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Chapter 28

Probiotic Biofilms

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1. Introduction

Microalgae are in global scale primary producers, they are involved in all marine and fresh waters ecosystems. The growth of microalgae is correlated directly with the chlorophyll *a* concentration, and the bacterial population, and both variables are tightly related with the number of planktonic cells [1, 2]. However, there are numerous studies completed at date about microalgae, often the associated communities of bacteria have not been considered. Recently it has been evidenced that there is not only a positive correlation between bacteria and microalgae concentration but there is also a positive correlation between the extracellular polymeric substances (EPS), which is bigger in bacteria-microalgae mixed cultures than in microalgae axenic cultures [3]. These bacterial communities play a critical role in modulating the population dynamic and the algal metabolism. The kinds of interactions between algae and symbiotic bacteria under photoautotrophic conditions may involve mutualism and commensalism [4]. The role of bacteria is important because they act as a source of inorganic nutrients, feeding, and in viral lysis in algal growth control, physiology, and events of cellular differentiation [5, 6]. Bacteria in microalgal phycosphere stimulate algal growth creating a favorable environment [figure 1; 7], regenerating organic and inorganic nutrients [8, 9], or producing growing factors, including trace metals, vitamins, phytohormones and chelates [10, 11]. Nevertheless, in some described cases microbiota can inhibit algal growth. Algaecide bacteria are investigated as a one of the key biological agents in the abrupt end of microalgae blooms [12]. Algaecide bacteria attack and kill directly the microalgae or produce special compounds to lyse these cells [13, 14, 15]. Other non-algaecide bacteria can inhibit the microalgal growth changing the microenvironment of the microalgae [16] or by competing with the microalgae for nutrients [17, 18].

Other described processes that occur between bacteria and microalgae involve various ecological relationships such as competence, parasitism and other important microbiological processes [19]. Thereby, the microalgae can inhibit and/or induce the bacterial growth due to

the production of organic exudates or toxic metabolites. Inversely, the bacteria can produce stimulating or inhibiting effects in microalgae through the production or absence of nutrients and/or stimulating or inhibiting substances which affect microalgae [20, 21, 22]. Delucca and McCracken (1977) [23] suggest that the interactions bacteria-algae are not randomly but highly specific. There are numerous data which report that the extracellular products from algae are capable to stimulate the growth of bacterial strains [21, 22] through the excretion of carbohydrates, organic acids, nitrogenous substances and vitamins [24]. Some studies in natural ecosystems have determined that organic substances derived from phytoplankton are used by bacteria as a substrate for growing. However, microalgae also inhibit bacterial growth by production of organic exudates or toxic metabolites. There are several reports suggesting a synergistic action between microalgae and its bacterial flora associated [figure 2; 25].

Most part of microbial life develops in biofilm form, either in surface or aggregates. In this ecosystem, bacteria and microalgae are the predominant components and they are the basis of the trophic chain and of the organic matter recirculation. A biofilm is a microbial consortium associated with EPS and other molecules attached to a submerged surface. The formation of a biofilm begins with the accumulation of organic molecules over a submerged surface, this physicochemical event occurs in a few seconds or minutes after the immersion of any surface in a liquid. Few hours later of the establishment of a macromolecular film, the bacterial colonization starts [26].

A mature biofilm is capable to maintain the concentrations of ammonium and phosphate present in the surrounding medium at low levels. Thompson et al. (2002) [27] determined that the decline of the ammonium concentrations is related with the increase of the chlorophyll *a* in biofilms, determining that the ammonium was absorbed mainly by the microalgae to produce new biomass. In Thompson et al. (2002) [27] experiments, most of the ammonium ingest in biofilm occurs at 10-15 days after the beginning of the experiment, when the chlorophyll *a* concentration reaches 5 μgcm^{-2}. In this case, the microalgae community is dominated by pennates diatoms (*Amphora*, *Campylopyxis*, *Navícula*, *Sinedra*, *Hantschia* and *Cylindrotheca*) and filamentous cyanobacteria (*Oscillatoria* and *Spirulina*). The fact that a biofilm effectively absorbs or transforms the ammonium present in the water column has important applications as probiotic for health of cultivable species such as juveniles of mollusks and crustaceans, including *Farfantepenaeus paulensis*, due to that shrimps tolerate high nitrate (>15000 μM) and nitrite (>1000 μM) concentrations [28], but ammonium in high concentrations is lethal, and can inhibit seriously the ingestion of food and growth [29, 30].

