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1	QUORUM SENSING AND QUORUM QUENCHING
2	IN THE PHYCOSPHERE OF PHYTOPLANKTON:
3	A CASE OF CHEMICAL INTERACTIONS IN ECOLOGY
4	
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26 ABSTRACT

27 The interactions between bacteria and phytoplankton regulate many important 28 biogeochemical reactions in the marine environment, including those in the global carbon, nitrogen and sulfur cycles. At the microscopic level, it is now well established 29 30 that important consortia of bacteria colonize the phycosphere, the immediate environment of phytoplankton cells. In this microscale environment, abundant bacterial 31 cells are organized in a structured biofilm and exchange information through the 32 33 diffusion of small molecules called semiochemicals. Among these processes, quorum 34 sensing plays a particular role as, when a sufficient abundance of cells is reached, it 35 allows bacteria to coordinate their gene expression and physiology at the population 36 level. By contrast, quorum quenching mechanisms are employed by many different 37 types of microorganisms to limit the coordination of antagonistic bacteria. This review 38 synthesizes quorum sensing and quorum quenching mechanisms evidenced to date in 39 the phycosphere of phytoplankton, emphasizing the implications that these signaling 40 systems have for the regulation of bacterial communities and their activities. The diversity of chemical compounds involved in these processes is examined. We further 41 42 review the bacterial functions regulated in the phycosphere by quorum sensing, which 43 includes biofilm formation, nutrient acquisition and emission of algaecides. Quorum 44 quenching compounds, their function in the phycosphere and their potential 45 biotechnological applications are also discussed. Overall, the current state of the art 46 demonstrates quorum sensing and quorum quenching regulate a balance between a 47 symbiotic and a parasitic way of life between bacteria and their phytoplankton host.

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51 **REVIEW**

52 **1. Definition of phycosphere of phytoplankton**

53 The term "phycosphere", was first used in the 70's (Bell and Mitchell 1972) to describe 54 the immediate region surrounding an algal cell, chain or colony. It describes a microbial 55 habitat deeply shaped by the alga. This microenvironment is composed of bacteria 56 measuring between 0.2 and 2µm, around algae varying in between 2 and 200µm 57 (Sieburth et al. 1978). The phycosphere supports bacteria in higher concentrations than 58 in the water column. For example, the concentration of 8.2×10⁸ to 2.6×10¹¹ bacteria ml⁻¹ 59 reported to surround the alga *Trichodesmium* (Paerl 1982; Sheridan et al. 2002) is much 60 higher than the average concentration of $5-6 \times 10^5$ cells ml⁻¹ found in the seawater. 61 Similarly, 10⁷–10⁸ culturable bacterial cells have been reported per gram of 62 Botryococcus braunii, a green microalga (Rivas et al. 2010). Several studies have 63 revealed that the phycosphere bacterial communities from different phytoplankton cells are complex and taxonomically diverse (Delong et al. 1993; Fandino et al. 2001; 64 65 Hasegawa et al. 2007; Rooney-Varga et al. 2005; Sapp et al. 2007; Tuomainen et al. 66 2006). In addition, this microscale environment is regulated by the release of 67 extracellular products by the algae, feeding a large consortium of inhabiting bacteria 68 (Bell and Mitchell 1972).

69

70 **2. Quorum sensing in the phycosphere of phytoplankton**

71 **2.1 Definition of quorum sensing**

Quorum sensing is a term describing a cell-to-cell bacterial communication system,
allowing bacteria to adapt their physiological response to the ambient bacterial density
(Fuqua et al. 1994; Nealson 1977). Bacteria engaged in quorum sensing emit small
semiochemicals named autoinducers (Schulz 2014) and simultaneously detect their

76 concentration. At low bacterial concentrations, few autoinducers are present in the 77 nearby environment and cells display individual phenotypes and behaviors. When the 78 concentration of autoinducers reaches a certain threshold, reflecting an increase in local 79 cell density, bacteria switch their genetic and physiological program and display 80 collective phenotypes and comportments (Fuqua et al. 1994). These communication 81 mechanisms induce numerous transduction cascades and regulate the expression of 82 many genes (Bassler 1999). They are known to influence bacterial metabolism and 83 phenotypes, including the establishment of biofilms (Parsek and Greenberg 2005), 84 bioluminescence (Nealson 1977; Waters and Bassler 2005) and virulence (Smith and 85 Iglewski 2003) among other functions (Diggle et al. 2007).

