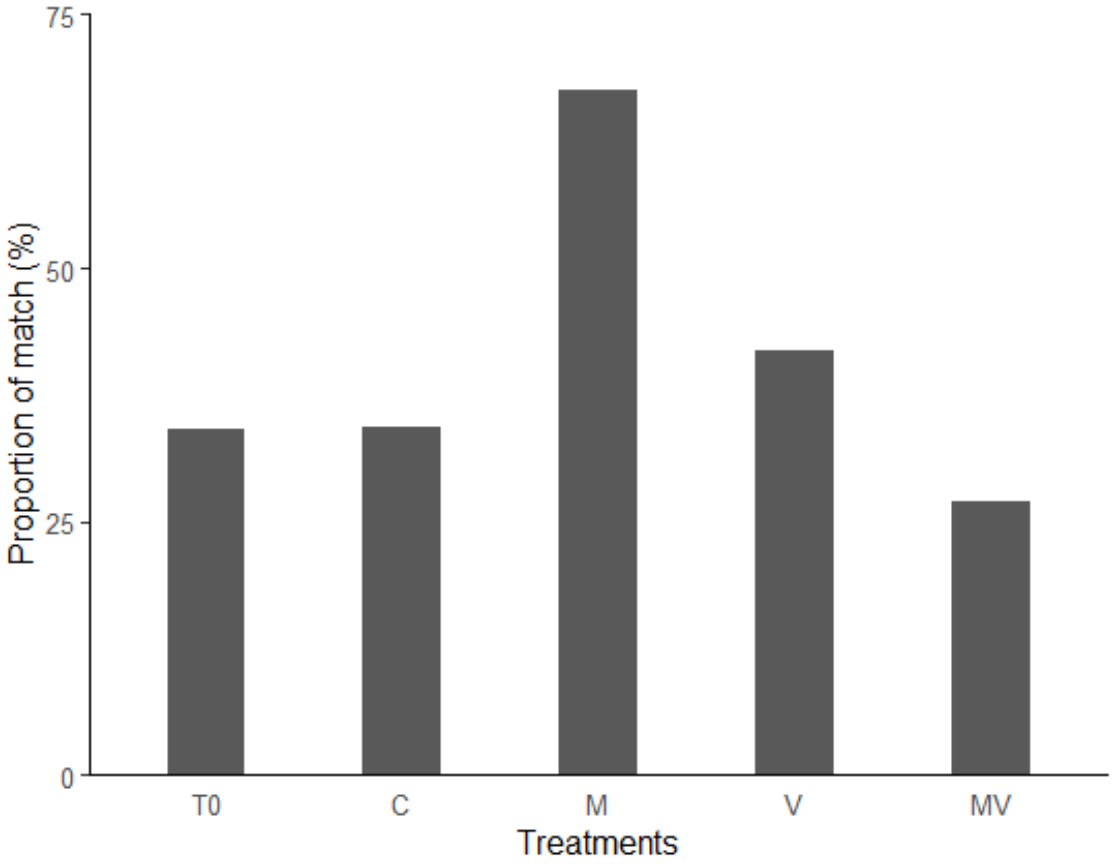

Figure 4: Proportion of matches against mismatches in the comparison of the communities of the D-larvae rearing medium and the larval microbiota for each treatment (C: control, M: marennine, V: vibrio and MV: marennine + vibrio) and the initial communities (T0).

Comparison of microbial communities

The simultaneous comparison of the initial community (T0), the control (C) and each treatment (M, V, and MV) allowed determining which OTUs were lost, conserved and gained among the different treatments. The treatment M, in regards of the larval microbiota and the rearing medium, was the one that lost the less OTUs that were either shared between T0 and C or unique to the treatment C (figure 5). The number of OTUs gained or conserved was dissimilar between the D-larvae microbiota and the rearing medium. For example, in the treatment M, 22 OTUs were conserved from the initial community in the rearing medium and only 4 in the larval microbiota. In terms of lost OTUs, the treatments V and MV were similar, except for the OTUs lost that were unique to the initial microbiota (T0). The challenged marennine-untreated D-larvae microbiota (V) conserved 7 OTUs from T0 and the challenged marennine-treated D-larvae (MV) conserved only 1 OTU from T0 (figure 6). This difference is less important in the rearing medium for the same treatments (figure 5). In the rearing medium, treatment M gained 10 unique OTUs while both V and MV gained 5 unique OTUs each (figure 5). The difference in the number of unique OTUs gained is even greater than in the microbiota for the treatment M. There were 19 unique OTUs found in the larval microbiota from the M treatment, 13 unique OTUs in the treatment V and 4 in the treatment MV (figure 5).

