Pakistan J. Zool., pp 1-10, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20230118140133

Review Article

Acyl Homoserine Lactone-Based Quorum Sensing and its Role in Regulation of Extracellular Hydrolytic Enzymes in Marine Snow Associated Bacteria

Abdul Nabi Jatt[1]*, Abdul Sattar Qureshi[2] and Abdul Sami Dahri[1]

[1]*Institute of Microbiology, University of Sindh, Jamshoro, Pakistan*
[2]*Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh, Jamshoro, Pakistan*

Article Information
Received 18 January 2023
Revised 20 April 2023
Accepted 16 May 2023
Available online 31 July 2023
(early access)

Authors' Contribution
ANJ designed the study. ANJ, ASQ and ASD collected the data and wrote the manuscript. All the authors read and approved the final version of the manuscript.

Key words
Quorum-sensing, AHLs, Extracellular enzymes, Marine snow

ABSTRACT

The discovery of quorum sensing (QS) amongst bacterial cells substantiates the coordinated activities in these microscopic organisms. QS is a system of cell-to-cell communication through which bacteria express certain genes to achieve various physiological and biological functions. Presently, several kinds of autoinducers have been reported in microbial cells, however *N*-acyl homoserine lactones (AHLs) are known as the most significant QS autoinducers, frequently produced by large numbers of Gram-negative bacteria. QS system in marine snow associated bacteria has a great concern with respect to regulate extracellular enzymatic activities. Substantially, marine aggregates are large sinking particles composed of both organic and inorganic detritus and living organisms including phytoplankton, zooplankton and bacteria, and perform a critical role in moving materials from upper levels to deep Ocean. Intriguingly, findings of AHL signaling molecules in marine snow aggregates and associated microbial cells have been attributed to play a crucial role in dissociation of these large carbon rich particles with a profound impact on marine food webs along with biogeochemical cycles in marine environment. This mini review highlights the role of AHL based QS system in marine snow with respect to enhanced enzymatic activities (EEA) of marine snow associated bacterial populations and hydrolysis of these carbon rich aggregates.

INTRODUCTION

Quorum sensing (QS) is a mechanism of detection and responding cell density population by regulation of specific genes (Lim *et al*., 2014). Bacteria assess cell density by sensing threshold concentration of QS signaling molecules produced by the same or neighboring bacterial cells (Platt and Fuqua, 2010). Principally, bacteria produce different types of signaling molecules also called as autoinducers; for instance, *N*-acyl homoserine lactones (AHLs) are the major autoinducers frequently produced by Gram-negative bacterial species, whereas small oligopeptides have been found as the QS autoinducers in several Gram-positive bacterial species (Jatt *et al*., 2015). Moreover, several enzymes have been reported in some bacterial species that disrupt particularly AHL based QS system and this whole mechanism is generally called as quorum-quenching (QQ) (Tang and Zhang, 2014). Predominantly, three major QQ enzymes have been shown to degrade AHL molecules such as AHL-oxidase-reductase, AHL-lactonase and AHL-acylase (Chu *et al*., 2014). Significantly, AiiA (AHL lactonase) enzyme produced by *Bacillus* species has been reported to degrade several types of AHL molecules (Dong *et al*., 2000). Heretofore AiiA protein has been widely used to degrade AHL signaling molecules without affecting microbial numbers and composition (Park *et al*., 2008; Bai *et al*., 2008).

QS bacteria associated with natural snow particles are of great importance in marine environments characterized with higher levels of extracellular enzymatic activity (Hmelo *et al*., 2011; Jatt *et al*., 2015). Marine snow

* Corresponding author: abdul.nabi@usindh.edu.pk
0030-9923/2023/0001-0001 $ 9.00/0

represents the sinking aggregates, composed of organic and inorganic detritus, fecal pellets and living organisms including phytoplankton, bacteria, and zooplankton, resulting in transporting organic matter from the surface to deep levels of ocean (Piontek *et al*., 2009). Natural marine snow aggregates are highly fragile and usually degraded quickly during sampling and handling processes. Marine snow degradation may be attributable to two physical and biological processes. Physical degradation is due to a shear stress that breaks down the marine aggregates, whereas biological degradation is due to a very high flora and fauna attached to marine aggregates being responsible for elevated heterotrophic activity (Kiorboe, 2001; Arnosti, 2004).