86

87 **2.2 Experimental approaches used to characterize quorum sensing in the**

88 phycosphere

89 Most of the studies reported in this review are based on a similar workflow. First, 90 bacterial strains are isolated from natural seawater sampled during a phytoplankton 91 bloom (Bachofen and Schenk 1998) or are isolated from algae cultures (Geng and Belas 92 2010; Gram et al. 2002; Schaefer et al. 2008; Wagner-Dobler et al. 2005). The capacity of 93 the isolated strains to communicate using quorum sensing is then tested using bacterial 94 whole cell sensing-systems (biosensors) (Patzelt et al. 2013; Rivas et al. 2010; Wagner-95 Dobler et al. 2005), such as Escherichia Coli [B523 (Andersen et al. 2001), Chromatium 96 violaceum CV026 (McLean et al. 1997) and Vibrio harvevi JMH612 (Henke and Bassler 97 2004) to name several examples among many others. These genetically modified 98 organisms encode the purple pigment violacein (1) or the Green Fluorescent Protein 99 (GFP) or and luminesce in the presence of exogenous acylhomoserine lactones (AHLs), 100 the most studied semiochemical. Such approach using genetically modified organisms to

101 detect AHLs expression is required, as these compounds are present in low 102 concentrations in water and technically very difficult to be directly quantified *in situ* in 103 bulk seawater (Hmelo and Van Mooy 2009). A few report mention the possibility to 104 detect AHLs directly in seawater particles (Hmelo et al. 2011), which can also avoid 105 potential false positive results acquired when using biosensors (Holden et al. 1999).

106 In a further step, AHLs can be characterized. In general, bacterial supernatants 107 containing AHLs are extracted, usually with ethyl acetate. Preliminary studies relied on 108 Thin Layer Liquid Chromatography (TLC) (Gram et al. 2002; Rivas et al. 2010). More 109 recent studies are frequently based on Liquid Chromatography and Mass Spectrometry 110 (LC-MS) (Schaefer et al. 2008), Gas Chromatography Mass Spectrometry (GC-MS) 111 (Wagner-Dobler et al. 2005) and MS/MS approaches (Van Mooy et al. 2012), sometimes 112 preceded by microfractionation, which allows greater separation and concentration of 113 the extracted metabolites. In some cases, double bond location has been determined by 114 derivatization with dimethyl disulfide (Neumann et al. 2013), and irrevocable 115 characterization can be achieved by 1D and 2D Nuclear Magnetic Resonance (NMR) 116 analyses.

117 The experimental approaches to evaluate the functional roles of quorum sensing 118 are more diverse. Most of studies have based their work on model strains where key 119 genes involved in autoinducer biosynthesis pathways are mutated, in particular in *lux*-120 like genes. Characterization of the growth and phenotypes of mutant strains elucidates 121 the potential physiological functions regulated by quorum sensing (Patzelt et al. 2013).

122

123 **2.3 Occurrence in the phycosphere**

124 There is strong evidence that quorum sensing occurs in microbial communities125 inhabiting the phycosphere of phytoplankton cells. A pioneering study published in

126 1998 revealed the presence of AHLs in cyanobacterial blooms (Bachofen and Schenk 1998), which is not surprising since the high bacterial densities required for quorum 127 128 sensing occur in the phycosphere. Since then, many reports have directly detected the 129 existence of quorum sensing in various phycosphere samples. These very diverse 130 bacteria were isolated from equally diverse phytoplankton, indicating that quorum 131 sensing is not restricted to a particular type of bacteria-algae interaction. This wide 132 diversity of phytoplankton includes dinoflagellates (isolated bacteria: Dinoroseobacter 133 shibae, Hoeflea phototrophica, Roseovarius mucosus), and other picoplankton cultures 134 (isolated bacteria: Sulfitobacter sp., Thalassospira lucentensis) (Wagner-Dobler et al. 135 2005). Similarly, another study revealed two bacterial strains (Pseudomonas and 136 *Rhizobium*) able to communicate by quorum sensing in a *Botryococcus braunii* 137 associated biofilm (Rivas et al. 2010). These results are in line with observations of 138 quorum sensing in bacterial communities attached to sinking particulate organic matter 139 (Gram et al. 2002; Hmelo et al. 2011), in the 0.8 – 3 μm fraction from filtered seawater 140 (Doberva et al. 2015) and in microbial mats, which include layers of cyanobacteria or 141 eukaryotic algae (Decho et al. 2009; McLean et al. 1997). Interestingly, a few reports also 142 noticed the capacity of certain bacterial phytoplankton (cyanobacteria) to produce AHLs 143 in their phycosphere (Sharif et al. 2008; Zhai et al. 2012).