Mainly, the use of bacteria-microalgae biofilms would be applicable to tanks of intensive cultures in which there are a great accumulation of dissolved nitrogen, especially ammonium, as a result of addition of food and excretion of organisms maintained in high density, being one of the most important problems in intensive culture of shrimp and other mollusks, affecting the ingestion of food, growth and survival [28, 30]. One alternative to maintain a high water quality is the biological treatment, based in the use of pre-colonized

filters by microorganisms that absorb the excess of nutrients from water. A similar process occurs in nature, where biofilms associated with a matrix of EPS attached are responsible of many biogeochemical cycles in aquatic ecosystems, especially the one of the nitrogen [31]. The eutrophication process accelerates if the main form of nitrogen inputted in the ecosystem is ammonium. This happens due to that the primary producers use less energy to incorporate this source of N into the amino acids and proteins, while the nitrate form must be transformed inside the cells to ammonium, with a higher cost of energy. Therefore, autotrophic cells grow faster in presence of ammonium forms than nitrate [27]. Thus, the presence of biofilms could reduce the eutrophication in the water mass that receives the effluents of aquaculture rich in ammonium through the absorption of this.

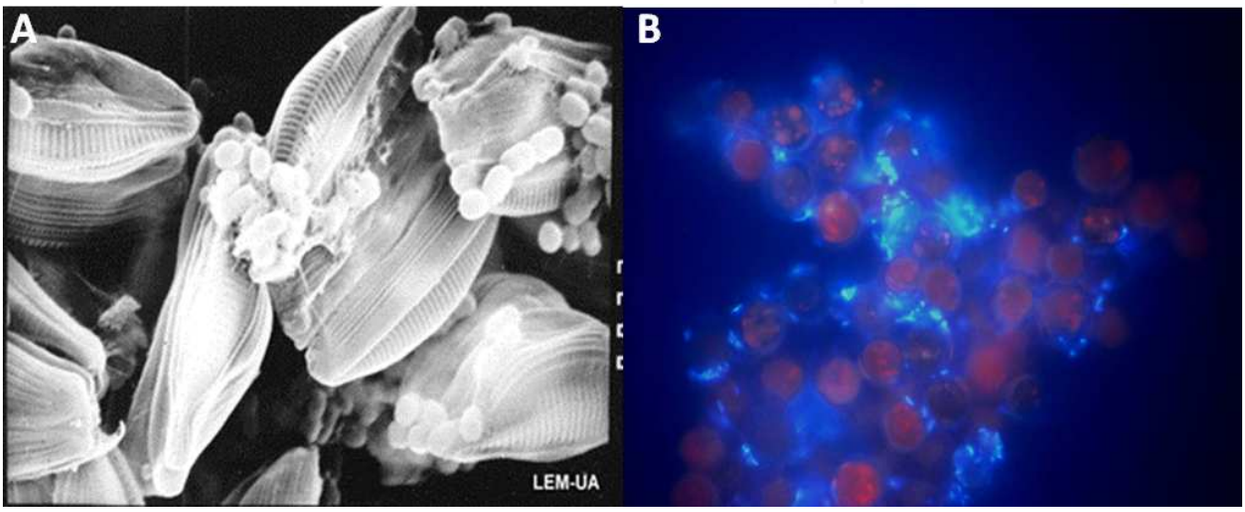

Figure 1. A, Biofilm from bacteria *Alteromonas* sp. and microalga *Navicula incerta*. B, Biofilm from microalga *Botryococcus braunii* and bacteria *Rhizobium* sp.

Nevertheless, a point to consider is that the biofilms have been thoughtful as reservoirs of pathogens bacteria, like *Vibrio harveyi*, which can affect crustacean's cultures such as shrimp. Pathogens bacteria present in biofilms are difficult to eliminate through the use of antibiotics, due to the hardness of the access of these molecules into the biofilms [32]. However, the results of Thompson et al. (2002) [27] indicate that the ingestion and transformation of nitrogen by the biofilm may help to reduce the occurrence of pathogens bacteria, due to that this microorganisms normally are present in situations where nitrogenous compounds are extremely high [33]. On the other way, lots of microalgae present in biofilms are capable to produce antibiotics that prevent the growing of pathogens bacteria [34, 35]. Protozoa that inhabit biofilms could also control abundance of pathogenic bacteria through the grazing [36]. Avila-Villa et al. (2011) [37] evaluate the presence of pathogen bacteria in microalgae, determining that species of these kind of bacteria such as NHPB (necrotizing hepatic pancreatitis bacteria) don´t attach to the surface of any microalgae and besides, they don´t survive in presence of these species, confirming the production of antibiotic substances by these microalgae species [38]. Respect to the benthic microalgae *Navicula* sp., this can easily form biofilms, and some bacteria thrive there using the exudates of the microalgae and the excreted extracellular products (carbohydrated