2.6 Discussion

The future of the development of shellfish aquaculture is often related to stable production juveniles potentially having controlled genetic characteristics. However, the larval rearing process can create an environment conductive to the development of opportunistic bacterial pathogens like *V. splendidus*. Controlling these pathogens in the rearing environment is crucial to ensure a stable juvenile production. As new alternative methods to the harmful usage of antibiotics in aquaculture, marennine, a biomolecule extracted from a diatom is becoming an interesting way of limiting the impact of pathogens (Gastineau et al.,

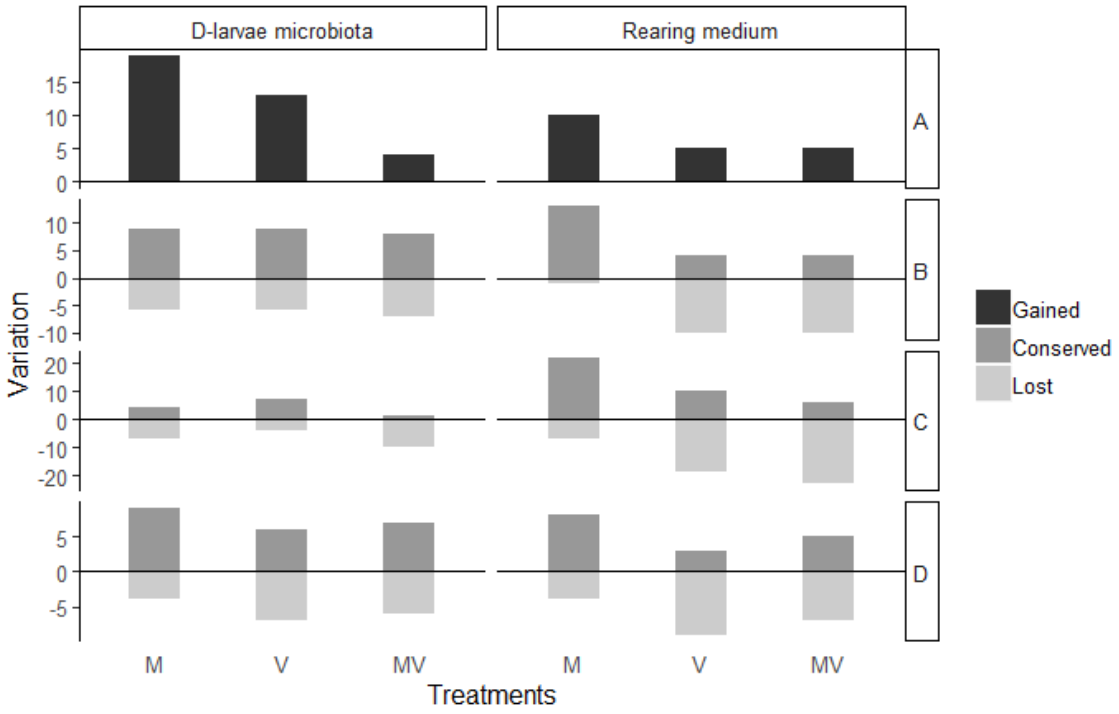

Figure 5: Simultaneous comparison of the initial community (T0) and the control (C) after 96 h of exposition with all other treatments (M, V and MV) in regard of a) the unique OTUs Gained in each treatment, b) the Conserved OTUs between T0 and the control (C), c) the Unique OTUs from the initial community (T0) and d) the unique OTUs from the control (C).

2014; Falaise et al., 2016; Turcotte et al., 2016). In our study, we confirm the beneficial effects of marennine on blue mussels larvae challenged with *V. splendidus* and investigated the marennine effect in regards of the response of the bacterial communities in the system.