144

145 **2.4 Nature of molecules involved in these chemical interactions**

Diverse secondary metabolites have been identified as quorum sensing mediators (Keller and Surette 2006). Among them, AHLs (or autoinducer type 1, AI-1), constitute a widespread class of quorum sensing molecules (Eberhard et al. 1981; Lazdunski et al. 2004). These semiochemicals are made of a lactonized homoserine ligated through an amide bond to a fatty acyl chain, which is of variable length (C₄ to C₁₈) and oxidization state and gives the signal its specificity. However, many other types of metabolites have
been identified that are involved in quorum sensing, including the *p*-coumaroyl
homoserine lactones (Schaefer et al. 2008), Furanosyl Diester Borate (FDB, autoinducer
type 2, AI-2) (Chen et al. 2002), quinolones (Pesci et al. 1999), peptides (Onaka et al.
1995) and γ-butyrolactones (Onaka et al. 1995).

156 Many different types of AHLs have been identified from bacteria isolated from the 157 phycosphere. Rivas et al. (2010) identified short chain AHLs (C4-AHL (2) and C8-AHL 158 (3)) in *Pseudomonas* sp. and *Rhizobium* sp. associated with the microalga *Botryococcus* 159 braunii. However, it appears that long-chain AHLs (more than 8 carbons in the acyl side 160 chain) tend to dominate in these cultures. This trend is sometimes interpreted as an 161 adaptation to increase the stability of these molecules in aquatic environments (Hmelo 162 and Van Mooy 2009; Yates et al. 2002). LC-MS analysis revealed that Dinoroseobacter shibae, isolated from the surface of the dinoflagellate Prorocentrum lima (Biebl et al. 163 164 2005), produces C18:1-AHL (or C18-en HSL) (4) and C18:2-AHL (or C18-dien HSL) (5), 165 including one to two unsaturated bonds in the acyl side chain. In addition, the strain DFL 166 18 is reported to produce a C8-AHL (3) (Neumann et al. 2013; Wagner-Dobler et al. 167 2005). Roseovarius mucosus strains, also isolated from dinoflagellate cultures, produce 168 C18:1 (4) and C14:1 (6) AHLs (Wagner-Dobler et al. 2005). Vibrio strains isolated from 169 Trichodesmium colonies produce 3-oxo-C8-AHL (7), while the Erythrobacter strains 170 obtained over the same experiment produce C14-HSL (Van Mooy et al. 2012). The AHLs 171 produced by a few cyanobacterial strains have also been characterized. Microcystis 172 aeruginosa is probably able to produce AHLs (Zhai et al. 2012) (8), while the 173 cyanobacterium *Gloeothece* PCC6909 produces a C8-AHL (3) (Sharif et al. 2008). The 174 AHLs are still the main quorum sensing molecules characterized in the bacteria isolated 175 from the phycosphere, probably because not many studies have focused on other

compounds. Two exceptions are the report of AI-2 (9) producing *Vibrios* in the epibiont
populations of *Trichodesmium* (Van Mooy et al. 2012) and the report of a potential role
for AI-2 in the control of the algaecide activity against the dinoflagellate *Gymnodinium catenatum* (Skerratt et al. 2002).