substances and with nitrogen, organic acids and lipids) as a source of nutrients [39]. Besides, it has been documented that predominant bacteria linked to biofilms of algae are γ-proteobacteria and α-proteobacteria [40]. Thus it is possible, on the contrary to the expected effect, that the elimination of a biofilm could increase the risk to develop pathogenic bacteria. Also, is important to note that biofilms are considered an important source of food for cultivable species such as *Daphnia* [41], Nile tilapia [42] and carpa [43]. Despite the low protein content measured in biofilms, the microorganisms in there can provide essential elements such as; polyunsaturated fatty acids, sterols, amino acids, vitamins and carotenoids [36]. Thus, the biofilm probably contribute to the increment of weight and total biomass of juvenile of crustaceans like *F. paulensis* [27]. On the other hand, biofilms are essential in crustacean´s cultures too like fresh water crab *Cherax quadricarinatus*, and also another kind of cultures, the presence of biofilms impact directly in water quality of cultures, increasing survival almost in 100% when they are feed with biofilms and also there is an increment in the growth of juveniles [44]. Different species of cultured crustaceans have improved their growth or survival when biofilms are used as a food source [27, 45, 46]. Moreover, water quality in culture systems is remarkably improved by the use of the biofilm [27, 47].

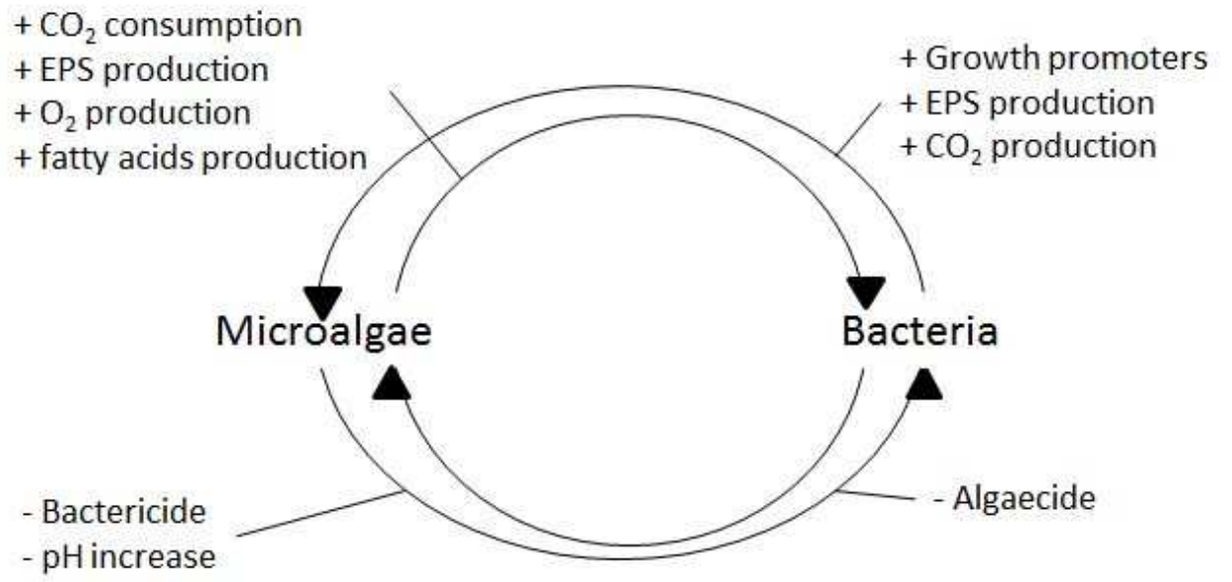

Figure 2. Interactions between microalgae and bacteria.

2. Probiotic role

Aquaculture is an important economic activity worldwide, in an attempt to improve the production of organisms it has been used a great quantity of antibiotics in an indiscriminate way for diseases control. Due to this, nowadays its use is questioned because the bacterial resistance generated and for the tons of antibiotics released to the biosphere during the last 60 years [48]. Recently, as an alternative for improve the growth of the cultured organisms, disease control and to improve the immune system it has been proposed the use of

probiotics [49, 50, 51, 52]. The term "probiotic" is defined as "live microorganisms administered in appropriated quantities as food or food supplement that have benefic effects in the intestinal microbiological equilibrium of the host" [53]. The benefits for the host consist in to optimize the degradation and absorption of the food, favoring the autochthonous microbiota balance [49] reducing the pathogenic load [50]. According to the literature, most of the probiotics proposed as agents of biological control in aquaculture are bacteria from genus *Vibrio* and *Bacillus* [50].

