Probiotics and Antimicrobial Proteins
https://doi.org/10.1007/s12602-019-09629-3

From Farm to Fingers: an Exploration of Probiotics for Oysters, from Production to Human Consumption

Heidi Yeh [1] · Sarah A. Skubel [2] · Harna Patel [2] · Denia Cai Shi [2] · David Bushek [1] · Michael L. Chikindas [3,4]

Abstract
Oysters hold a unique place within the field of aquaculture as one of the only organisms that is regularly shipped live to be consumed whole and raw. The microbiota of oysters is capable of adapting to a wide range of environmental conditions within their dynamic estuarine environments; however, human aquaculture practices can challenge the resilience of this microbial community. Several discrete stages in oyster cultivation and market processing can cause disruption to the oyster microbiota, thus increasing the possibility of proliferation by pathogens and spoilage bacteria. These same pressure points offer the opportunity for the application of probiotics to help decrease disease occurrence in stocks, improve product yields, minimize the risk of shellfish poisoning, and increase product shelf life. This review provides a summary of the current knowledge on oyster microbiota, the impact of aquaculture upon this community, and the current status of oyster probiotic development. In response to this biotechnological gap, the authors highlight opportunities of highest potential impact within the aquaculture pipeline and propose a strategy for oyster-specific probiotic candidate development.

Keywords Oysters · *Crassostrea* · Aquaculture · Microbiota · Probiotics

Introduction: Oyster Industry Growth and the Challenges Posed by Disease

Aquaculture has become an economically important activity around the world with production now exceeding wild fishery harvests [1]. In comparison to fishing, aquaculture allows for selectively increasing production of species used for industry and consumption by humans [2]. The shellfish industry, including cultured shellfish (*Crassostrea gigas* and *Crassostrea virginica*), is valued at $323 million and comprises 35% of total industry value [3]. New Jersey, the authors' home state, has a growing oyster aquaculture industry that is supported by the Haskin Shellfish Research Laboratory (HSRL) at the Agricultural Experiment Station of Rutgers University [4]. In a survey conducted by HSRL staff, participants reported that they sold 2,029,500 cultivated oysters in 2016 at a total farm gate value of $1,370,060 [5]. With natural production being limited, depleted or declining, an increasing demand for seafood has created a large market opportunity for aquaculture products that have characteristically been more expensive to produce. Loss of these valuable cultured stocks to infectious diseases is an important issue affecting aquaculture production worldwide. For example, in 2005, a mass mortality event of Pacific oysters (*C. gigas*) took place on the East Frisian coast in Germany due to conditions that were favorable for infection e.g., reduced oxygen content, limited water exchange, reduced food availability, and higher pollution levels [6]. In addition, the changing climate has resulted in marine heatwaves that alter the abundance of opportunistic *Vibrio* species, specifically *Vibrio harveyi* and *Vibrio fortis*, associated with the Pacific oyster leading to mass mortalities where the oysters are farmed around the world [7]. Interventions that could prevent or reduce these significant losses of aquaculture stock to disease and heat shock could boost productivity significantly.

✉ Heidi Yeh
heidi.yeh@marine.rutgers.edu

1 Haskin Shellfish Research Laboratory, Rutgers State University, 6959 Miller Avenue, Port Norris, NJ 08349, USA

2 Department of Plant Biology, Rutgers State University, New Brunswick, NJ 08904, USA

3 Health Promoting Naturals Laboratory, School of Environmental and Biological Sciences, Rutgers State University, New Brunswick, NJ 08904, USA

4 Don State Technical University, Rostov-on-Don 344002, Russia

Published online: 13 February 2020

Oysters are one of the few animal foods consumed whole and raw by humans in western culture; therefore, the safety of this product can have significant health consequences for the consumer. Incidence of acute gastroenteritis—a *Vibrio parahaemolyticus*-related illness—has been increasing, with the majority of these cases occurring in association with the consumption of raw oysters [8]. In the summer of 2015, the largest outbreak associated with consumption of raw oysters harvested from British Columbia coastal waters occurred in Canada [9]. Although fully cooking oysters does eliminate the threat of bacterial illness, demand for raw oysters remains high. The risks associated with raw oyster consumption are relatively well-known but are insufficient to motivate consumers to choose processed options that carry a reduced risk of food-borne illness; consumers are unwilling to opt for postharvest-processed oysters if the taste, texture, smell, and esthetics of the oyster are altered [10]. Disease poses a health threat to both the oyster and the consumer, so significant care and attention are warranted to develop prophylactic and therapeutic methods of managing bacterial pathogens.

Probiotics for Farmed Oysters

Although used in some forms of aquaculture, antibiotics are not used in the cultivation of bivalve shellfish such as oysters. Given growing consumer concerns over the use of antibiotics in food production, the lack of antibiotics in shellfish aquaculture is a point of pride for the industry. In the absence of antibiotics, probiotics offer an alternative means by which pathogens can be inactivated. These live microbial supplements deliver in vivo benefits to the host through their specific attributes such as antimicrobial substance production, competitive exclusion of pathogens, immunomodulation, and commensal microbiota modulation [2]. When administered alive and at adequate concentrations, probiotics can have a favorable impact on the eukaryotic host health [2]. For example, an inefficient immune response was associated with juvenile oyster susceptibility to mass mortality events in Europe [11]. Developing probiotics for oyster immune support is a topic worth investigating to achieve benefits such as increased stock resilience to mortality events, hatchery water quality control, successful transition between growing environments, and enhanced growth at every life stage.

Candidate bacteria strains with probiotic potential are often derived from the digestive tract of the host as well as from its environment [12]. Presently, there are commercial probiotics prepared from different bacterial species such as *Lactobacillus* spp., *Bacillus* spp., *Carnobacterium* spp., *Enterococcus* spp., and the yeast *Saccharomyces cerevisiae* among others that are available for aquaculture use [2]. Unfortunately, the transfer of this technology to bivalve aquaculture as reliable products targeted to oysters has been slow. For example, probiotic candidate OY15—isolated from the oyster gut by NOAA Fisheries Milford Laboratory—is a promising supplement that has been shown to boost larval oyster survival [13]. However, the road to bringing OY15 to market includes challenges such as gaining FDA approval—which may prove difficult for this benign *Vibrio* strain—and finding a sponsor for commercial-scale production [14]. An organism-specific approach with natural isolates may be the key to unlocking the benefits of probiotics for a new market, so efforts such as these could provide large benefits to the oyster aquaculture industry.

The Oyster Microbiota

Current Understanding of Natural Oyster Microbiota

Oysters are sessile invertebrates that feed primarily on phytoplankton collected from the marine environment through filter-feeding activity. As a result of this connection with the surrounding water, the marine environment is an important determinant of oyster microbiota composition and function. A milliliter of seawater typically contains 10^4 CFU bacteria, 10^3 CFU fungi, and around 3×10^6 viruses [15]. Free-floating microbes are ingested by oysters, which filter up to 10 L of water per hour [16]. Microbes entrained in the feeding current of oysters may only impact the oyster temporarily, passing through the digestive system along with food particles and exiting the oyster as feces/pseudofeces. Those microbes that colonize the various organs and surface of the oyster exert a more persistent influence as part of the resident microbiota.

Despite this intimate connection with the surrounding seawater, oysters maintain a microbial community that is different from that of its environment. The unique environmental conditions within the oyster internal tissues foster anoxic/hypoxic conditions and combine with a high concentration of labile organic carbon from the oyster diet [17, 18] to create unique niches for microbial communities to form that differ from the source water. However, the oyster microbiota does respond to variation in its environment, to which the processes involved in aquaculture may add additional variability and stress or stability (e.g., constant temperature and salinity within a hatchery compared to field conditions). Studies of oysters for the market tend to sample the entire adult oyster meat [19, 20], which is a logical strategy for a product that is intended to be consumed whole. However, there are bacterial dynamics specific to each life stage and the various tissues of the oyster body that are useful to consider for effective probiotic application before and after harvest. Organs of the oyster differ in their exposure to the outside environment, richness of organic substrates, and aerobic/anaerobic conditions. The unique set of conditions in each organ selectively creates a characteristic microbial composition [21]. Researchers have used all of the tissues summarized in Fig. 1 to characterize the natural oyster microbiota [22].

Oysters can live in the higher salinities of the open ocean but are much more likely to thrive in the dynamic conditions of coastal estuaries. The activity of the oyster immune system can be compromised by exposure to high salinity conditions [23], manifesting as a positive correlation between salinity and disease/mortality in *C. virginica* [24]. Additionally, oyster predators and competitors are more abundant in the open ocean, so oysters are unable to effectively compete for their ecological niche in these marine environments. Therefore, both wild and cultured oysters grow optimally in coastal estuaries; this dynamic environment experiences daily shifts in variables including salinity, temperature, nutrients, and microbial composition. Significant evolutionary pressure has been placed on oysters growing in these environments to remain resilient to a broad range of conditions [25]. The oyster microbiota may be a part of the equation that has brought the oyster success in this highly variable, challenging, and competitive environment.

Natural variation in the oyster microbiota in response to environmental factors was recently reviewed with a focus on the changes that functional and genetic diversity drive in both transient and resident communities [26]. The majority of oyster microbiota studies have focused on the microbial composition of *C. gigas*, which is the most widely cultivated species and comprises over half of papers in the published literature (Fig. 2a). Its congener *C. virginica*, and multi-species studies, come in with the 2nd and 3rd largest number of studies, followed by a mixture of other species that collectively comprise 15% of the current literature on the oyster microbiota. Genetic methods such as 16S rRNA metabarcoding have facilitated a recent rapid rise in studies on the microbiota of all organisms, with a notable increase in studies being published in the last decade (Fig. 2b). Although our knowledge of oyster microbiota is rapidly advancing, much remains to be explored concerning their microbial interactions with the environment [18], as well as the impact of human activity (including unintentional pollution and intentional aquaculture practices) on these natural processes.