180 Some recent publications also highlighted the role of tropodithietic acid (TDA) 181 (10) as an autoinducer in many *Rhodobacterales* species (Geng and Belas 2010). This 182 molecule is produced by different bacterial strains, including the genus *Phaeobacter*, 183 Silicibacter and Ruegeria, known to be frequently associated to unicellular algae 184 (Brinkhoff et al. 2004; Bruhn et al. 2005; Geng et al. 2008; Porsby et al. 2008). A series of 185 genetic-based experiments demonstrated that TDA acts as an autoinducer in Silicibacter 186 sp. TM1040 (Berger et al. 2011; Geng and Belas 2010), an isolate from the phycosphere 187 of the dinoflagellate, Pfiesteria piscicida (Alavi et al. 2001; Miller and Belas 2006). These 188 approaches demonstrated that TDA induces the transcription of *tda* genes, and that the 189 production of TDA is density dependent (Geng and Belas 2010), two key conditions in 190 recognizing TDA as a quorum sensing mediator. Interestingly, TDA production has been 191 shown to be 3.7 to 17.4 times greater in standing compared to shaking cultures, 192 suggesting that it plays an important role in bacterial biofilm function within the 193 phytoplankton phycosphere (Geng and Belas 2010).

194

195 **2.5 Roles of quorum sensing in the phycosphere**

196 **2.5.1 Formation of biofilms**

197 Quorum sensing is commonly hypothesized to play a role in niche colonization, in 198 particular because quorum sensing is known to be involved in biofilm formation and 199 surface attachment (Davies et al. 1998; Labbate et al. 2007; Nadell et al. 2008). Some 200 studies support the idea that quorum sensing may help bacteria to colonize particles of

201 organic matter or the phytoplankton phycosphere in the marine environment (Gram et 202 al. 2002; Hmelo et al. 2011). The capacity of *Roseobacter* to attach to surfaces has been 203 linked with an ability to communicate by quorum sensing (Rao et al. 2006). Similarly, 204 the emission of AHLs by the cyanobacterium *Microcystis* correlates with the switch to a 205 biofilm lifestyle (Zhai et al. 2012). The same hypothesis has been proposed to interpret 206 the capacity of *Phaeobacter* (isolated from a dinoflagellate culture) to produce TDA as an 207 autoinducer, as the expression of *tda* genes coincided with biofilm-formation (Geng and 208 Belas 2010). However, the relationship between biofilm formation and emission of 209 quorum sensing compounds is more complex. For example, *Silicibacter lacuscaerulensis* 210 and *Silicibacter pomeroyi* both harbor quorum sensing systems, but do not present the 211 same traits for surface colonization (Slightom and Buchan 2009). Other authors 212 demonstrated that biofilm formation is not necessary for TDA production in 213 Phaeobacter inhibens (Prol Garcia et al. 2014). Also, to our knowledge, no study has 214 demonstrated that quorum sensing facilitates biofilm formation within the phycosphere.

215

216 **2.5.2 Acquisition of nutrients**

The hypothesis that quorum sensing may favor nutrient acquisition in bacteria has been well supported from experiments on model strains in niches other than the phycospshere (Popat et al. 2015; Rosenberg et al. 1977). For example, it has been shown in *Pseudomonas aeruginosa* that the secretion of proteases confers a greater benefit to the whole population (Darch et al. 2012).

The phycosphere, as initially described, is a source of algal nutrients available to bacteria in its immediate environment (Bell and Mitchell 1972). Thus, it is not surprising that one of the hypothesized functions of quorum sensing in the phycosphere is that cell coordination favors nutrient acquisition. A recent article clearly showed that epibionts

226 of *Trichodesmium* use quorum sensing to up-regulate phosphate acquisition by alkaline 227 phosphatases. AHLs were involved in this process, while AI-2 (9) lead to a decrease of 228 phosphate uptake (Van Mooy et al. 2012). Similarly, it has been shown that *Ruegeria* 229 pomeroyi overproduces N-(3-oxotetradecanoyl)-L-homoserine lactone (11) when 230 grown with dimethylsulfoniopropionate (DMSP) as an energy source, which is 231 metabolized into dimethyl sulfide (DMS) (Johnson et al. 2016). This AHL production is 232 also consistent with important modifications in the cell metabolome, suggesting that in 233 the presence of algal DMSP, Roseobacter switches to a cooperative lifestyle (Johnson et 234 al. 2016). Interestingly, it has been shown that *p*-coumaric acid (12), a product of algal 235 lignin degradation released by decaying phytoplankton cells, is also the precursor of the 236 *p*-coumaroylhomoserine lactone (13) involved in *Rhodopseudomonas palustris* quorum 237 sensing (Schaefer et al. 2008). Thus, the emission of semiochemicals linked with the 238 release of phytoplanktonic molecules may also convey information about environmental 239 conditions in the phycosphere, such as the availability of exogenously supplied 240 substrates (Buchan et al. 2014; Schaefer et al. 2008). In a similar vein, it has been shown 241 in the algae symbiont, Dinoroseobacter shibae, that quorum sensing controls flagellar 242 biosynthesis (Patzelt et al. 2013), potentially enabling chemotaxis to microalgae and 243 thus favoring nutrient acquisition.