In natural habitats, most bacteria are associated to algae and can have both effects in the algal growth, beneficial or deleterious. The interaction between algae and bacteria are complex and include competition for resources [54], production of antimicrobial agents [55, 56], stress protection through the production of extracellular polymeric substances, and the junction of metals or transformation through the production of exudates [57]. The algal cells can associate with a range of bacterial communities [58, 59] and this association vary from to share the general habitat, to a colonization of bacteria in the algal surface (epiphytic biofilm) and the endophytic association of bacteria inside de algal cells. There are reports that show that the presence of a large number and diversity of bacteria associated with algal cultures enhances the growth of algal species [table 1; 60]. This increase in growth rate suggests that the relationship between algae and bacteria in these cultures is beneficial to algae. Grossart et al. (2006) [59] also found that the cell density of *Skeletonema costatum* in the exponential phase of growth was significantly higher in the presence of bacteria. The ability of bacteria to increase algal growth depends on the growth phase of algae in which is added [59]. It has been determined that the cell densities of *Thalassiosira rotula* remain higher when is exposed to bacteria in the exponential phase of growth, but if is exposed in the stationary phase, the algal cell densities decrease rapidly. The response of the algae will then depend on the species of bacteria and the medium in which the algae obtain their nutrients and vitamins [5, 61]. It has been observed that bacteria specifically isolated from the surface of marine diatoms have a greater positive effect on algal growth than those isolated from the ocean [54], suggesting that the spatial relationships between bacteria and algae can be important. Rier and Stevenson (2002) [62] suggest that bacteria tend to be effective competitors for resources because they have (i) rapid growth rate, (ii) a ratio of volume per surface area larger (iii) rapid rates of phosphorus intake. In the oligotrophic conditions of the open sea the algae-bacteria relationship is consolidated because the concentration of the non-algal dissolved organic matter is very low and bacteria prefer carbon derived from algae as an energy source. This was verified in laboratory bioassays in which dissolved organic matter decreases rapidly when bacteria are present, demonstrating that they have a rapid dissolution and decomposition of organic matter [59].

There are many studies reporting the growth promoter effect on microalgae by bacteria (table 1). Induction of bacterial growth in specific cultures has been reported for a few species of microalgae such as *Chlorella vulgaris*, *C. sorokiniana* and *B. braunii*, and growth promoter bacterial strains are mainly of *Azospirillum spp* and a *Rhizobium sp.* [63, 64, 65, 66, 67]. Induction of growth in plants used in agriculture through the use of plant growth promoter bacteria (PGPB) [68] is an established fact, involving the use of different

mechanisms between plants and bacteria, in which the final product of these many associations is to improve a characteristic of the plant, usually depending on the uses of the plant for human consumption [69]. On the other hand, induction of aquatic microalgae by bacteria, although it was discovered decades ago, is an emerging field in which the majority of studies have been performed in recent years [65, 70,71]. The main interest in this artificial association between algae and bacteria is due to obtaining a community associated with better characteristics than the microalgae alone [73] for applications such as removal of contaminants from wastewater [8], or use as food [74] or as a probiotic. The mechanisms by which growth-promoter bacteria in plants (PGBP) [68] affect the growth of plants vary widely. PGPB directly affect the metabolism of plants giving substances that are usually of low availability. These bacteria are capable of fixing atmospheric nitrogen, solubilize phosphorus and iron, and produce plant hormones such as; auxins, giggerelins, cytokinins, ethylene, nitrite and nitric oxide. Additionally, they improve stress tolerance in plants (drought, high salinity, metal toxicity and the presence of pesticides). One or more of these mechanisms may contribute to increase the growth and development of plants, higher than normal in standard culture conditions [69, 75]. Most PGPB are *Bacillus* spp. that work by diseases control [76], however some species of *Bacillus* promote the absence of disease by stimulating the immune system [77]. Possible interactions between *Bacillus* spp. with microalgae are unknown. Thereby, *Azospirillum* is one of the few genera of bacteria known to promote the growth of microalgae (Microalgae growth promoter bacteria, MGPB) [65]. *Azospirillum* is the most studied PGPB in agriculture [77]. Its habitat is the rhizosphere, N_2-fixing bacteria that is very versatile in its nitrogen transformations. In addition to fix N_2 under microaerobic conditions, act as denitrifying under anaerobic or microaerobic conditions, and can assimilate NH_4^+, NO_3^-, o NO_2^- and acts as a general PGPB for many species of plants, including the microalgae *Chlorella* [65]. *Azospirillum* spp. significantly alters the metabolism of microalgae, mainly producing indole-3-acetic acid (IAA) [78] and increasing the nitrogen cycle enzymes in these algae [73]. Although several studies described that inoculation of marine phytoplankton and freshwater bacteria sometimes increase their productivity [74], these studies are descriptive and exploratory and there is no mechanism described or demonstrated by which the phenomenon occurs. Despite the induction of microalgal growth by bacteria, not all interactions are positives; interaction of *C. vulgaris* with their associated bacteria *Phyllobacterium myrsinacearum* induces culture senescence [65, 79]. In a study by Hernández et al. (2009) [66] was employed the PGPB *Bacillus pumilus* Es4, originally isolated from the rhizosphere. This PGPB fix atmospheric nitrogen, produce IAA in vitro in the presence of tryptophan, besides to efficiently produce siderophores and increase growth in a cactus for long periods of time. *B. pumilus* Es4 also induces the growth of the microalga *C. vulgaris* acting as a MGPB, but this occurs only in the absence of nitrogen. *Chlorella* spp. is able to grow without nitrogen by a limited period of time, using ammonium that can be produced and recycled within the organism by a variety of metabolic pathways, such as photorespiration, phenylpropanoid metabolism, use of compounds of nitrogen transport, and amino acids catabolism [66, 80]. In this regard, *Chlorella* growth in the absence of other microorganisms can be explained by the differential activity of the enzyme glutamate dehydrogenase. This enzyme serves as a bond between the