The Impact of Aquaculture on Oyster Microbiota

Each step in the aquaculture process introduces the potential for oyster microbiota disruption, as well as opportunities for remediation with probiotics. Young oysters may be collected from the environment as juveniles (aka "spat") when larval stages metamorphose and attach to hard surfaces. Alternatively, these can be produced in hatcheries from broodstock that have been induced to spawn to produce free swimming larvae that are typically cultured in static tanks containing sterilized seawater and fed mixtures of axenically cultured phytoplankton. The spat produced in the hatchery or collected from the wild are transplanted to containment systems such as cages or bags that allow the free inflow of phytoplankton-laden waters and outflow of wastes as the oysters develop further in the natural environment, where they are

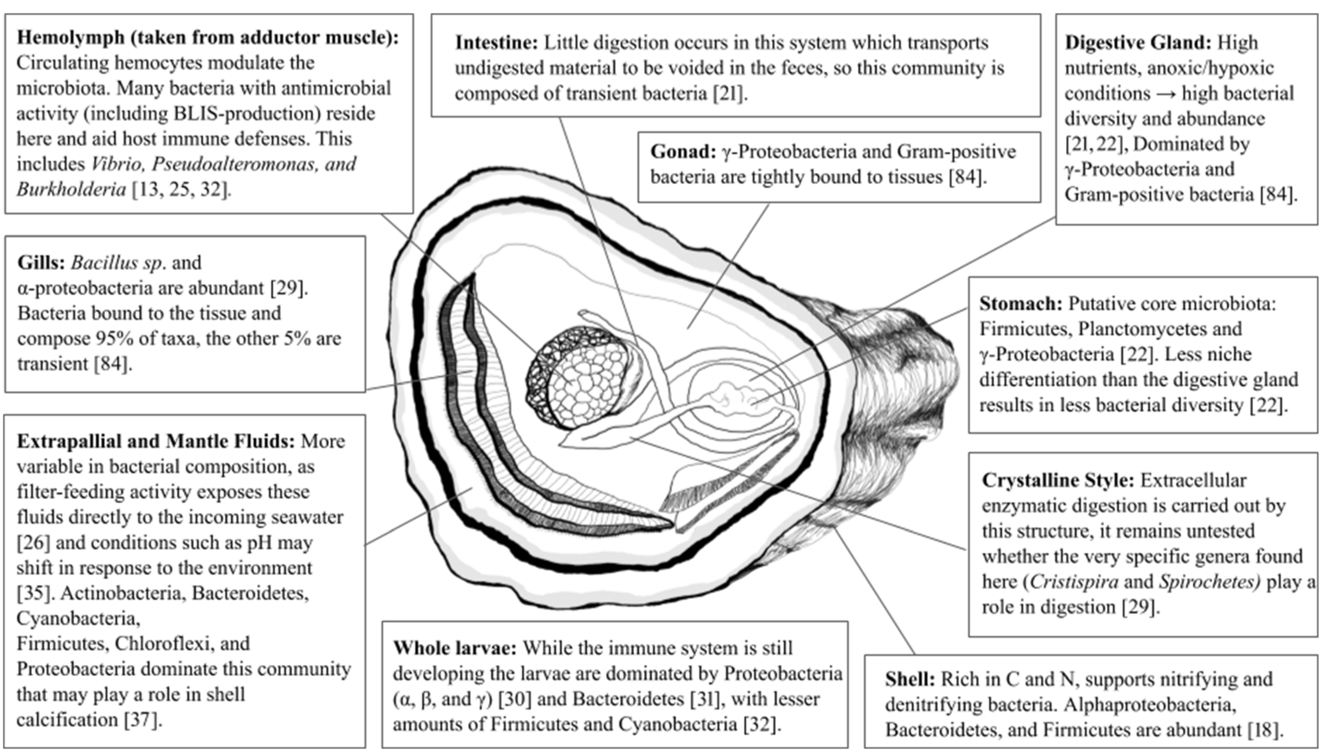

Fig. 1 Oyster microbiota variation by tissue. Anatomical representation of the various body locations of the oyster and the typical microbiotas that are associated with them. Tissue-specific factors that influence the composition and activity of transient and resident microbiota are also noted

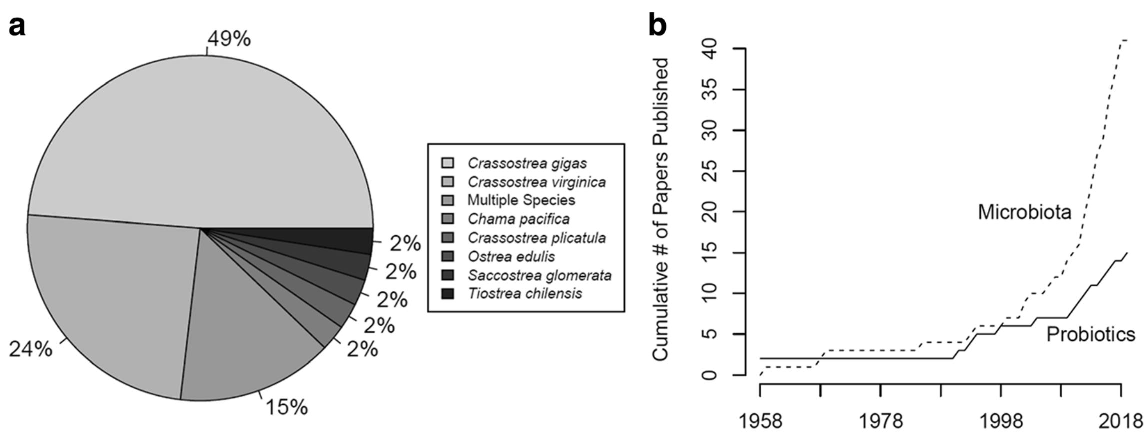

Fig. 2 Summary of the literature published on oyster microbiota and probiotics using data collected from 38 microbiome studies published between 1959 and 2018 (**a**) and cumulative number of non-review studies published in the field pertaining to the oyster microbiome and probiotics over time (**b**)

exposed to the ambient bacteria regimes of estuaries [27–29]. When the mature adult oysters reach market size, they are collected and may be pre-treated for marketing in a process called depuration to eliminate bacteria that is potentially harmful to humans. Following these treatments, or as soon as possible after harvest, the oysters are placed under refrigeration and shipped alive in containers for consumer consumption. Throughout these processes, the oysters are challenged by different stressors (such as physical, mechanical or environmental stress) that can disturb their microbiota, potentially resulting in dysbiosis. The microbial community changes occurring as a result of these processes are summarized in Figs. 3 and 4.

The microbial community varies through the lifespan and commercial processing of oysters (Fig. 3). It is important to highlight that microbial diversity could be low during hatchery growth due to the homogeneous environment in which the oysters are typically cultivated. Additionally, the risk of *Vibrio* infection remains high from conception through the juvenile stage, as the immune system is not yet fully developed [27]. A transplantation event can occur during the juvenile or adult stages, in which young oysters are moved from a hatchery culture environment to estuaries for "grow-out." As the oysters grow in size, their immune system and the composition of their microbiota also mature, allowing colonization by Actinobacteria which are found predominantly in the adult life

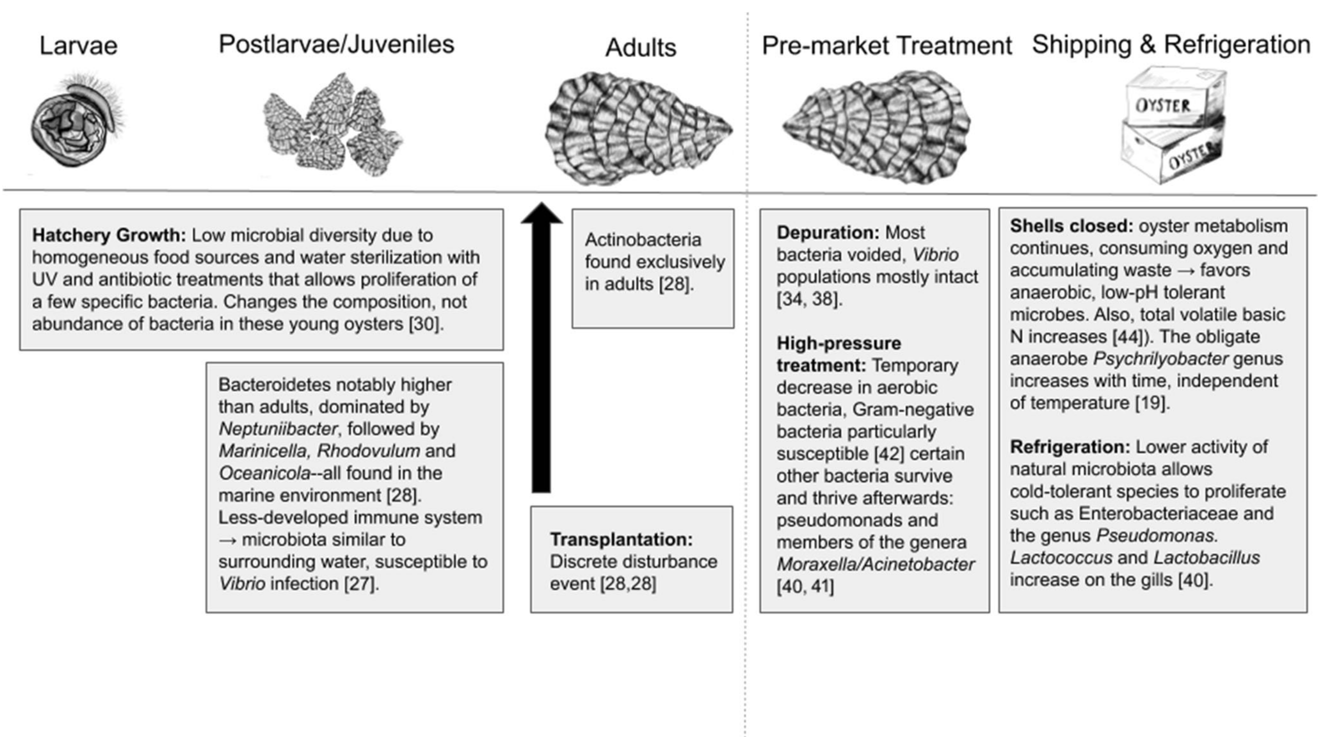

Fig. 3 Where it starts and where it ends: microbiota shifts through the oyster's development and preparation for consumer consumption