The large-scale biogeochemical consequences of bacterial coordination for nutrient acquisition within the phycosphere remain poorly explored. A few articles explored this question by focusing on particulate organic carbon and marine snowassociated communities, which may provide some pieces for a relevant conceptual framework. For example, some AHLs were detected in organic particles collected near Vancouver Island (Hmelo et al. 2011). They also reported an enhancement of hydrolytic enzyme activities in microcosms when adding synthetic AHLs to particulate organic

carbon collected in seawater (Hmelo et al. 2011). Similarly, alkaline phosphatase activity
is enhanced by C10-AHL (14) in a *Pantoea ananatis* isolated on marine snow (Jatt et al.
2015). Such results reveal that quorum sensing in particle-attached bacteria may drive
oceanic mineralization kinetics. However, more research is needed to better
characterize biogeochemical implications of quorum sensing expression (and more
generally the importance of cell-cell interactions) in such microenvironments, including
in the phycosphere (Moran et al. 2016).

258

259 **2.5.3 Regulation of microbial population dynamics**

It is well established that quorum sensing is involved in antimicrobial compound synthesis (Bainton et al. 1992; Wood and Pierson 1996), and this activity has been documented in strains directly isolated from the phycosphere or known to be able to colonize such microenvironments (Bruhn et al. 2005; Gram et al. 2002; Wagner-Dobler et al. 2005). It has also been reported that quorum sensing regulates the production of different algaecides compounds.

TDA (**10**), reported above as an autoinducer (Berger et al. 2011; Geng and Belas 2010) inducing its own synthesis, acts as an antimicrobial molecule (Berger et al. 2011; Bruhn et al. 2005; Geng et al. 2008; Porsby et al. 2008). Its synthesis is also controlled by quorum sensing AHLs in many *Roseobacter* (Berger et al. 2011; Rao et al. 2007; Thole et al. 2012). The production of TDA by *Silicibacter* may protect dinoflagellates from pathogen attack (Bruhn et al. 2005; Geng et al. 2008).

Similarly, *Phaeobacter gallaeciencis* BS107, associated with *Emiliana huxleyi*, provides the alga with growth inducers like auxins during bloom conditions and produces antibiotics like TDA, which fight algal pathogens (Geng et al. 2008; Greer et al. 2008; Thiel et al. 2010). In return, *Phaeobacter gallaeciencis* beneficiates of the DMSP

276 produced by the algae as a sulfur source (González et al. 1999; Newton et al. 2010). 277 Thus, it at first engages in a symbiotic relationship with *Emiliana huxleyi*. By contrast, at 278 the end of the phytoplankton bloom, Phaeobacter gallaeciencis liberates diverse 279 roseobacticides (troponoids) that act as algaecides. These metabolites are emitted in 280 response to *p*-coumaric acid (12), sinapic acid, ferulic acid and cinnamic acid (all lignin 281 precursors) released by decaying *Emiliana huxleyi* cells (Seyedsayamdost et al. 2011a; 282 Sevedsayamdost et al. 2011b). At this stage, Phaeobacter gallaeciencis switches to 283 becoming a parasite of its host algae (Seyedsayamdost et al. 2011a; Seyedsayamdost et 284 al. 2011b).