nitrogen and carbon metabolism due to its ability to assimilate ammonium to glutamate or to deaminate the glutamate to 2-oxoglutarate and ammonium under stress conditions [80, 81]; thus, the ammonium may be re-absorbed by *Chlorella* and used to a limited growth.

De Bashan and Bashan (2008) [78], proposed and studied a model of microalgae and bacteria immobilized in alginate to analyze and evaluate their possible interactions. In their study described the following sequence of events occurring during the interaction between the two microorganisms. Randomly immobilization of *Chlorella* spp. occurs first with a PGPB strain within a matrix and nutrients are in the surrounding medium that diffuses freely. In a given time (from 6 to 48 hours), depending on the bacteria-microalgae combination, both microorganisms are in the same cavity of the sphere, mainly in the periphery [79]. Here the bacteria secrete indole-3-acetic acid (IAA) and other undefined signal-molecules, possibly near the microalgal cells. At this stage, the activity microalgal enzyme (glutamine synthetase and glutamate dehydrogenase) does not increase. In the next phase of interaction, after 48 h occurs the increment of the enzymatic activity, production of photosynthetic pigments, and nitrogen and phosphorus intake. It also occurs releasing of oxygen as a byproduct of photosynthesis [for review see 65]. The most notable effect is the increasing by 2 to 3% on growth of microalgae with PGPB on those without PGPB [65]. This model proposed by Bashan and Bashan (2008) [78] has been evaluated in various combinations of microalgae-PGPB demonstrating the induction of growth in *C. sorokiniana* and *B. pumilus*, and others *C. vulgaris* and *A. brasilense* Sp6 [table 1; 78]. At cell and culture level there is an increase in the absorption of ammonium. The addition of exogenous tryptophan (precursor of the phytohormone IAA and the main mechanism by which *Azospirillum* affects the growth of *Chlorella* [64]) also induces a significant increase in the growth of microalgae. It also increases the activity of glutamate dehydrogenase, a key enzyme in ammonium assimilation in plants. Other PGPB such as *B. pumilus* and other microalgae, such as *C. sorokiniana* have been tested successfully (table 1). These options create opportunities for many combinations of microalgae and PGPB. Similarly, different alginates and derivatives from many macroalgae are commercially available [72] and to design the necessary combination and entrapment schemes. Because the immobilization of microorganisms is commonly used with other polymers [83], this model is not restricted to alginates, but each polymer has its advantages and disadvantages to be studied in future studies.

The EPS (a heterogeneous mixture of polysaccharides, proteins, nucleic acids, lipids and humic acids [84]) have a key role in biofilms, recently defined as a stabilization mechanism in mixed biofilms of bacteria and microalgae and present in a significantly higher percentage only when microalgae are associated with bacteria [3]. Furthermore, EPS are also important for the recycling of trace metals in aquatic systems, favoring metal binding to bacterial and algal agglomerates, and colloidal material/EPS, allowing the removal from surface waters and large particles [57]. Bacterial colonization is superior in stressed algal cells more than in healthy algal cells [54], which can be related to the release of organic material from the cell after cell lysis as part of a process of senescence, or under conditions of induced stress, such as exposure to contaminant metals [60]. The inability to detect visually bacteria from axenic cultures may be due to a very close association of the bacteria in the algal phycosphere or in the cell wall, or

bacteria are in endophytic form in the algal cell, making it impossible to remove the bacteria from the algae using physical techniques. What's more, it appears that algal species benefit from the presence of bacteria, increasing their growth rate [60, 67]. The production of exudates of communities in bacteria/microalgae mixed biofilm increase in exposure to metals [85]. These exudates may be produced from algae or bacteria, but they are used as a mechanism of survival and resistance to stress for entire biofilm [60].