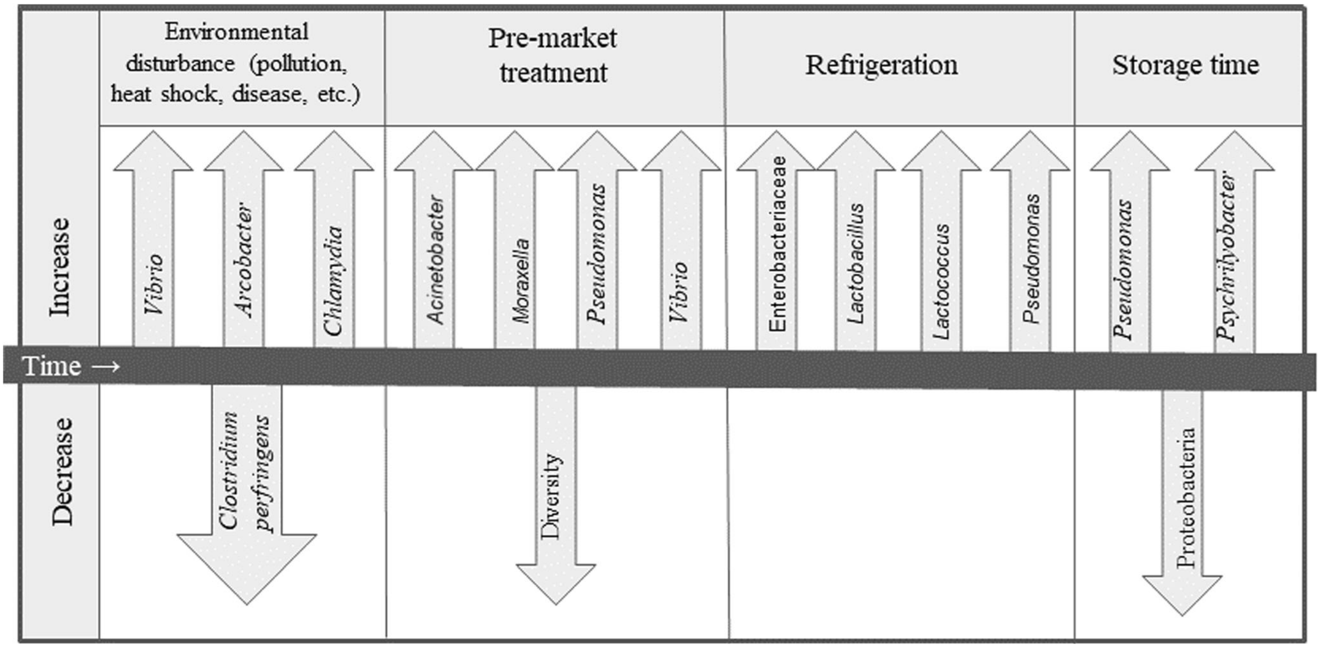

Fig. 4 Oyster microbiota response to aquaculture processes. Specific species and genera changes in response to environmental variables manipulated as part of the aquaculture process. Probiotics can be used to correct compositional shifts and restore the natural healthy microbiota

stage of oysters [28]. Knowledge of the immune status and microbial dynamics in each stage of oyster growth could be leveraged to develop microbiota-aware aquaculture practices.

Hatchery Growth

Early development stages are an important period for microbiota establishment that can have lasting effects with varying levels of resilience to change. Larvae in hatcheries have limited exposure to the natural microbial assemblage of marine environments, as they are typically raised in treated water with low microbial diversity [30, 31]. Factors contributing to this environmental state of low microbial diversity include water and tank sterilization processes such as UV and antibiotics treatments as well as the administration of homogenous, axenically cultivated diets. UV treatment typically kills 90% of the bacteria present—a sizeable, but incomplete, reduction in the standing stock of bacteria. This leaves an "ecological vacuum" that is quickly filled again by the few bacteria that survive UV treatment [30] or bacteria that are introduced by aquaculture husbandry practices. Although bacterial numbers and diversity may remain high, this does not necessarily indicate that the treatments are ineffective: a study of recirculating systems found that the bacterial community maintained throughout the study did not contain any pathogens [30]. Interestingly, decreases in water microbial diversity did not cause a parallel decrease in oyster larvae microbiota diversity, suggesting that water sanitation methods do not drastically change the microbial diversity of the oyster larvae that the systems are supporting, although changes in the specific taxa may have occurred [30]. A study that compared hatchery larvae, tank biofilm, and tank water found that the biofilm had a bacterial composition intermediate to the other two compartments [32]. Experimental antibiotic treatments have been shown to have a lasting effect on the microbiota of young oysters which persists for several weeks following transplantation to grow-out sites [33]. The microbiota will eventually acclimate and reflect the conditions of the new growth environment, but the oyster could temporarily experience decreased fitness and ability to control its own microbiota [33]. Dysbiosis at this early stage of development can have consequences that reverberate throughout the lifespan of the oyster, as it may be unprepared for the challenges presented by the microbial diversity of later growing environments.

Transplantation to Marine Environments

During the postlarvae and juvenile stages, the microbiota continues to change as the oyster tissues and immune system develop and shape the resident microbiota. During this life stage, the oysters are typically transplanted to nursery and then grow-out sites in natural estuary environments where they will complete maturation to market-size, fed by the natural marine plankton assemblage. This drastic environmental change is a unique disturbance experience for sessile organisms like oysters. Only a few studies have looked at the effect of transplantation on the oyster microbiota. Translocation of adult oysters between two natural environments resulted in a higher diversity of the microbiota, which happened to track a rise in *Vibrio* spp. along with temperature [33]; although not all members of this genus are pathogenic, this suggests that the immune system of the translocated oysters was less capable of eliminating external pathogens. If the diversity of the hemolymph microbiota has low species evenness prior to a move, then *Vibrio* will proliferate [27]. Current methods of probiotic treatment typically focus on protecting oysters against pathogen challenge inside the hatchery, but little attention has been paid to potentially easing transitions between growth environments in later stages of development. How this transplantation process affects the microbiota of the oyster has yet to be given much consideration and could be a significant point for intervention.

Aquacultured oysters are subject to many of the same environmental stresses that affect wild oysters as they are grown in aquaculture gear that is open to the natural environment. Seasonal variability (which includes changes in temperature, salinity, turbidity, and primary production) impacts the microbial composition of the marine environment that oysters are exposed to, while also influencing the immunity and internal growth environment of the oyster as a microbial host [7, 34]. Variations in oxygenation of the water can exert a significant influence on oyster microbial composition, with hypoxia events causing significant stress to oysters that is reflected in the microbiota [21]. Extreme temperature highs can cause heat stress for the oysters and change the internal growth conditions such as pH for the bacteria [35]. Heat stress is correlated with increases in certain bacteria (e.g., Fig. 4) and pathogen-induced mass-mortalities [36]. Biofouling organisms from the marine environment will grow on the shells of oysters unless oystermen regularly perform mechanical cleaning techniques; whether microbiota dynamics play a role in biofouling has yet to be explored. Species-specific factors such as water filtration rate can also influence the relative exposure of oysters to bacteria from the surrounding environment; for example, *C. gigas* has a significantly higher water filtration rate than *Ostrea lurida*, which is thought to explain the higher similarity between *C. gigas* and the microbial composition of the surrounding water [37]. Oyster microbiota fluctuations in response to the environment are complex and require an understanding of the oyster as an organism as well as an environment for microbial growth.

Increasing our understanding of natural impacts on the wild oyster microbiota would inform farming practices for aquacultured stocks and could serve to identify promising probiotic candidate strains that could be applied during the grow-out stage. Although oyster grow-out occurs in the natural environment, oystermen interact with their stocks regularly; oysters may be periodically removed from their cages and tumbled to mechanically remove epibionts and shape the growth of the shell. This practice presents a potential opportunity for the regular application of probiotics to support oyster health and growth during the grow-out period. This temporary removal of oysters from the grow-out environment is also an occasion for microbiota disruption; subtidally grown oysters that would otherwise live their entire lives submerged must hold their valves shut when removed from the water. While intertidal oysters do this routinely, the process may involve holding oysters for extended periods above the surface and exposing them to elevated temperatures if not held under refrigeration. Whether this mechanical or environmental disruption also impacts the microbial community of the oyster has yet to be explored.