285 Various alagecides have been identified from bacteria inhabiting the 286 phycosphere, and sometimes a regulation of their emission by quorum sensing has been 287 either demonstrated or is highly likely (Nakashima et al. 2006; Paul and Pohnert 2011; 288 Skerratt et al. 2002). For example, the strain Kordia algicida, although isolated from a 289 red tide composed of the diatom Skeletonema costatum (Sohn et al. 2004), demonstrated 290 an algicidal activity against the diatoms *Skeletonema costatum*, *Thalassiosira weissflogii*, 291 Phaeodactylum tricornutum, but not against Chaetoceros didymus. This algaecide activity 292 is mediated by the excretion of proteases, which experimental data suggest is regulated 293 by quorum sensing based on AHLs (Paul and Pohnert 2011). In a similar vein, a potential 294 AI-2 based regulation of bacterial algaecide activity against the dinoflagellate Gymnodinium catenatum has also been suggested (Skerratt et al. 2002). 295

In a similar way, the recently isolated 2-heptyl-4-quinolone (**15**) emitted by *Pseudolateromonas piscicida* induces mortality of the marine coccolithophore *Emiliana huxleyi* (Harvey et al. 2016). The 2-heptyl-4-quinolone is also reported as an antibiotic and as a precursor of the quorum sensing mediator 2-heptyl-3-hydroxy-4-quinolone and designated the «*Pseudomonas* quinolone signal » (PQS). However, interestingly, this

quorum sensing compound is not produced by *Pseudoalteromonas piscicida* (Harvey etal. 2016).

303

304 2.5.4 Induction of phenotypic heterogeneity

305 Another potential function of quorum sensing in the phycosphere is the preservation of 306 population heterogeneity which is thought to be a survival strategy in fluctuating and 307 unpredicted environment. It has been recently highlighted in *Dinoroseobacter shibae* 308 (Patzelt et al. 2013), a strain isolated from a dinoflagellate culture (Biebl et al. 2005) that 309 the combination of genetic and transcriptomic analysis revealed that the lack of AHL 310 production deeply affects cell physiology, with 344 genes differentially transcribed. This 311 research reveals that quorum sensing is implied in many physiological activities, 312 including cell division, flagellar biosynthesis, sigma factor synthesis, as well as T4SS 313 production, a protein implied in both DNA and protein secretion systems (Christie et al. 314 2005). Also, the authors demonstrated the implication of quorum sensing in the 315 induction of individual morphological heterogeneity within a single population of 316 Dinoroseobacter. The luxI mutant led to single ovoid morphology of Dinoroseobacter 317 cells, while the wild-type phenotype, restored with C18-AHL, included ovoid, rod-318 shaped and very elongated cells. The maintenance of such heterogeneity in a population 319 may convey ecological advantages at the population level, for example during 320 phytoplankton blooms where cell shape dependent processes such as grazing may be 321 more intense (Patzelt et al. 2013). A population might enhance its fitness by allowing 322 individual cells to stochastically transition among multiple phenotypes, thus ensuring 323 that some cells are always prepared for an unforeseen environmental fluctuation (Acar 324 et al. 2008). The authors in 2013 suggest that quorum sensing induced heterogeneity

ensures at least a subpopulation of cells maintains a high fitness under constantlychanging environment such as seasonal planktons bloom.

327

328 **2.6 Microalgal stimulation of quorum sensing**

329 Enhancements of quorum sensing activities have been observed, in particular in the 330 freshwater alga Chlamydomonas reinhardii. This alga synthesizes a dozen chemical 331 compounds that mimics AHL activity, and so is able to readily stimulate various types of 332 quorum sensing receptors (Teplitski et al. 2004). Also, colonies of both Chlamydononas 333 reinhardtii and Chlorella sp. were able to enhance the quorum sensing dependent 334 luminescence of Vibrio harveyi (Teplitski et al. 2004). Some of these compounds emitted 335 by *Chlamydomonas* have been identified: the vitamin riboflavin (16) and its derivative 336 lumichrome (17) are able to stimulate Las R receptors in *Pseudomonas aeruginosa* and 337 thus are the first eukaryotic quorum sensing agonists (Rajamani et al. 2008). The 338 ecological function of these metabolites remains to be investigated. Collectively, these 339 data reveal that microalgae chemically influence bacterial quorum sensing in the 340 phycosphere.