Type of study	Microalga species	Bacterial strain (s)	Reference (s)
Growth promotion	*Oscillatoria* sp.	*Pseudomonas* sp., *Xanthomonas* sp., *Flavobacterium* sp.	23
Growth promotion (dry wt, cell no., colony size, cell size)	*Asterionella gracilis*	*Pseudomonas* sp., *Vibrio* sp.	20
Antibacterial activity	*Chattonella marina*	*Pseudomonas*	20
Growth promotion	*Asterionella gracilis*	*Flavobacterium* NAST	20
Antibacterial activity	*Skeletonema costatum*	*Vibrio* sp., *Listonella anguillarum, Vibrio fisheri*	108
Growth promotion	*Isochrysis galbana*	*Vibrio* sp. C33, *Pseudomonas* sp. 11, *Arthrobacter* sp. 77	22
Antibacterial activity	*Tetraselmis suecica*	*Listonella anguillarum, V. alginolyticus, V. salmonicida, V. vulnificus, Vibrio* sp.	34
Growth promotion (dry wt, cell no., colony size, cell size)	*C. vulgaris*	*A. brasilense* Cd. Sp6, Sp245; A. lipoferum JA4	65, 70
Delayed senescence	*C. vulgaris*	*A. brasilense* Cd; P. myrsinacearum	79
Population control	*C. vulgaris*	*A. brasilense* Cd; P. myrsinacearum	59, 79
Lipids	*C. vulgaris*	*A. brasilense* Cd	126
Modification of fatty acids	*C. vulgaris*	*A. brasilense* Cd	126
Cell-cell interactions	*C. vulgaris*	*A. brasilense* Cd	126
Mitigation of heat and intense sunlight	*C. Sorokiniana*	*A. brasilense* Cd	126
Population dynamics	*C. vulgaris*	*A. brasilense* Cd	63
Mitigation of tryptophan inhibition	*C. vulgaris*	*A. brasilense* Cd	63
Mitigation of pH inhibition	*C. vulgaris*	*A. brasilense* Cd	8
Photosynthetic pigments	*C. vulgaris*	*A. brasilense* Cd, *Phyllobacterium myrsinacearum, B. pumilus*	8, 66, 72, 105, 126
Nutrient starvation	*C. Sorokiniana*	*A. brasilense* Cd	70
Enzymes in the nitrogen cycle	*C. vulgaris*	*A. brasilense* Cd	70
Hormones	*C. Sorokiniana*	*A. brasilense* Cd; *B. pumilus*	66, 70
Absortion of nitrogen and phosphorus	*C. vulgaris, C. Sorokiniana*	*A. brasilense* Cd, Sp6, Sp245; FAJ0009, SpM7918; *A. lipoferum* JA4, JA4::ngfp15	73
Growth promotion	*Botryococcus braunii*	*Rhizobium* sp.	67

Table 1. Studies of paired microalga-bacteria interactions.

3. Induction of larval settlement

Benthic diatoms present in the biofilm plays an important role in the marine ecosystem not only serve as food for advanced stages of development of marine invertebrate larvae [86], but also with bacteria and other microorganisms, form an attractive site for larval settlement in the process of metamorphosis [87]. There are numerous studies which have determined the characteristics that make a substrate optimal for larval settlement, and which are the effects of various biofilms in controlling larval settlement events [87, 88, 89, 90]. In the natural environment, the development of a biofilm formed by diatoms and other organisms is preceded by primary colonization of bacteria [91] aided by the EPS which act as "glue" and work at the cellular and molecular level to establish a strong and irreversible binding to a given substrate [92]. This succession of microorganisms often precedes the subsequent stages in a substrate, in which the macroorganisms eventually begin to be dominant [26].