Pre-market Treatment

Once oysters have reached market size and are ready for commercial sale, postharvest processes may be carried out with the goal of extending shelf life and preventing spoilage. Following an external cleaning process, oysters are typically subjected to one of two processes meant to flush the internal tissues and remove or kill the resident microbiota. A depuration process may occur which facilitates the purging of biological contaminants by placing oysters in closed, recirculating tanks. This process strips the oyster of much of its bacterial diversity, but surface-attached bacteria such as resident *Vibrio* populations tend to remain intact [34, 38]. Probiotic candidate trials of *Streptomyces* strains have lead to the conclusion that these probiotics could be effective coadjuvants for the oyster depuration process due to the observed effect on bacterial microbiota modulation [39]. Oysters subjected to high pressure treatments (aka high pressure processing or HPP), may show a temporary decrease in total aerobic bacteria, but are soon overrun by the few bacterial species that survived the treatment, even when stored in refrigerated environments [20]. Microbes that are well-suited to survive and thrive in these conditions are pseudomonads and members of the genera *Moraxella*/*Acinetobacter* [40, 41], whereas Gram-negative and aerobic bacteria are particularly susceptible to the high pressure treatment process [42]. HPP oysters were found to have higher bacterial counts after several days than untreated control oysters that were used for comparison [20], possibly because HPP kills the oysters and thus prevents any bacterial control that may have otherwise been exerted by a living oyster.

This potential resurgence in bacteria following postharvest treatment can include human pathogens of concern *V. parahaemolyticus*, *Vibrio alginolyticus*, and *Aeromonas hydrophila*; although these may appear to be killed or inactivated by HPP at the time of measurement and certification, the meat can recover its bacteria populations soon thereafter [20]. Therefore, consumers have reason to be skeptical of the claims being made by high pressure-treated products, which may not be as free of pathogens as claimed. However, this finding of a resurgence in HPP oysters was contradicted by earlier studies which found reduced and/or consistent bacterial counts during posttreatment storage [42, 43]. All studies of the effect of HPP on the oyster microbiota have relied on culture-dependent methods, so the discrepancies found therein could be attributed to the well-established biases associated with cultivation steps. Pathogenicity aside, the chemical changes brought about by altering the oyster microbiota can impact the organoleptic properties of the final product, making them potentially less palatable to the consumer [44]. The opportunity remains for next-generation sequencing techniques to compare the full diversity of the HPP oyster microbiota with depurated oysters and non-depurated oysters, potentially identifying probiotic treatments that could alter flavor profiles, extend shelf-life, and protect consumers, as discussed below.

Shipping and Refrigeration

Acknowledging the changes that occur in the oyster microbiota between harvest and market—despite attempts to sterilize the oyster tissues—is essential to designing commercial systems that maximize the shelf-life and safety of the product. When removed from the water, the oyster shell closes, trapping seawater and microbes inside the extrapallial space of the oyster. While the shell remains sealed, oxygen continues to be used by the oyster, thus depleting the oxygenation of the trapped seawater. Cut off from its means of water exchange, wastes accumulate in the internal environment of the oyster shell while the total volatile basic nitrogen increases [44]. Independent of storage temperature, the obligate anaerobe *Psychrilyobacter* increases with storage time as Proteobacteria decreases, rendering the ratio of these two groups a potentially useful indicator of shelf-life and storage times [19]. Oysters that have spoiled, regardless of their storage temperature, are dominated by *Pseudomonas* [44]. The action of fermenting bacteria was thought to cause the pH drop that is observed in the gills during storage, which were dominated by *Lactococcus* and *Lactobacillus* at the start of refrigeration. The chemical composition of the internal environment in live oysters is constantly changed by its continuing metabolism. In shucked oyster meats, refrigerated conditions allow the proliferation of Enterobacteriaceae and *Pseudomonas* even at temperatures as low as 4 °C [40]. Whether the oyster product is alive or dead, refrigerated shipping and storage conditions reduce the activity of natural microbiota and allows cold-tolerant species to proliferate. Sensitivity analyses showed that the processing and transportation temperatures are significant risk factors; in order to significantly reduce annual incidences of *V. parahaemolyticus* infections, maintaining product temperatures below 12 °C is key to reducing *V. parahaemolyticus* infections [45]. Additional protective effects within this refrigerated environment may be achieved through the application of cold-tolerant candidate probiotics from the natural microflora of the oyster. A robust natural microbiota may help circumvent the proliferation of pathogens and spoilage bacteria during the storage and transportation processes.

Developing Probiotic Candidates for Every Stage of the Aquaculture Pipeline

Research Progress

The majority of oyster probiotic studies have focused on early development of larvae, often beginning experiments within 2 days of the start of development [13, 46–52]. Significant losses are typically observed in this first stage, so the focus on this life stage is practical. Embryos are more susceptible to bacterial disease than the subsequent veliger stage, which itself is more susceptible than the presetting larvae stage [53]. Oysters in the hatchery are typically provided with live phytoplankton feeds, so care must be taken to ensure that probiotic candidates will have no adverse impact on either the oysters or the organisms upon which they feed [13]. A study of juveniles that followed their transplantation into grow-out sites demonstrated that natural probiotics isolated from oysters, shrimps, and scallops enhanced growth and survival over non-sterilized controls, as well as a commercial probiotic product, which only fared better than the antibiotic control [54]. This suggests that antibiotic-treated oyster juveniles are poorly prepared for the transition to the natural environment and stand to benefit from any treatment other than antibiotics. The limited number of studies completed on oyster probiotics has been focused on the larval stage, but there is also great potential to leverage the protective effect of probiotics to ease the transition of hatchery-raised oysters to their grow-out sites.

Setting and Achieving Aquaculture Production Goals with Probiotics

Multiple goals can be achieved with probiotics such as enhanced growth, increased survival in early life stages and transplantation, or the elongation of product shelf-life. However, a single strain is not guaranteed to achieve all of these goals; researchers should be careful not to overlook truly valuable strains that offer a narrower range of benefits that fall short of a panacea solution. The in vivo effectiveness of probiotics may differ significantly from the in vitro trials typically used for the initial screening stages, so care must be taken to give isolates due consideration through multiple assays. Most studies on probiotic candidates for larvae have focused on survival, with less studies additionally monitoring the impact of the treatment on oyster size distribution [52, 54] or other potentially important factors such as settlement and shell development. The CA2 strain (identified as a member of the *Alteromonas* genus) was found to improve larval growth and even sustain the larvae in starvation conditions, with possible explanations being that the probiotic provided a nutritional supplement to the algae feed, aided in digestion, or were involved in bivalve waste remediation [52].

Choosing the Right Probiotic Candidate

Shrimp farms have held the lead in testing and applying new aquaculture probiotics [55, 56]. However, success achieved in the cultivation of other species does not transfer easily to the context of the oyster. Different priorities such as control of pathogen growth in seawater and the specific control of *Vibrio* populations are goals unique to bivalve aquaculture [48]. Table 1 lists the probiotic candidate strains that have been tested in the context of oyster aquaculture. Members of many genera are represented in the literature for having probiotic potential in oyster aquaculture: *Alteromonas*, *Phaeobacter*, *Enterococcus*, *Pseudoalteromonas*, *Aeromonas*, *Vibrio*, and several bacilli (Table 1). This diversity of candidates mostly results from the isolation of bacteria from the oyster itself and subsequent screening for effective pathogen growth inhibition in the pathogen-inhibition plate test. Nearly all studies were targeted at the inhibition of *Vibrio* pathogens [13, 32, 46–49, 54, 57–60]. Although members of the *Vibrio* genus typically play the role of villain in the context of shellfish aquaculture, heroes may be found in their midst as specific strains have been found in possession of abilities to hinder the success of their congeners [13]. In some cases, chemical mechanisms by which bacteria inhibit pathogen growth have been identified: bacteriocin-like inhibitory substance (BLIS) production by *Aeromonas media* as well as the production of the antibiotics trophoditietic acid by *Phaeobacter* sp. and amicoumacin by *Bacillus pumilus* (Table 1). They may also be effective as competitors for surface-attachment, as in the case of *Pseudoalteromonas* sp. *D41*, thus limiting opportunities for pathogens to colonize surfaces in the oyster and its growing environment (Table 1). Inclusion in the summary in Table 1 does not imply effective inhibition of the pathogen in question. Furthermore, all of the probiotic

Table 1 Summary of probiotic candidate strains tested on oyster species

Probiotic candidate	Source	Target host	Tested against	Strain characteristics	Reference
Aeromonas media strain A199	Aquatic environment	*C. gigas* (larvae)	*Vibrio tubiashii*	BLIS-producing	[49]
Alteromonas macleodii 0444	Larvae hatchery environment	*C. gigas*, and *Ostrea edulis*	*Vibrio coralliilyticus* and *Vibrio splendidus*	Antimicrobial activity, mechanism unknown	[48]
B. pumilus RI06-95	Sponge	*C. virginica* (larvae)	*Vibrio tubiashii* and *Roseovarius crassostreae*	Produces the antibiotic amicoumacin	[32, 46, 47, 57, 58]
Burkholderia cepacia and *Pseudomonas aeruginosa* mixture	Scallops, oyster, shrimp	*Pinctada mazatlanica* (juveniles)	*V. alginolyticus* and *V. harveyi*		[54]
CA2 (*Alteromonas* sp.)	Algae or oyster larvae (not clear which)	*C. gigas* (larvae)	No specific pathogen	Exopolysaccharide synthesis, inability to utilize inorganicsources of nitrogen	[50–52]
Enterococcus faecium HL7	Oyster	*C. gigas*	*V. parahaemolyticus*, *Streptococcus iniae*, and *Edwardsiellatarda*	High resistance to environmental stressors (salt, ethanol, gastric conditions, and oxidative hydrogenperoxide)	[59]
Lactobacillus sp.	Scallops, oyster, shrimp	*P. mazatlanica* (juveniles)	*V. alginolyticus* and *V. harveyi*	Improved growth and survival, mechanism unknown	[54]
Phaeobacter inhibens/gallaeciensis S4	Oyster	*C. virginica*, *C. gigas*, and *O. edulis* (larvae)	*V. coralliilyticus*, *V. splendidus*, *V. tubiashii*, and *R. crassostreae*	Produces TDA (trophoditietic acid)	[46–48, 57, 58]
Pseudoalteromonas sp. D41	Marine biofilm (coastal area of Brest, France)	*C. gigas, and O. edulis*	*V. coralliilyticus* and *V. splendidus*	Strong adhesive capabilities and a protein-enriched cell wall surface	[48]
Streptomyces N7 and RL8	Marine sediments	*Crassostrea sikamea* (juvenile)	*No specific pathogen*	Produce antibiotics and extracellular enzymes	[39]
Unidentified P02-45 and P02-1	Shellfish production facilities	*C. gigas* (larvae)	*V. tubiashii*	Improved resistance to pathogenic *Vibrio* sp., mechanism unknown	[60]
Vibrio spp. strain OY15	oysters, scallops, green algae culture	*C. virginica* (larvae)	*V. alginolyticus*, *Vibrio* spp., *V. coralliilyticus*	Control of pathogenic *Vibrio* sp., unidentified mechanism	[13]