2.6.1 Antibacterial activity of marennine on *V. splendidus* 7SHRW

When investigating the beneficial effects of a new biomolecule against bacterial diseases, one of the first hypotheses to be tested would be that the molecule of interest exerts an antibacterial effect. Our results do not show a direct antibacterial effect of marennine at a concentration of 500 µg L^{-1} and therefore an antimicrobial effect on the pathogen *V. splen-*

didus itself is unlikely to contribute to the observed beneficial effects documented by Turcotte et al. (2016). It has been previously reported that marennine demonstrates an inhibitor effect on the growth of several *Vibrio* species in previous experiments. Falaise et al. (2016) assessed the sensitivity of various species from the *Vibrio* genus (*V. tasmaniensis*, *V. aestuarianus*, *V. coralliitycus* and *V. tubiashii*) to marennine at different concentrations ranging from 1 mg L^{-1} to 100 mg L^{-1}. Results showed concentration-dependant inhibitor effects on the growth kinetics of all the tested species for marennine concentrations higher than the one used in our experiments. Hence, the main objective of the present study which is to investigate a potential larval microbiota modification leading to a better resistance against the pathogen *V. splendidus*. Turcotte et al. (2016) observed that marennine had a lethal effect on *M. edulis* D-larvae at a concentration as low as 1000 µg L^{-1}. The marennine concentration used during their bacterial challenge experiments was therefore reduced to 500 µg L^{-1} to avoid the lethal effect of the pigment on the larvae during the rearing procedure.

2.6.2 Observed beneficial effects of marennine during bacterial challenges

The exposition of the larvae to marennine during bacterial challenges did not result in a measurable inhibition or an enhancement of the larval growth unlike the results obtained by Turcotte et al. (2016). The effect of marennine on larval growth might only be observable after a longer exposure to the bioactive molecule. Turcotte et al. (2016) exposed D-larvae to marennine for 20 days and observed significantly bigger shell when the larvae were exposed to marennine at 100 µg L^{-1} and accumulation of energetic lipids.

The effect of marennine on the D-larvae survival after 96 h of exposition was similar to the results published by Turcotte et al. (2016) with no significant difference between the survival rate of the control (C) and the corresponding treatments M (marennine) and MV (marennine and Vibrio). In the same way, the survival rate of the marennine untreated challenged D-larvae with Vibrio (V) was lower than the other treatments. Thus, our result confirms that marennine had a beneficial effect on the larval survival rate of the D-larvae

when challenged against the opportunistic pathogen *V. splendidus*. The survival rates from the bacterial challenge experiment conducted with post-larvae were not significantly different between the treatments including the control (C). The lethal effect from the exposition to a pathogen known to cause mass mortality in the rearing systems was not observed for that ontogenic stage, suggesting a better resistance to pathogens after metamorphosis, as suggested in the genomic studies of Bassim et al. (2014, 2015a). As an example, the susceptibility of invertebrates to pathogens before and after metamorphosis is suggested to be partially linked with the prophenoloxidase (proPO) system which is recognized as an important component of the innate immune system (Smith and Söderhäll, 1991). This system is responsible for the non-self-recognition and some components of the cascade-like phenoloxidase have been linked with the opsonisation of foreign cells in invertebrates. Dyrynda et al. (1995) has demonstrated that phenoloxidase was active in the blue mussel larvae's cells and that the latter cells were capable of phagocytosis when exposed to *E coli*. The phagocytosis rate of the bacterial cells within the larvae was much lower than the rate measured in post-larvae and adults. Similar observations were made with the Pacific oyster (*Crassostrea gigas*) by Thomas-Guyon et al. (2009). The activation of this innate defence against pathogens after the blue mussel's metamorphosis might contribute the higher survival rate observed for the challenged post-larvae during our experiments whether or not marennine-treated.

The beneficial effect of marennine was only observed when exposing mussel D-larvae to *V. splendidus* and marennine at $500\,\mu g\,L^{-1}$ for 96 h. The larval survival rate increased without a significant difference in the final shell length indicating that the beneficial effect of marennine is unlikely to be due to a change in the physiological state of the larvae that impacts the shell's growth. However, Turcotte et al. (2016) observed an accumulation of 40% more triacylglycerol in blue mussel larvae exposed to marennine for 20 days. This trend suggests that marennine has a slight effect on the larvae physiological state, but these authors were not able to link this physiological change to an effect of marennine on neither the feeding behaviour of the larvae nor the accumulation of lipids reserves. A change in the larval microbiota might be another explanation for the observed beneficial effects of marennine

on the larval survival rate against a pathogen than a direct influence of the biomolecule on the host's physiology.