Avendaño-Herrera and Riquelme (2007) [87] showed how optimize the production of a biofilm formed by the diatom *Navicula veneta* and a bacterium of the genus *Halomonas* sp., proposed model for the use in the induction of larval settlement. When the strain of *Halomonas* spp. was added to the diatom occurs an acceleration of growth of *N. veneta* [87], this occurs only when adding live bacteria, indicating the requirement of precursors of extracellular products excreted by the bacteria. Without the presence of *Halomonas* the microalgal biomass obtained is 65% lower. Is important to note that the diatom-bacteria biofilm can be used efficiently to provide food for species such as, abalone or scallop juvenile stages, and/or to colonize substrates that are used for adhesion, favoring larval settlement and reducing production time in macroorganisms cultures [93]. In addition, phytoplankton cultures are widely used in the aquaculture industry for a variety of purposes; these cultures are described as "green water" because they contain high levels of phytoplankton species such as *Nannochloropsis* sp. and *Chlorella* sp. The "green water" is added to the tanks with fish larvae and to enrich zooplankton, and provide a direct and indirect nutrition for the larvae. Moreover, the "green water" reduces water clarity, minimizing larval exposure to light, which acts as a stressor [94]. According to this, the presence of phytoplankton improves water quality by reducing the ammonium ion concentrations and increasing concentrations of dissolved oxygen through photosynthesis. Notably, phytoplankton also produces antibacterial substances that can prevent disease outbreaks [95, 96, 97, 98]. Among these, important are some members of the *Roseobacter* clade (Alphaproteobacteria) such as *Phaeobacter* and *Ruegeria* that suppress the growth of the fish pathogen *Vibrio anguillarum* by producing tropodithietic acid (TDA) [98, 99, 100, 101]. Also the abundance of bacteria from *Roseobacter* clade is highly correlated with phytoplankton blooms [102].

4. Chemical signals in bacteria-microalgae biofilms

According to the study of Sharifah and Eguchi (2011) [94] there is synergy and beneficial contribution by using bacteria belonging to the *Roseobacter* clade together with phytoplankton like *N. oculata*. In their study they used approximately between 11.4 to 13.2%

of bacteria in indoor cultures of *N. oculata*. These levels are comparable to the concentration of bacteria in coastal sea water (<1-25%) [102, 103]. Most of the cultivable bacteria in the *Roseobacter* clade corresponding to the genera *Phaeobacter*, *Silicibacter*, *Sulfitobacter*, *Roseobacter* and *Roseovarius*, which have potentially probiotic properties [99, 100, 102]. When these species are adding with phytoplankton to the tanks with fish larvae increased larval survival [95, 96, 97, 104] for growth inhibition of pathogenic bacteria. This process could be mediated by at least two possible mechanisms. The first one involves the preferential entry of nutrients or competition for nutrients, by bacteria. The second one, and more complex, involves a direct interaction between phytoplankton and microbes such as phytoplankton and pathogenic bacteria, probiotic bacteria and pathogenic bacteria, and phytoplankton-probiotic bacteria and pathogenic bacteria. Regarding the first mechanism, competition for entry of nutrients, the abundance of the *Roseobacter* clade in the coastal sea is correlated with the release of organic substances from natural phytoplankton blooms such as dimethylsulfoniopropionate (DMSP) and amino acids [105, 106]. In turn *N. oculata* may also excrete some substances similar to DMSP or amino acids that support more optimally bacterial growth of the clade [94]. Referring to the second mechanism described above, involving complex interactions, there is no direct inhibition of fish pathogens by phytoplankton, in contrast to other studies [107, 108]. As there is no difference in the viability of *V. anguillarum* by using probiotic bacteria it was concluded that there is no direct inhibition on the viability of *V. anguillarum*. In contrast, a study of the diatom *Skeletonema costatum* and the macroalgae *Ulva clathrata*, they produce organic compounds that inhibit the growth of *V. anguillarum* directly [107, 108].

From this point of view, the *Roseobacter* clade is beneficial and acts as a probiotic to induce the spread of scallop [109] and larvae of turbot [110] by removing fish pathogens. Other studies show that bacterial cell density of the clade in the range of 10^6-10^9 CFUml^{-1} is needed to reduce pathogenic bacterial population by 10% [94]. Added to this, the static conditions favor culture biofilm formation by allowing bacteria of the genera *Phaeobacter*, *Silicibacter*, *Sulfitobacter*, *Roseobacter*, *Pseudoalteromonas* and *Roseovarius* produce tropodithietic acid (TDA), antibacterial compound produced by *Phaeobacter* spp., *Silicibacter* sp. and *Ruegeria* sp. [100, 111]. Static culture conditions and the presence of a brown pigment are indicators of the production of TDA [100]. However, in the study of Sharifah and Eguchi (2011) [94] *Roseobacter* clade members produced different antibacterial compounds to TDA, and the cultures were incubated under agitation and did not produce brown pigment. Interestingly, the previous study demonstrated that agitated *Roseobacter* cultures are able to eliminate *V. anguillarum* only in the presence of substances excreted from phytoplankton, and none of these species belongs to *Phaeobacter* sp. previously described [101]. The Inhibitory activity *of Sulfitobacter* sp., *Thalassobius* sp., *Rhodobacter* sp. and *Antarctobacter* sp., is significantly affected by the thermostable substances excreted by *N. oculata* [94]. Microalgae *N. oculata*, *N. granulata*, *N. oceanica* and *N. salina* produce putrescine, a thermostable polyamine [112]. Moreover, *N. oculata* CCMP525 produces signaling molecules like low molecular weight *n*-acyl-homoserine lactones which are produced by bacteria to the communication system cell-