candidates were not tested against all pathogen species/strains of interest, so there is still potential for more permutations of these probiotic and pathogen combinations to reveal even more probiotic potential. The majority of effective strains were isolated from the oyster microbiota or close relatives of the oyster and consistently outperformed commercially available probiotic products [46, 59]. Why do commercial probiotic strains that are developed for general aquaculture applications have limited effectiveness when applied to oyster culture? Probiotic candidates isolated from distantly related organisms may not be adapted well enough to the unique conditions in the oyster tissues or have properties needed to effectively target their specific pathogens. Experiments that tested probiotic candidates isolated from the target host alongside commercially available products originally developed for other organisms found no significant protective effect in the latter [46]. Considering this phenomenon through the lens of the "hologenome" concept, the aquacultured organism and its natural microbiota would be recognized as a superorganism (a holobiont) that has evolved as a single entity [61]. Seeking probiotic candidates that leverage this evolutionary history between oysters and their symbionts maximizes the benefits of probiotics to aquaculture, so oyster host biology should be a priority in the development of probiotics.

Additional Variability to Consider

Treatment factors such as dosage frequency, concentration, and environmental temperatures can significantly impact probiotic effectiveness. The formulation of a probiotic product must be considered for effective treatment; for example, probiotics lyophilized with 100 mM sucrose were found to eliminate any protective effect for oyster larvae [47, 57], whereas granulation was found to be an effective solution for oyster hatchery probiotics [57]. Additionally, a range of bacterial concentrations must be tested for a probiotic candidate, as effectiveness can vary greatly depending on dosage, appearing ineffective at low concentrations [47] or even introducing deleterious effects at concentrations that are too high [52]. The margins for effective treatment have been demonstrated to be as narrow as a 1-fold change in bacterial concentration in some agricultural contexts [62]. High concentrations of even neutral bacteria are thought to reduce growth of oysters by inhibiting efficient feeding [50]. The temperature at which the system is maintained can make a large difference in probiotic effectiveness, as a temperature difference of 5 °C erased the significant benefits that were seen in one treatment [52]. Dosage frequency is also an important factor for effective protection. Although certain candidate probiotic strains have been shown to benefit the target oyster, the presence of these probiotic bacteria may be short-lived, as populations have been observed to drop precipitously after just 24 h and were undetectable after 72 h [49]. Similarly, when complete water changes were performed to remove the probiotic bacteria from the tank environment of the oysters, the benefits conferred by the bacteria did not have a lasting effect on the oysters [46]. If the benefit of the probiotics could be as transient as the bacteria themselves, then probiotic replenishment is required to maintain these benefits. It is critical that the optimal dose of probiotic bacteria be administered and sustained in the tank environment to maintain the beneficial effects of these probiotics beyond a 24-h time period. Creative solutions for probiotic administration are required if probiotics are to be used to address oyster aquaculture needs beyond the hatchery. Achieving any benefits for oysters with probiotics in the grow-out environment beyond the initial period of transition is a challenge because oystermen exert no control over the oyster growth environment; however, regular interaction with oyster stocks to perform cleaning processes could be an opportunity for the external application of probiotics.

When designing trials for the selection of probiotic candidates based on antagonistic action towards pathogens, researchers should consider the timing in which the probiotic candidate and the pathogen are introduced into the ecological niche. The most common use of probiotics in oyster cultivation is the prophylactic prevention of disease in oysters by pathogenic bacteria infection. Figure 5a shows a timeline in which probiotics could be introduced in the development and processing of oysters. Additionally, a simplified design schematic for in vivo trials is shown in Fig. 5b to emphasize the difference between prophylactic and therapeutic uses of probiotics. All studies listed in Table 1 with pathogen-challenge components were conducted by applying pathogens some period of time after the application of probiotics to achieve prophylactic effects. No published studies have yet tested the potential for therapeutic treatment of diseases already in progress (only applying probiotics after pathogen introduction, Fig. 5b), but experience from the greater field of probiotics suggests that therapeutic treatment would be unlikely to be effective.

The intense focus on probiotic development for oyster larval stages has resulted in studies that are shorter than—or barely longer than—these life stages themselves, with many studies only tracking probiotic effect for 2–10 days after their application [13, 51, 52, 58]. Such studies miss the potential for probiotics to confer long-term benefits to the oysters that go beyond enhanced larval survival. The potential for probiotic benefits to follow hatchery-raised oysters to their grow-out locations was tested by removing free-floating probiotics from the tank with a water change after an initial incubation period, and then challenging oysters with pathogens at successive time periods to observe if the probiotics still exerted an effect on oyster disease susceptibility [58]. For longer studies in the hatchery environment with no specific pathogen challenge, researchers have reapplied probiotics following regular water changes [52] or feedings [54]. Increased understanding of the

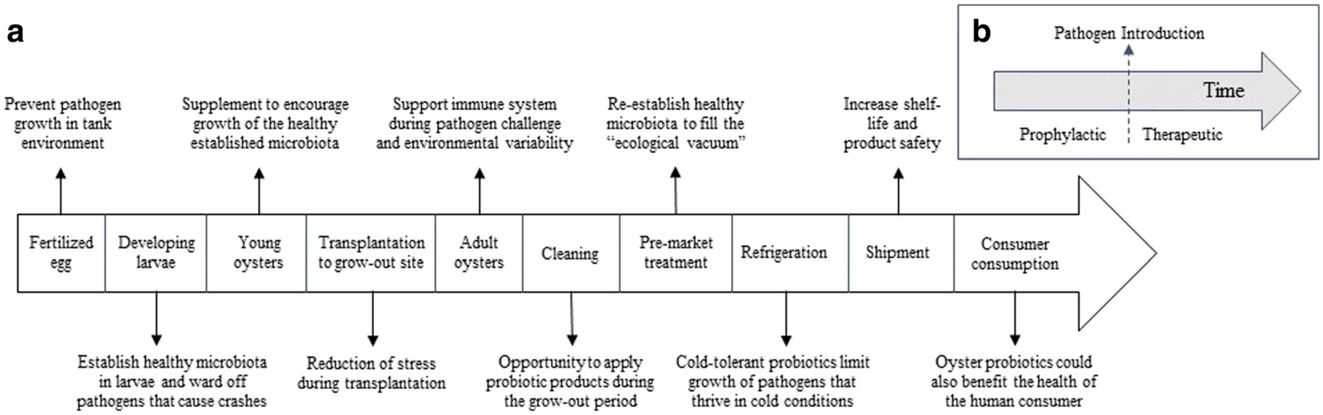

Fig. 5 Potential objectives timeline for probiotics in oysters. Timeline showing the steps where potential probiotics could be applied and their purpose of application (**a**) and a design scheme for trials and tests, especially for in vivo tests (**b**). The timing of pathogen introduction determines whether the probiotic is applied for prophylactic measurement or as a treatment/cure

dynamics governing microbiota changes throughout the aquaculture process could inform the appropriateness of study timelines to achieve different beneficial outcomes for oyster production.

Working with the Natural Immune System of Oysters

Age-Specific Dynamics

The conditions for microbial growth can change with oyster host life stage, largely driven by changes in oyster immune function and behavior. Regardless of species, oyster postlarvae have microbiotas very similar to that of the surrounding water [29] and are particularly susceptible to *Vibrio* infections [27], suggesting that the less-developed immune systems of juveniles is a major determinant of their microbiota composition. The immune response of juvenile oysters was found to be an inefficient means of eliminating both bacterial and viral infections [11]. The immune system of oysters selectively agglutinates specific *Vibrio* spp. [47], ideally causing a beneficial shift in the ratio of pathogenic and beneficial taxa. Oysters become more selective feeders with age, which has been correlated with a loss in microbiota diversity [28]. Although all of the causes are not well-understood, other life stage patterns have been observed, such as a much higher proportion of *Bacteroidetes* in the postlarvae stage and finding Actinobacteria almost exclusively in adults [28]. Actinobacteria isolates are often used as probiotics in aquatic systems for their diverse secondary metabolites that act as antimicrobials and combat *Vibrio* growth [56]. Studies cataloging isolates from aquatic ecosystems have found Actinobacteria in biofilters [63, 64], indicating that it is also naturally present in the hatchery environment. Actinobacteria have even been shown to increase shrimp growth when introduced to their marine environment [56] and thus may be considered as a potential probiotic for adult oysters as well.