Even though marennine did not show an antimicrobial effect on a pure culture of *V. splendidus*, the effects of the same bioactive molecule might differ when exposing it to a complex community. It could modify the taxonomic diversity or the cell abundance of the indigenous bacterial communities already in place by directly interacting with the bacteria or by modifying the conditions in the rearing medium thus modifying the bacterial assemblage. In the rearing medium of the unchallenged larvae (C and M), the increase of the bacterial abundance between 1 h and 96 h of exposition is most likely due to the normal development of the bacterial community in UV-treated and filtered sea water used to perform the experiments. The rearing medium was probably colonized by the microorganisms from the rejection of larvae's feces and microorganisms attached to the shell of the larvae.

In the rearing medium of the challenged larvae (V and MV), the high abundance of bacterial cells after 1 h of exposition is most likely due to the artificial addition of *V. splendidus* cells. Notably, the abundance of bacterial cells decreased after 96 h to reach the same abundance detected in the rearing medium of the other treatments (C and M) suggesting a potential ingestion of the suspended cells by the larvae. Dubert et al. (2016) demonstrated that shellfish larva tissues were colonized within 2 h during a challenge experiment against the bacterial pathogen *V. splendidus*. The observed beneficial effects of marennine on challenged larvae survival rate might come from a modification of the diversity of bacteria composing the larval microbiota and the larvae themselves, hence the main objective of this study.

The analysis of the bacterial diversity in the rearing medium and the larval microbiota from the larvae exposed to marennine and the pathogen revealed high dissimilarity between the control (C) and the treatments (M, V and MV) and between each treatment. These results suggest that each treatment from the challenge experiment induced a microbial diversity modification (a different balanced state compared to microbial communities from healthy individuals) in both the rearing medium and the larval microbiota. The modification

that occurred in the larval microbiota from the challenged larvae (V) most likely induced a microbial dysbiosis which is known to occur in invertebrates' microbiota when exposed to pathogenic *Vibrio* sp. (Rungrassamee et al., 2016; Xia et al., 2018). These pathogens-induced imbalances in the host microbiota can potentially impair the physiology of the host and therefore modulate its immune response against infections (De Schryver and Vadstein, 2014). During an infection, opportunistic bacterial pathogens like *V. splendidus* outcompete other taxa and cause a microbial imbalance modifying the interactions between the host, the microbial communities (in the rearing medium and the microbiota) and the environment.

Interestingly, the exposition of mussel larvae to marennine seems to be related to a modification of the bacterial community in the rearing medium and in the larval microbiota as well when compared to the control. The latter results suggest that marennine has an impact on the environmental conditions of the rearing medium thus modifying the community contained within. A change of diversity in the bacterial community in the rearing medium or in the larval physiological state due to the use of marennine might result in an observable change in the selection of the bacterial taxa recruited to form the larval microbiota. Surprisingly, the treatment combining an exposition to *V. splendidus* and to marennine exerted a significantly important dissimilarity of the bacterial diversity of both the rearing medium and the larval microbiota compared to the other treatments. In other words, the simultaneous exposition to the bacterial pathogen and marennine (MV) resulted in a different bacterial community diversity compared to those sampled from the treatments M and V. A synergetic effect between *V. splendidus* and marennine might contribute to the observed beneficial effect of marennine on the larval survival rate of the challenged marennine-treated D-larvae.

Assessing the link between the microbial diversity in the rearing medium and the larval microbiota allow to determine how the bacterial community in the rearing medium influences the composition of the larval microbiota or vice versa. The proportion of match-mismatch of OTUs detected in the samples from the rearing medium and the D-larvae microbiota assess how the link between those two communities changes under different con-

ditions. Surprisingly, the treatments M and V seemed to increase the resemblance between the rearing medium and the larval microbiota and the treatment MV seemed to decrease that proportion slightly under the one found in the control (C) and the initial time point (T0). Somehow, the combination of the exposition of D-larvae to *V. splendidus* and marennine (MV) resulted in a weaker link between the rearing medium bacterial community and the larval microbiota. Because mussel larvae are filter feeders, a modification of the bacterial community in the rearing medium should influence the composition of the larval microbiota assuming that larvae harvest and maintain environmental bacteria to form their microbiota. In contrast, our data suggest that mussel larvae maintain a uniquely composed microbial community as their microbiota. This trend in our data should be taken carefully since there was no replication. However, it is known that mussels maintain a different bacterial community from the rearing environment as their microbiota. Prieur (1982) presented data on the differences between the cultivable bacterial community of the normal microflora of *M. edulis* larvae and the rearing medium showing a clear dissimilarity between the two communities. Nevertheless, these results need to be cautiously interpreted due to the cultivation techniques used during the experiments. More recently, many studies using cultivation-free molecular techniques showed that the link between the surrounding environment (natural waters or rearing mediums) and shellfish microbiota is not as direct as previously thought (Chauhan et al., 2018). The host tissues should be considered as a unique niche with unique conditions (physiological state of the host and bacterial interactions balance) that constraint the bacterial settlement and colonization processes leading to a different community structure and diversity from the surrounding water.