341

342 **3. Quorum quenching in the phycosphere**

343 **3.1 Definition of quorum quenching**

By contrast to quorum sensing, quorum quenching describes the mechanisms which inactivate quorum sensing (Dong et al. 2001; Givskov et al. 1996). A few potential functions of quorum quenching have been characterized so far, including the inactivation of pathogens, virulence or competitors by the bacteria producing these quorum quenching molecules, or self modulation of quorum sensing signals (Romero et al. 2008; Zhang et al. 2002). However, many functional effects of quorum quenching remain unknown. Quorum quenching based mechanisms are highly diverse and include, for example, AHLs degradation by oxidization (Borchardt et al. 2001) and enzymatic hydrolysis of AHLs lactones by either lactonases or acylases (Dong et al. 2007; Romero et al. 2008). The synthesis of inhibitors or analogs of quorum sensing signals by algae, invertebrates, plants and bacteria have also been described as quorum quenching mechanisms (Gao et al. 2003; Givskov et al. 1996; Kim et al. 2007).

356

357 **3.2 Occurrence in the phycosphere of microalgae**

358 Quorum quenching activities have been previously detected in marine bacteria (Hmelo 359 and Van Mooy 2009; Hmelo et al. 2011; Romero et al. 2011; Van Mooy et al. 2012) and in 360 macroalgae (Rasmussen et al. 2000). The presence of quorum quenching molecules in 361 the phytoplankton phycosphere has also been verified. Chlorella saccharophila, Chlorella 362 vulgaris, Nannochlororopsis sp., Isochrisis sp., Tetraselmis suecica and Tetraselmis striata 363 were identified as a quorum quenching compounds producers, inhibiting color and 364 fluorescence produced by the AHL-based reporting strains Chromatium violaceum and 365 Escherichia coli JB523 (Natrah et al. 2011). Interestingly, it has also been shown that 366 Chlorella saccharophila, Nannochloris atomus and Nannochloropsis oculata inhibit the 367 AHL based quorum sensing reporter strain Vibrio harveyi JMH612, revealing that 368 biosensors detect different types of algae quorum quenching activities. However, the 369 molecules responsible of the observed quorum quenching effect are still unknown. 370 Similarly, a novel acylase has been identified in *Anabaena* sp. PCC7120 and homologous 371 sequences of this enzyme were also detected in the filamentous cyanobacteria, Nostoc 372 punctiforme, Gloeobacter violaceus and Synechocystis sp. (Romero et al. 2008). 373 Halogenation of the acyl chains in AHLs can also inhibit quorum sensing mechanisms.

374 Interestingly, sequences encoding potential halogenases were found in public databases

375 containing the genome of the diatom *Fragilariopsis cylindrus* (Amin et al. 2012).

376

377 **3.3 Control of pathogenic bacteria virulence**

378 Quorum sensing controls many genes involved in virulence. Thus, inhibiting quorum 379 sensing can help to limit bacterial induced diseases. In light of the increasing concern 380 over widespread antibiotic resistance, an understanding of other strategies to limit 381 bacterial growth and virulence is invaluable. From this point of view, quorum quenching 382 molecules appear very promising for biotechnological purposes (Singh 2015), in 383 particular to fight bacterial diseases in aquaculture (Defoirdt et al. 2011). For example, 384 microalgae producing quorum quenching metabolites have the potential in aquaculture 385 to protect against aquatic pathogens (Natrah et al. 2011). A few studies have shown that 386 marine cyanobacteria have quorum quenching activity against Vibrio spp., one of the 387 most important pathogens in aquaculture, but also against other pathogens including 388 Pseudomonas aeruginosa (Dobretsov et al. 2010; Kwan et al. 2011). Cyanobacteria of the 389 genus Lyngbya appear to be particularly active emitters of quorum quenching 390 compounds that include amides, peptides and lipids (Dobretsov et al. 2011; Meyer et al. 391 2016). Similarly, *Leptolyngbya crossbyana* produces (S)-3-hydroxy-y-butyrolactones 392 (Honaucines (**18,19,20**)), which inhibits quorum sensing-dependent bioluminescence of 393 *Vibrio harveyi* BB120. Similar quorum quenching activity by the microalga *Picochlorum* 394 sp. S1b against Vibrio is also suspected (Kuo et al. 2014), and the cyanobacterium 395 Blennothrix cantharidosmum produces tumonoic acids which moderately inhibits 396 bioluminescence of a wild strain of *Vibrio harveyi* without affecting growth. Along these 397 biotests, tumonoic acid F (21) appears to be the most active (Choi et al. 2012).