Complementing the Oyster Immune System

A mechanistic description of the oyster immune system and the role of the microbiota within it was recently reviewed [65]. The oyster immune system shapes the microbial community found within oysters through the production of antimicrobial proteins (AMPs) and reactive oxygen species (ROS) as well as the phagocytic activity of hemocytes that selectively neutralize potential pathogens [66]. In turn, the oyster microbiota can influence the immunocompetence of its host; oyster hemolymph was found to host resident bacteria that confer some health benefits such as antimicrobial activity [15]. A multi-tissue study found that the microbiota associated with the hemolymph played the largest role in the fitness of oysters undergoing transplantation, compared to the relative influence of microbiota in the mantle, gill, and gut [67]. Oyster immune particles found in the hemolymph such as defensins are most effective against Gram-positive bacteria, but most oyster pathogens are actually Gram-negative [68]. Partnering with bacteria (5 species identified as *Vibrio* and *Pseudoalteromonas*) that produce bacteriocin-like inhibitory substances (BLIS) helps to complete the immunological arsenal of oysters; although these bacteria species compose only ~2% of the total microbiota for oyster hemolymph, their impact on the internal growth environment is powerful [15]. These BLIS compounds detected in vivo likely modulate the hemolymph microbiota and provide defense against pathogenic infection. *Vibrio* and *Pseudoalteromonas* persist in oyster hemolymph and likely contribute to its defense, displaying anti-bacterial, bacteriolytic, and algicidal activities [38]. Due to their bioactive molecules preventing pathogen infection of fish eggs, these genera have been used in aquatic biofilms as a probiotic [69]. The hemolymph is also home to *Alteromonas*, a genus of Proteobacteria which has been used in aquaculture for the anti-algal properties of some of its members [70]. The apparent cooperation between oysters and their microbiota to achieve effective immune defenses could be leveraged as a probiotic candidate that has essentially already been identified by the oyster itself.

Including the Human Consumer in Probiotic Design

As oysters are shipped live and consumed whole, it is important to understand not only what compromises and influences the oyster microbiota, but also how it relates to the human microbiota. Probiotic treatments applied in later stages in the aquaculture process, such as pre-market treatment, could potentially provide an additional health benefit to the human consumer. For example, cyanobacteria, a familiar microorganism to marine environments, are found in the pallial fluids of oysters [37] and can also be found in minute amounts within humans [71, 72]. Reported health benefits attributed to spirulina include immunomodulation and protection against influenza virus as it was shown in model studies with mice [73], reduction of anti-tuberculosis treatment induced hepatotoxicity in rats [74], antioxidant and immunomodulatory activities in animal models and in fermented food products [75], and anti-diabetic activity in model rat studies [76]. Often sold as a dietary supplement for humans, the introduction of cyanobacteria as a probiotic to oysters may have the potential to become an indirect source of cyanobacteria supplementation for humans. In the case of oysters, spirulina was reported as contributing to the improved growth, modulated immune system, and antioxidant protection in juveniles [77]. Cyanobacteria already well-adapted to the aquatic environments found in hatcheries and estuary grow-out sites could provide benefits to both oysters and their human consumers.

Another group of microorganisms with potential probiotic benefit for oysters and humans is bacilli, which has been described as "the friendliest to humans, animals, and plants" [78]. *Bacillus* is the genus of spore-forming bacteria that produces antimicrobials and has been increasingly used in probiotic formulations. Bacilli are transient colonizers in humans, with essential responsibilities in modulating human health [79–81] and the potential to increase the longevity of its eukaryotic host [82]. *Bacillus* strains are used in various probiotic cocktails for humans; consuming this *Bacillus* along with oysters could have positive health benefits for humans [83]. *Caenorhabditis elegans* treated with *B. subtilis* was found to have dramatically increased life expectancy, so researchers hope to transfer this beneficial effect to human applications [82]. Bacilli could be a good match for the oyster aquaculture environment, as *Bacillus* spp. have been found to be abundant in the gills of oysters because these bacteria readily attach to surfaces [29, 84]. They are likely to persist in hatchery environments with treated water, as spores from this genus have been shown to survive through the UV radiation that is commonly employed in hatcheries [78]. To maximize the potential for benefit to oysters and humans while ensuring success in the aquaculture environment, genera found at the confluence between these three microbiotas should be given special consideration.

Regardless of whether oysters someday become a vehicle for probiotic delivery to humans, there are many other potential benefits that humans can derive from oyster probiotics. Restoring the non-pathogenic portion of the oyster microbiota through probiotic supplementation could further enhance the unique *merroir* of regional products, while also improving oyster health and tissue preservation in transport. The microbial contribution to oyster flavor and texture merits further exploration for potential product improvement.

Conclusion

Oyster product losses that occur throughout the aquaculture production cycle may be rooted in changes to the oyster microbiota; using probiotics to limit oyster stock losses due to mortality could increase the yield of aquaculture activities. The harvest of wild and cultured oysters for human consumption supports millions of dollars of economic activity in the USA alone, providing significant economic impetus for these stocks to be protected. Antibiotic usage is associated with potential adverse effects on the health of humans and environment, which necessitates a search for alternative methods. Probiotics can be developed for oysters that address the needs of these populations in various stages of the aquaculture process. Consideration should also be given to improving the health and safety of the human consumer, as oyster products are typically consumed whole and raw. By considering the taxonomic groups shared between oysters, humans, and the environment, probiotic candidates can be chosen that maximize health benefit to all three and potentially improve the experience of the finished product.

Funding Information MLC was supported by the Ministry of Science and Higher Education of the Russian Federation (Project Number 075-15-2019-1880).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. FAO (2018) The state of world fisheries and agriculture - meeting the sustainable development goals. Rome. http://www.fao.org/3/i9540en/i9540en.pdf. Accessed 14 Aug 2019
2. Cruz PM, Ibáñez AL, Hermosillo OAM, Saad HCR (2012) Use of probiotics in aquaculture. ISRN Microbiol:1–13. https://doi.org/10.5402/2012/916845
3. Olin P, Smith, J, Nabi, R (2011) Regional review on status and trends in aquaculture development in North America – 2010 FAO Fisheries and Aquaculture Circular No. 1061/2. Rome, FAO. 2011. 84 pp. http://www.fao.org/3/i2163e/i2163e00.pdf. Accessed 14 Aug 2019
4. Haskin Shellfish Research Laboratory at Rutgers NJAES. https://hsrl.rutgers.edu/. Accessed 10 Feb 2019
5. Calvo LM (2018) New Jersey shellfish aquaculture situation and outlook report 2016 production year. Rutgers NJ Agric. Experiment Station. http://njseagrant.org/wp-content/uploads/2018/04/NJAquacultureSurvey2016.pdf. Accessed 13 Aug 2019