Comparing the sampled bacterial communities of each treatment (M, V and MV) after 96 h of exposition with the initial community (T0) and the community found in the control (C) after 96 h allows characterizing the microbial imbalance in terms of OTUs gained, conserved and lost. In the rearing medium, marennine seemed to cause an important gain of unique OTUs and the conservation of a more important proportion of OTUs compared to the other treatments, which are similar. This similarity between the bacterial communi-

ties from the treatment V and MV suggests that the synergetic effect previously mentioned do not take place in the rearing medium but rather in the larval microbiota. The community from the challenged marennine-treated D-larvae microbiota (MV) gained less unique OTUs and conserved less OTUs from the initial microbiota (T0) compared to the two other treatments (M and V). Surprisingly, a different pattern in the rearing medium was observed compared to the larval microbiota. These data support the idea of a potential synergetic effect of marennine with the opportunistic bacterial pathogen *V. splendidus* on the microbial diversity of the D-larvae resulting in an observable beneficial effect on the larval survival rate. The influence of the composition of one's microbiota on the host health status has been extensively studied in many types of organisms. Zilber-Rosenberg and Rosenberg (2008) developed the hologenome theory of evolution that considers the host and its associated microorganisms as a whole unit, the holobiont, uniquely responding to biotic and abiotic stressors. In addition, the high genetic diversity of the microbiota is described as a component capable of extending the range of environments in which the holobiont is able to establish in and thrive. The presence of marennine in the rearing medium of the D-larvae exposed to the pathogen might have modified the larval microbiota's diversity via direct interactions of marennine with the microbial cells or by modifying the microbial recruitment by the larvae, resulting in a modification of the larvae sensibility toward *V. splendidus* infections.

2.6.3 Conclusion and perspectives

To conclude, our study demonstrated that marennine, a bioactive molecule extracted from the diatom *H. ostrearia* culture, did have a beneficial effect on the larval survival rate of blue mussel D-larvae when challenged with the opportunistic bacterial pathogen *V. splendidus*. Moreover, our data clearly demonstrate that the exposition of D-larvae to marennine and pathogen was accompanied by a modification of the microbial diversity in the rearing medium and in the larval microbiota. To go further, metagenomics analyses would

allow investigating the latter modification even deeper and identifying in detail how microbial diversity is modified and how this modification may affect the host immune response. Nonetheless, our data strongly suggest that a coupled effect of marennine and *V. splendidus* contributes to the increase of the larval survival rate of the D-larvae. A better understanding of the mode of action of the bioactive molecule marennine is crucial to expand and regulate its usage in shellfish aquaculture. Recent work on the influence of the environment on the fluidity and dynamics of phospholipids in the cell membrane of *V. splendidus* by Bouhlel et al. (accepted for publication 2019) will certainly benefit to our understanding of the mode of action of marennine.

Notably, only the survival rate of the challenged D-larvae was lower indicating that post-larvae are able to survive the exposition to *V. splendidus*. Marennine seems useful preferentially with the larval stages before the metamorphosis. These results are of interest for the development of marennine-based prophylactic treatments and optimize its application to prevent mass mortality events caused by the opportunistic bacterial pathogen *V. splendidus* in blue mussel hatcheries.