398

399 **4. Controversies**

400 The role of quorum sensing in microbiomes remains controversial (Cornforth et al. 401 2014; Platt and Fuqua 2010; West et al. 2012), and its function in the phycosphere is still 402 a large and open field of investigation (Bachofen and Schenk 1998; Decho et al. 2009; 403 Hmelo et al. 2011). Clearly, AHLs carry information and act as semiochemicals (Dicke 404 and Sabelis 1988). However, additional functions of these compounds have also been 405 described, including antimicrobial activity and iron chelation (Kaufmann et al. 2005; 406 Schertzer et al. 2009). This is also the case for TDA, which was first known in 407 *Rhodobacterales* as an antibacterial agent (Brinkhoff et al. 2004) before being described 408 as a quorum sensing autoinducer (Geng and Belas 2010). Clearly, quorum sensing 409 molecules display versatility and play different roles, depending on both the emitting 410 and targeted cells. .

Another source of controversy is whether sensing autoinducer concentrations 411 412 actually functions to detect the rate mass transfer in the environment ("diffusion-413 sensing") rather than the local density of other bacteria ("pure" quorum sensing) (Redfield 2002). The concept of "efficiency-sensing" unifies diffusion-sensing and 414 415 quorum sensing as specific cases within a more general framework whereby 416 autoinducer sensing allows bacteria to infer, in combination, mass transfer, cell density 417 and spatial distribution of other cells (Hense et al. 2007). This concept of efficiency-418 sensing could aid in the interpretation of signaling events observed in the complexity of 419 the phycosphere where the environment is structured on a microscale by physical 420 barriers (cells are embedded in a complex matrix of polymers), subject to fluid motions 421 (Amin et al. 2012) and variable spatial clustering of bacteria and cell abundance 422 (Doucette 1995). By contrast, the "pure" quorum sensing framework arose from well-423 controlled laboratory studies on clonal populations (Hense et al. 2007).

425 **Conclusions and perspectives**

426 The objective of this review was to shed light on quorum sensing communication in the 427 phycosphere of microalgae. Many reports have revealed the presence of this 428 communication system in bacteria isolated from phytoplankton blooms or associated 429 with microalgae cultures. It appears from the literature that quorum sensing is a 430 common type of bacterial communication in the phycosphere. It is performed by diverse 431 bacteria within the phycosphere of a large diversity of microalgae. The metabolites 432 involved seem dominated by long-chain AHLs, however some reports mention the 433 potential importance of AI-2. The emerging picture greatly needs further research to 434 evaluate the extent of bacterial and chemical diversity involved in quorum sensing 435 processes within the phycosphere. Little is known about the potential involvement of diverse types of AHLs and other quorum sensing molecules, including gamma-436 437 butyrolactones, quinolones, and others.

438 Already, very diverse functions have been identified in the phycopshere involving 439 quorum sensing. These functions include biofilm formation and nutrient acquisition, as 440 well as the regulation of algaecide production, antibiotic synthesis and cell shape. 441 Probably, this list remains far for the real extent of biological functions governed by 442 quorum sensing within the phycopshere. Recent advances coupling genomics with 443 metabolomics approaches will give interesting new insights in this field of research. A 444 promising experimental approach to answer these questions is simultaneous tracking of 445 transcriptomic and metabolomic variations within model organisms grown in co-446 culture. This method enables the characterization of metabolic pathways affected by 447 quorum sensing expression in both algae and bacteria. Furthermore, the large scale

448 impacts of quorum sensing in the phycosphere remain very poorly characterized,449 particularly in terms of biogeochemistry and evolution.

This review also described the importance that quorum quenching has in the phycosphere of microalgae. Many studies revealed the emission of quorum quenching molecules either by algae or diverse bacteria in the phycospshere. These molecules have important biotechnological potential, as they are able to control growth and virulence of microorganisms. Again, more research is needed to characterize new molecules with such activities, and to better characterize their mechanism of action.

456

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460

461 **FIGURE LEGENDS**

462 Figure 1 to 7: Major compounds cited in the text

463 Figure 8: Synthetic view of quorum sensing processes in the phycopshere of464 phytoplankton

465 Figure 9: Synthetic view of quorum quenching processes in the phycosphere of466 phytoplankton

467

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