6. Watermann BT, Herlyn M, Daehne B, Bergmann S, Meemken M, Kolodzey H (2008) Pathology and mass mortality of Pacific oysters, *Crassostrea gigas* (Thunberg), in 2005 at the East Frisian coast, Germany. J Fish Dis 31:621–630. https://doi.org/10.1111/j.1365-2761.2008.00953.x
7. Green TJ, Siboni N, King WL, Labbate M, Seymour JR, Raftos D (2018) Simulated marine heat wave alters abundance and structure of *Vibrio* populations associated with the Pacific oyster resulting in a mass mortality event. Microb Ecol 77:736–747. https://doi.org/10.1007/s00248-018-1242-9
8. Newton AE, Garrett N, Stroika SG, Halpin JL, Turnsek M, Mody RK, Centers for Disease Control and Prevention (CDC) (2014) Increase in *Vibrio parahaemolyticus* infections associated with consumption of Atlantic coast shellfish — 2013. Morb Mortal Wkly Rep 63:335–336 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5779391/pdf/335-336.pdf
9. Taylor M, Cheng J, Sharma D, Bitzikos O, Gustafson R, Fyfe M, Greve R, Murti M, Stone J, Honish L, Mah V, Punja N, Hexemer A, McIntyre L, Henry B, Kendall P, Atkinson R, Buenaventura E, Martinez-Perez A, Galanis E, Team TOI (2018) Outbreak of *Vibrio parahaemolyticus* associated with consumption of raw oysters in Canada, 2015. Foodborne Pathog Dis 15:554–559. https://doi.org/10.1089/fpd.2017.2415
10. Bruner DM, Huth WL, McEvoy DM, Morgan OA (2014) Consumer valuation of food safety: the case of postharvest processed oysters. Agric Resour Econ Rev Durh 43:300–318. https://doi.org/10.1017/S1068280500004330
11. de Lorgeril J, Escoubas J-M, Loubiere V, Pernet F, le Gall P, Vergnes A, Aujoulat F, Jeannot JL, Jumas-Bilak E, Got P, Gueguen Y, Destoumieux-Garzón D, Bachère E (2018) Inefficient immune response is associated with microbial permissiveness in juvenile oysters affected by mass mortalities on field. Fish Shellfish Immunol 77:156–163. https://doi.org/10.1016/j.fsi.2018.03.027
12. Newaj-Fyzul A, Al-Harbi AH, Austin B (2014) Review: developments in the use of probiotics for disease control in aquaculture. Aquaculture 431:1–11. https://doi.org/10.1016/j.aquaculture.2013.08.026
13. Lim HJ, Kapareiko D, Schott EJ et al (2011) Isolation and evaluation of new probiotic bacteria for use in shellfish hatcheries: I. Isolation and screening for bioactivity. J Shellfish Res 30:609–615. https://doi.org/10.2983/035.030.0303
14. (2018) Good news, bad news for next-gen probiotic. In: https://www.aquaculturenorthamerica.com. https://www.aquaculturenorthamerica.com/good-news-bad-news-for-next-gen-probiotic-1972/. Accessed 12 Aug 2019
15. Defer D, Desriac F, Henry J, Bourgougnon N, Baudy-Floc'h M, Brillet B, le Chevalier P, Fleury Y (2013) Antimicrobial peptides in oyster hemolymph: the bacterial connection. Fish Shellfish Immunol 34:1439–1447. https://doi.org/10.1016/j.fsi.2013.03.357
16. Kassaify ZG, Hajj RHE, Hamadeh SK et al (2009) Impact of oil spill in the Mediterranean Sea on biodiversified bacteria in oysters. J Coast Res 25:469–473. https://doi.org/10.2112/07-0962.1
17. Stief P, Poulsen M, Nielsen LP, Brix H, Schramm A (2009) Nitrous oxide emission by aquatic macrofauna. Proc Natl Acad Sci U S A 106:4296–4300. https://doi.org/10.1073/pnas.0808228106
18. Arfken A, Song B, Bowman JS, Piehler M (2017) Denitrification potential of the eastern oyster microbiome using a 16S rRNA gene based metabolic inference approach. PLoS One 12(9):e0185071. https://doi.org/10.1371/journal.pone.0185071
19. Fernandez-Piquer J, Bowman JP, Ross T, Tamplin ML (2012) Molecular analysis of the bacterial communities in the live Pacific oyster (*Crassostrea gigas*) and the influence of postharvest temperature on its structure. J Appl Microbiol 112:1134–1143. https://doi.org/10.1111/j.1365-2672.2012.05287.x
20. Prapaiwong N, Wallace RK, Arias CR (2009) Bacterial loads and microbial composition in high pressure treated oysters during storage. Int J Food Microbiol 131:145–150. https://doi.org/10.1016/j.ijfoodmicro.2009.02.014
21. Khan B, Clinton SM, Hamp TJ, Oliver JD, Ringwood AH (2018) Potential impacts of hypoxia and a warming ocean on oyster microbiomes. Mar Environ Res 139:27–34. https://doi.org/10.1016/j.marenvres.2018.04.018
22. King GM, Judd C, Kuske CR, Smith C (2012) Analysis of stomach and gut microbiomes of the eastern oyster (*Crassostrea virginica*) from coastal Louisiana, USA. PLoS One 7(12):e51475. https://doi.org/10.1371/journal.pone.0051475
23. Goedken M, Morsey B, Sunila I et al (2005) The effects of temperature and salinity on apoptosis of *Crassostrea virginica hemocytes* and *Perkinsus marinus*. J Shellfish Res 24:177–183. https://doi.org/10.2983/0730-8000(2005)24[177:TEOTAS]2.0.CO;2
24. Bushek D, Ford SE, Burt I (2012) Long-term patterns of an estuarine pathogen along a salinity gradient. J Mar Res 70:225–251. https://doi.org/10.1357/002224012802851968
25. Gavery MR, Roberts SB (2010) DNA methylation patterns provide insight into epigenetic regulation in the Pacific oyster (*Crassostrea gigas*). BMC Genomics 11:483. https://doi.org/10.1186/1471-2164-11-483
26. Pierce ML, Ward JE (2018) Microbial ecology of the *Bivalvia*, with an emphasis on the family *Ostreidae*. J Shellfish Res 37:793–806. https://doi.org/10.2983/035.037.0410
27. King WL, Jenkins C, Go J, Siboni N, Seymour JR, Labbate M (2019) Characterisation of the Pacific oyster microbiome during a summer mortality event. Microb Ecol 77(2):502–512. https://doi.org/10.1007/s00248-018-1226-9
28. Trabal Fernández N, Mazón-Suástegui JM, Vázquez-Juárez R, Ascencio-Valle F, Romero J (2014) Changes in the composition and diversity of the bacterial microbiota associated with oysters (*Crassostrea corteziensis*, *Crassostrea gigas* and *Crassostrea sikamea*) during commercial production. FEMS Microbiol Ecol 88:69–83. https://doi.org/10.1111/1574-6941.12270
29. Trabal N, Mazón-suástegui JM, Vázquez-juárez R et al (2012) Molecular analysis of bacterial microbiota associated with oysters (*Crassostrea gigas* and *Crassostrea corteziensis*) in different growth phases at two cultivation sites. Microb Ecol Heidelb 64:555–569. https://doi.org/10.1007/s00248-012-0039-5
30. Asmani K, Petton B, Le Grand J et al (2016) Establishment of microbiota in larval culture of Pacific oyster, *Crassostrea gigas*. Aquaculture 464:434–444. https://doi.org/10.1016/j.aquaculture.2016.07.020
31. Laroche O, Symonds JE, Smith KF et al (2018) Understanding bacterial communities for informed biosecurity and improved larval survival in Pacific oysters. Aquaculture 497:164–173. https://doi.org/10.1016/j.aquaculture.2018.07.052
32. Stevick RJ, Sohn S, Modak TH, Nelson DR, Rowley DC, Tammi K, Smolowitz R, Markey Lundgren K, Post AF, Gómez-Chiarri M (2019) Bacterial community dynamics in an oyster hatchery in response to probiotic treatment. Front Microbiol 10:1060. https://doi.org/10.3389/fmicb.2019.01060
33. Lokmer A, Goedknegt MA, Thieltges DW, Fiorentino D, Kuenzel S, Baines JF, Wegner KM (2016) Spatial and temporal dynamics of Pacific oyster hemolymph microbiota across multiple scales. Front Microbiol 7:1367. https://doi.org/10.3389/fmicb.2016.01367
34. Wang D, Zhang Q, Cui Y, Shi X (2014) Seasonal dynamics and diversity of bacteria in retail oyster tissues. Int J Food Microbiol 173:14–20. https://doi.org/10.1016/j.ijfoodmicro.2013.12.008
35. Nash S, Rahman MS (2019) Short-term heat stress impairs testicular functions in the American oyster, *Crassostrea virginica*: molecular mechanisms and induction of oxidative stress and apoptosis in spermatogenic cells. Mol Reprod Dev 86:1444–1458. https://doi.org/10.1002/mrd.23268