CONCLUSION GÉNÉRALE

L'objectif de cette étude était de déterminer si l'augmentation du taux de survie des larves de moules bleues en aquaculture par l'utilisation de la marennine s'accompagnait d'une modification de la composition spécifique du microbiote larvaire. Les travaux de Turcotte et al. (2016) ont démontré l'effet bénéfique de la marennine sur la survie larvaire en aquaculture. Toutefois, les données de Turcotte et al. (2016) n'ont pas permis d'inférer le mode d'action de la molécule derrière les effets observés. Lors de notre étude, l'utilisation de tests de provocation bactérienne a permis de caractériser la dynamique des communautés bactériennes associées au milieu de culture des larves et au microbiote larvaire en présence ou non d'un pathogène opportuniste et de marennine. Premièrement, il a été déterminé que la marennine à des concentrations variant de 100 $\mu g\ L^{-1}$ à 1000 $\mu g\ L^{-1}$, n'avait pas d'effet antibactérien sur *V. splendidus*. L'effet bénéfique de la marennine ne semble donc pas provenir d'une diminution de la charge bactérienne pathogène dans le milieu de culture. Les résultats des analyses d'abondance bactérienne dans le milieu de culture lors des tests de provocation bactérienne concordent avec cette observation : la présence de marennine dans le milieu de culture ne concorde pas avec une diminution de l'abondance de cellules bactériennes. Ces observations appuient l'hypothèse de départ stipulant que l'effet bénéfique de la marennine est lié à une modification de la composition spécifique des communautés microbiennes dans le milieu et plus particulièrement au microbiote larvaire, et non à une réduction de la charge microbienne.

L'analyse de la composition des communautés bactériennes du milieu de culture et du microbiote larvaire a révélé d'importantes différences entre les communautés dans chaque traitement, mais également entre le milieu de culture et le microbiote larvaire. Ces observations démontrent clairement que non seulement l'augmentation de la survie larvaire lors de l'utilisation de la marennine est accompagnée d'une modification des communautés bactériennes, mais également que les effets bénéfiques ne proviennent pas du maintien

d'une communauté semblable à celle obervée dans le contrôle. En effet, les communautés bactériennes exposées simultanément à *V. splendidus* et à la marennine sont fortement dissimilaires de celles observées dans les autres traitements. Les effets bénéfiques de la marennine pourraient donc être attribuables à un effet synergique entre la marennine et les communautés bactériennes dysbiotiques menant à une meilleure résistance des larves contre les pathogènes. Nos résultats permettent de confirmer l'hypothèse principale de ce travail en plus d'appuyer l'intérêt pour l'utilisation de la marennine comme technique de contrôle de l'occurrence des évènements de mortalité de masse en écloserie de bivalves.

Afin de mieux comprendre les effets de la marennine sur le microbiote larvaire des bivalves, il est essentiel de caractériser les processus biologiques intervenant dans cette interaction. À la lumière de nos résultats et de ceux de Turcotte et al. (2016), le mode d'action de la marennine semble être un processus complexe impliquant plusieurs niveaux d'interactions dont l'étude demande un plan expérimental permettant d'analyser la diversité taxonomique conjointement à la divertsité métabolique du microbiote. Il serait donc intéressant de compléter cette étude par une approche multi-omiques dans un plan expérimental permettant de construire des séries temporelles pour chaque traitement. L'analyse simultanée de plusieurs couches omiques comme le métagénome (diversité spécifique du microbiote), le métatranscriptome (réponses fonctionnelles) et le protéome ou le métabolome (changements physiologiques) permettrait de mieux caractériser l'impact de la marennine dans les systèmes d'aquaculture. En effet, l'utilisation d'analyses en réseaux et l'investigation des fluctuations dans le temps de ces derniers permettraient d'identifier les facteurs importants influençant les interactions entre les différents taxons bactériens en se basant sur les liens de causalité obtenus par des mesures empiriques. Cette technique d'analyse est notamment utilisée en gestion des ressources halieutiques afin d'identifier les principaux facteurs influençant les relations interspécifiques et la stabilité des communautés d'espèces d'intérêt commercial (Ushio et al., 2018). Établir ces liens de causalité entre la composition du microbiote et l'état physiologique de l'hôte demeure un défi important dans les travaux portant sur le microbiote. En effet, avec les techniques d'analyse actuelles se basant principalement sur

des corrélations et des similarités, il est difficile d'inférer les relations directes entre la composition spécifique d'un microbiote et l'état physiologique de l'hôte. Plus spécifiquement, l'étude des effets bénéfiques d'un traitement qui, supposément interagit avec les communautés bactériennes, est contrainte à demeurer descriptive ou sommairement quantitative à cause de la limitation de l'inférence des processus écologiques à partir de corrélations. L'analyse de ces réseaux dans un contexte d'étude du microbiote permettrait de lier les changements dans les communautés bactériennes à des modifications phénotypiques des larves de moules bleues. De tels résultats permettraient de bonifier l'utilisation de la marennine dans un contexte de production larvaire à vocation aquacole.

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