to-cell regulating gene expression [quorum sensing; 113]. The analogues of *n*-acyl-homoserine lactones are thermostable. These compounds can be secreted by *N. oculata* and act as signaling molecules for communication with *Sulfitobacter* sp. RO3 resulting in growth inhibition of *V. anguillarum*. These results demonstrate that phytoplankton cultures used as "green water" for the production of fish larvae have a key role in enhancing the inhibitory effect of *Roseobacter* clade against *V. anguillarum*. A similar inhibitory effect was also observed in *Chlorella* sp., other marine microalgae used in aquaculture [94].

5. Other applications

Immobilization of microorganisms on polymers because the production of different products and environmental and agricultural applications is well known and have increased in the last two decades [93, 114, 115]. The immobilization of microalgae is a common approach for many applications of bioremediation [66]. Immobilization in several substances provides to the microorganisms several advantages over free-living microorganisms. These advantages include: (i) a continuous source of nutrients without competition with other microorganisms [116] and (ii) protection against environmental stress [66, 117], bacteriophages, toxins, and UV irradiation [118]. A recently developed treatment for tertiary domestic wastewaters uses the green microalga *Chlorella spp*. and the plant growth promoter bacteria (PGPB) *Azospirillum brasilense*, both bound and immobilized in alginate beads [116]. Each unit in this technological model, a single polymer sphere, contains within cavities that serve as matrix for the folding of microalgae and bacteria [66, 78, 119]. Additionally, the entrapment of microorganisms may also be within the solid matrix polymer of the polymeric sphere. In some cases, microbial cells are on the surface or partially in or out of the gel matrix. During the formation of alginate spheres the number of organisms is higher outside than inside. However, this approach can be used in aquaculture as a feeding method for growing mollusks such as *Haliotis rufescens* [120].

The algae are the organisms most commonly used to assess metal contamination and bioavailability in aquatic systems, are highly sensitive to heavy metals such as Cu, Fe and Cd in environmentally relevant concentrations. Algae are primary producers and affect nutrient cycling in marine and fresh water ecosystems, and in aquaculture [121]. As such, the algae are considered ecologically significant organisms and the ideal candidates for ecotoxicological studies. However, algae are rarely isolated in the environment, but are part of complex planktonic communities and biofilms. The alteration of community structure may influence the overall function (e.g. respiration, photosynthesis) and community sensitivity to toxicants. Although the tests of toxicity for single-species used in microalgae are highly sensitive and reproducible, they do not have a realistic environment. Interactions between algae and associated bacteria, in plankton or in biofilms, may alter algal sensitivity to pollutants. Recent research has attempted to develop multi-species algal test in the evaluation of metals based on toxicity [122, 123]. These studies explored the toxicological

response of individual algal species when they are exposed in combination with one or other species of algae.

Bacteria can have both positive and negative effects on algae in polluted environments. For example, the tolerance of the green macroalga *Enteromorpha compressa* to copper in a coastal environment in Chile attributed to an epiphytic bacterial community colonizing the surface [1]. Bacterial biofilms can mediate metal toxicity to the host organism by limiting the diffusion of toxins, protective effects of high concentrations of extracellular polymeric substances, protective effects of stored nutrients trapped, and effects due to a larger surface area (less toxic per cell). While the effects of metals in biofilms are widely reported [85, 124, 125], there are few studies on the effects of metal toxicity to algae biofilms.

6. Conclusions

Since the first studies of bacteria-microalgae interactions decades ago, it has been elucidate and discovered several events in which the close connection between these two heterotrophs and autotrophs components is evidenced. Showing that the coupling of microalgae-bacteria produces changes in the excreted compounds in the surrounding environment, that affects positively or negatively to others organisms.

Most of the interactions are strongly regulated by chemical signals. Although it has been described lots of phenomena in positive and negative interactions in biofilms, there are a few investigations that explore the chemical and molecular nature of chemical compounds involved in this interactions which are produced by microorganisms, this is why in the future will be required to deepen in the study of mechanisms involved in the growth of mixture biofilms.

The use of this biofilms in nature can be easily developed in the laboratory; they can be used increasing and affecting some specific compounds which are useful for a third organism of commercial interest. As well, in phenomena like larval settlement, induction of growth and increment of biomass rich in lipids has revealed a great potential probiotic use, particularly in aquatic industry which require more attention to the involved mechanisms in the action of this beneficial biofilms. These uses will allow us to get a better understanding of the role of these microbial consortiums in nature, and also a biotechnological orientation could be spread for the production of these beneficial biofilms in a stable and standard form.

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