36. Wegner KM, Volkenborn N, Peter H, Eiler A (2013) Disturbance induced decoupling between host genetics and composition of the associated microbiome. BMC Microbiol Lond 13:252. https://doi.org/10.1186/1471-2180-13-252
37. Banker R, Vermeij GJ (2018) Oyster microbial communities and implications for chalky deposit formation. Hydrobiologia 816:121–135. https://doi.org/10.1007/s10750-018-3569-0
38. Vezzulli L, Stagnaro L, Grande C, Tassistro G, Canesi L, Pruzzo C (2018) Comparative 16SrDNA gene-based microbiota profiles of the Pacific oyster (*Crassostrea gigas*) and the Mediterranean mussel (*Mytilus galloprovincialis*) from a shellfish farm (Ligurian Sea, Italy). Microb Ecol 75:495–504. https://doi.org/10.1007/s00248-017-1051-6
39. Bernal MG, Fernández NT, Lastra PES et al (2017) *Streptomyces* effect on the bacterial microbiota associated to *Crassostrea sikamea* oyster. J Appl Microbiol 122:601–614. https://doi.org/10.1111/jam.13382
40. Chen H, Liu Z, Wang M, Chen S, Chen T (2013) Characterisation of the spoilage bacterial microbiota in oyster gills during storage at different temperatures. J Sci Food Agric 93:3748–3754. https://doi.org/10.1002/jsfa.6237
41. Ashie INA, Smith JP, Simpson BK, Haard DNF (1996) Spoilage and shelf-life extension of fresh fish and shellfish. Crit Rev Food Sci Nutr 36:87–121. https://doi.org/10.1080/10408399609527720
42. Linton M, Clements JMJM, Patterson MF (2003) Changes in the microbiological quality of shellfish, brought about by treatment with high hydrostatic pressure. Int J Food Sci Technol 38:713–727. https://doi.org/10.1046/j.1365-2621.2003.00724.x
43. He H, Adams RM, Farkas DF, Morrissey MT (2002) Use of high-pressure processing for oyster shucking and shelf-life extension. J Food Sci 67:640–645. https://doi.org/10.1111/j.1365-2621.2002.tb10652.x
44. Cao R, Xue C-H, Liu Q, Xue Y (2009) Microbiological, chemical, and sensory assessment of Pacific oysters (*Crassostrea gigas*) stored at different temperatures. Czech J Food Sci 27:102–108. https://doi.org/10.17221/166/2008-CJFS
45. Huang Y-S, Hwang C-A, Huang L et al (2018) The risk of *Vibrio parahaemolyticus* infections associated with consumption of raw oysters as affected by processing and distribution conditions in Taiwan. Food Control 86:101–109. https://doi.org/10.1016/j.foodcont.2017.10.022
46. Abd Karim MM (2012) Mechanisms of protection of probiotics against bacterial pathogens in oyster aquaculture. Diss Masters Theses Campus Access 1–157. https://digitalcommons.uri.edu/dissertations/AAI3546827/. Accessed 14 Aug 2019
47. Sohn S (2016) Evaluation of the efficacy of candidate probiotics for disease prevention in shellfish hatcheries. Ph.D. Dissertation, University of Rhode Island. https://digitalcommons.uri.edu/cgi/viewcontent.cgi?article=1470&context=oa_diss. Accessed 14 Aug 2019
48. Kesarcodi-Watson A, Miner P, Nicolas J-L, Robert R (2012) Protective effect of four potential probiotics against pathogen-challenge of the larvae of three bivalves: Pacific oyster (*Crassostrea gigas*), flat oyster (*Ostrea edulis*) and scallop (*Pecten maximus*). Aquaculture 344–349:29–34. https://doi.org/10.1016/j.aquaculture.2012.02.029
49. Gibson LF, Woodworth J, George AM (1998) Probiotic activity of *Aeromonas media* on the Pacific oyster, *Crassostrea gigas*, when challenged with *Vibrio tubiashii*. Aquaculture 169:111–120. https://doi.org/10.1016/S0044-8486(98)00369-X
50. Douillet PA (1991) Beneficial effects of bacteria on the culture of larvae of the Pacific oyster *Crassostrea gigas* (Thunberg). Ph.D. Dissertation, Oregon State University. https://ir.library.oregonstate.edu/downloads/bz60d0505. Accessed 14 August 2019
51. Douillet P, Langdon CJ (1993) Effects of marine bacteria on the culture of axenic oyster *Crassostrea gigas* (Thunberg) larvae. Biol Bull 184:36–51. https://doi.org/10.2307/1542378
52. Douillet PA, Langdon CJ (1994) Use of a probiotic for the culture of larvae of the Pacific oyster (*Crassostrea gigas* Thunberg). Aquaculture 119:25–40. https://doi.org/10.1016/0044-8486(94)90441-3
53. Brown C (1973) The effects of some selected bacteria on embryos and larvae of the American oyster, *Crassostrea virginica*. J Invertebr Pathol 21:215–223. https://doi.org/10.1016/0022-2011(73)90206-1
54. Aguilar-Macías OL, Ojeda-Ramírez JJ, Campa-Córdova AI, Saucedo PE (2010) Evaluation of natural and commercial probiotics for improving growth and survival of the pearl oyster, *Pinctada mazatlanica*, during late hatchery and early field culturing. J World Aquac Soc 41:447–454. https://doi.org/10.1111/j.1749-7345.2010.00386.x
55. Vargas-Albores F, Martínez-Porchas M, Arvayo MA et al (2016) Immunophysiological response of Pacific white shrimp exposed to a probiotic mixture of Proteobacteria and Firmicutes in farm conditions. North Am J Aquac 78:193–202. https://doi.org/10.1080/15222055.2016.1167797
56. Tan LT-H, Chan K-G, Lee L-H, Goh B-H (2016) *Streptomyces* bacteria as potential probiotics in aquaculture. Front Microbiol 7:79. https://doi.org/10.3389/fmicb.2016.00079
57. Dao CA (2015) Chemical investigation of candidate probiotics in aquaculture and formulation of a probiotic agent for oyster larviculure. Ph.D. Dissertation, University of Rhode Island. https://digitalcommons.uri.edu/cgi/viewcontent.cgi?referer=https://www.google.com/&httpsredir=1&article=1317&context=oa_diss. Accessed 13 Aug 2019
58. Karim M, Zhao W, Rowley D et al (2013) Probiotic strains for shellfish aquaculture: protection of eastern oyster, *Crassostrea virginica*, larvae and juveniles against bacterial challenge. J Shellfish Res 32:401–408. https://doi.org/10.2983/035.032.0220
59. Kang C-H, Gu T, So J-S (2018) Possible probiotic lactic acid bacteria isolated from oysters (*Crassostrea gigas*). Probiotics Antimicrob Proteins 10:728–739. https://doi.org/10.1007/s12602-017-9315-5
60. Elston R, Humphrey K, Gee A et al (2004) Progress in the development of effective probiotic bacteria for bivalve shellfish hatcheries and nurseries. J Shellfish Res 23:288–289
61. Zilber-Rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiol Rev 32:723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x
62. Ribeiro V, Albino LFT, Rostagno HS et al (2014) Effects of the dietary supplementation of *Bacillus subtilis* levels on performance, egg quality and excreta moisture of layers. Anim Feed Sci Technol 195:142–146. https://doi.org/10.1016/j.anifeedsci.2014.06.001
63. Huang Z, Wan R, Song X, Liu Y, Hallerman E, Dong D, Zhai J, Zhang H, Sun L (2016) Metagenomic analysis shows diverse, distinct bacterial communities in biofilters among different marine recirculating aquaculture systems. Aquac Int 24:1393–1408. https://doi.org/10.1007/s10499-016-9997-9
64. Schreier HJ, Mirzoyan N, Saito K (2010) Microbial diversity of biological filters in recirculating aquaculture systems. Curr Opin Biotechnol 21:318–325. https://doi.org/10.1016/j.copbio.2010.03.011
65. King WL, Jenkins C, Seymour JR, Labbate M (2019) Oyster disease in a changing environment: decrypting the link between pathogen, microbiome and environment. Mar Environ Res 143:124–140. https://doi.org/10.1016/j.marenvres.2018.11.007
66. Schmitt P, de Lorgeril J, Gueguen Y et al (2012) Expression, tissue localization and synergy of antimicrobial peptides and proteins in

the immune response of the oyster *Crassostrea gigas*. Dev Comp Immunol 37:363–370. https://doi.org/10.1016/j.dci.2012.01.004
67. Lokmer A, Kuenzel S, Baines JF, Wegner KM (2016) The role of tissue-specific microbiota in initial establishment success of Pacific oysters. Environ Microbiol 18:970–987. https://doi.org/10.1111/1462-2920.13163
68. Schmitt P, Wilmes M, Pugnière M, Aumelas A, Bachère E, Sahl HG, Schneider T, Destoumieux-Garzón D (2010) Insight into invertebrate defensin mechanism of action: oyster defensins inhibit peptidoglycan biosynthesis by binding to lipid II. J Biol Chem 285: 29208–29216. https://doi.org/10.1074/jbc.M110.143388
69. Wesseling W, Wittka S, Kroll S et al (2015) Functionalised ceramic spawning tiles with probiotic *Pseudoalteromonas* biofilms designed for clownfish aquaculture. Aquaculture 446:57–66. https://doi.org/10.1016/j.aquaculture.2015.04.017
70. Cho JY (2012) Algicidal activity of marine *Alteromonas* sp. KNS-16 and isolation of active compounds. Biosci Biotechnol Biochem 76:1452–1458. https://doi.org/10.1271/bbb.120102
71. Sankar SA, Lagier J-C, Pontarotti P, Raoult D, Fournier PE (2015) The human gut microbiome, a taxonomic conundrum. Syst Appl Microbiol 38:276–286. https://doi.org/10.1016/j.syapm.2015.03.004
72. D'Argenio V, Salvatore F (2015) The role of the gut microbiome in the healthy adult status. Clin Chim Acta 451:97–102. https://doi.org/10.1016/j.cca.2015.01.003
73. Pugh ND, Edwall D, Lindmark L, Kousoulas KG, Iyer AV, Haron MH, Pasco DS (2015) Oral administration of a *Spirulina* extract enriched for Braun-type lipoproteins protects mice against influenza A (H1N1) virus infection. Phytomedicine 22:271–276. https://doi.org/10.1016/j.phymed.2014.12.006
74. Martin SJ, Baskaran UL, Vedi M, Sabina EP (2014) Attenuation of anti-tuberculosis therapy induced hepatotoxicity by *Spirulina fusiformis*, a candidate food supplement. Toxicol Mech Methods 24:584–592. https://doi.org/10.3109/15376516.2014.956910
75. Varga L, Szigeti J, Kovács R, Földes T, Buti S (2002) Influence of a *Spirulina platensis* biomass on the microflora of fermented ABT milks during storage (R1). J Dairy Sci 85:1031–1038. https://doi.org/10.3168/jds.S0022-0302(02)74163-5
76. Oriquat GA, Ali MA, Mahmoud SA et al (2018) Improving hepatic mitochondrial biogenesis as a postulated mechanism for the antidiabetic effect of *Spirulina platensis* in comparison with metformin. Appl Physiol Nutr Metab 44:357–364. https://doi.org/10.1139/apnm-2018-0354
77. Yang C, Hao R, Deng Y, Liao Y, Wang Q, Sun R, Jiao Y, du X (2017) Effects of protein sources on growth, immunity and antioxidant capacity of juvenile pearl oyster *Pinctada fucata martensii*. Fish Shellfish Immunol 67:411–418. https://doi.org/10.1016/j.fsi.2017.06.037
78. Mongkolthanaruk W (2012) Classification of *Bacillus* beneficial substances related to plants, humans and animals. J Microbiol Biotechnol 22:1597–1604. https://doi.org/10.4014/jmb.1204.04013
79. Hong HA, Khaneja R, Tam NMK, Cazzato A, Tan S, Urdaci M, Brisson A, Gasbarrini A, Barnes I, Cutting SM (2009) *Bacillus subtilis* isolated from the human gastrointestinal tract. Res Microbiol 160:134–143. https://doi.org/10.1016/j.resmic.2008.11.002
80. Ilinskaya ON, Ulyanova VV, Yarullina DR, Gataullin IG (2017) Secretome of intestinal bacilli: a natural guard against pathologies. Front Microbiol 8:1666. https://doi.org/10.3389/fmicb.2017.01666
81. Elshaghabee FMF, Rokana N, Gulhane RD, Sharma C, Panwar H (2017) *Bacillus* as potential probiotics: status, concerns, and future perspectives. Front Microbiol 8:1490. https://doi.org/10.3389/fmicb.2017.01490
82. Donato V, Ayala FR, Cogliati S, Bauman C, Costa JG, Leñini C, Grau R (2017) *Bacillus subtilis* biofilm extends *Caenorhabditis elegans* longevity through downregulation of the insulin-like signalling pathway. Nat Commun 8:14322–14315. https://doi.org/10.1038/ncomms14332
83. Ayala FR, Bauman C, Cogliati S et al (2017) Microbial flora, probiotics, *Bacillus subtilis* and the search for a long and healthy human longevity. Microb Cell 4:133–136. https://doi.org/10.15698/mic2017.04.569
84. Hernández-Zárate G, Olmos-Soto J (2006) Identification of bacterial diversity in the oyster *Crassostrea gigas* by fluorescent in situ hybridization and polymerase chain reaction. J Appl Microbiol 100: 664–672. https://doi.org/10.1111/j.1365-2672.2005.02800.x