Extracellular hydrolytic enzymes produced by marine snow associated bacteria are considered as beneficial tools for breaking down these large aggregates full of nutrients and resulting as transmission of these nutrient rich compounds to free-living communities in marine environment (Ziervogel *et al*., 2010; Jatt, 2021). Bacterial populations take the components of the dissolved organic matter (DOM) directly and use extracellular hydrolytic enzymes to cleave high molecular weight compounds prior to uptake (Arnosti, 2004). The QS system has been indicated mainly to regulate the production of large numbers of extracellular hydrolytic enzymes in marine aggregates and plays a beneficial role in degradation of these large particles with a pronounced impact on carbon dioxide moving from atmosphere to deep Ocean (Hmelo *et al*., 2011; Jatt *et al*., 2015).

QUORUM-SENSING (QS) SYSTEM

The term QS refers to a conversation mechanism in microorganisms based on production, secretion, and detection of signals. QS system regulates expression of specific genes in contradiction of cell density in several types of microorganisms (Myszka and Czaczyk, 2012; Lim *et al*., 2014). Initially, QS was observed in marine bacterium *Vibrio fischeri* that produces signaling molecules during its colonization and growth in light organs of cephalopods and marine fish, coordinating the expression of bioluminescence encoded genes in response to cell density (Nealson *et al*., 1970). Bacterial cells assess the cell density when threshold concentration of QS signaling molecules induced by same type of cells or neighboring bacterial cells (Platt and Fuqua, 2010). Several kinds of QS signal molecules have been detected in bacterial species. i.e., AHLs, oligopeptides, quinolones and furanosyl-borate diesters (Chen *et al*., 2002; Jatt, 2021). These all signaling molecules are secondary metabolites, which are not involved in primary metabolism.

Biosynthesis of AHL- signal molecule

In AHL biosynthesis, *S*-adenosyl methionine (SAM) binds to an active site of enzyme; an acyl carried protein (ACP) transfer proper acyl-group to this composite, which creates bond (amide) with amino-group of SAM. This action of complex acyl-ACP and SAM is conserved in QS systems of bacterial populations belonging to different species (Myszka and Czaczyk, 2012). A general structure of AHL molecule produced by bacteria has been shown in Figure 1. Bacterial species characterized with AHL based QS system usually recognize AHL signal molecules through a protein (LuxR) that works as receptor of AHL molecules (Zheng *et al*., 2006). While, enzyme AHL synthase (LuxI type protein) is involved to synthesize AHL signal molecules. AHL synthases in various bacterial species share an analogues sequence homology with *luxI* gene of luminescence bacterium *V. fischeri*. AHL signal molecules produced by *luxI* homologues consist of ring of homoserine lactone, which is bound with acyl-chain by an amide bond that differentiated into carbon atoms of 4 to 18 carbons and with variable fatty acyl groups such as carbonyl or hydroxyl group.

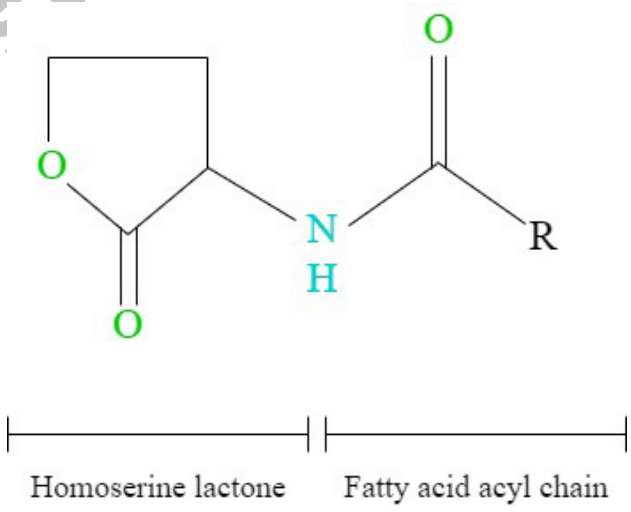

Fig. 1. A general structure of *N*-acyl homoserine lactone (AHL) signal molecule.

Quorum sensing in gram-negative bacterial species

Gram-negative bacterial species frequently use AHLs as QS signaling molecules to regulate various biological and physiological activities (Christopher and Bassler, 2005; Jatt, 2021). Essentially, LuxR/I type regulators are involved to produce AHL based QS signals in Gram negative bacterial cells (Fig. 2). AHLs are very crucial QS signaling molecules, usually used in communication between bacterial populations of same and even in

different bacterial species, genera, and families (Myszka and Czaczyk, 2012). AHL signal molecule in Gram-negative bacterial species have been classified as short and long chain AHLs based on length of acyl groups with carbon 4 to carbon 18 acyl side chains particularly with groups of either oxo or hydroxyl or even no substitution at carbon 3. Substantially, proteobacterial Gram-negative bacterial species belonging with alpha, beta and gamma sub-groups have been confirmed with AHL based QS mechanism (Chhabra *et al*., 2005).

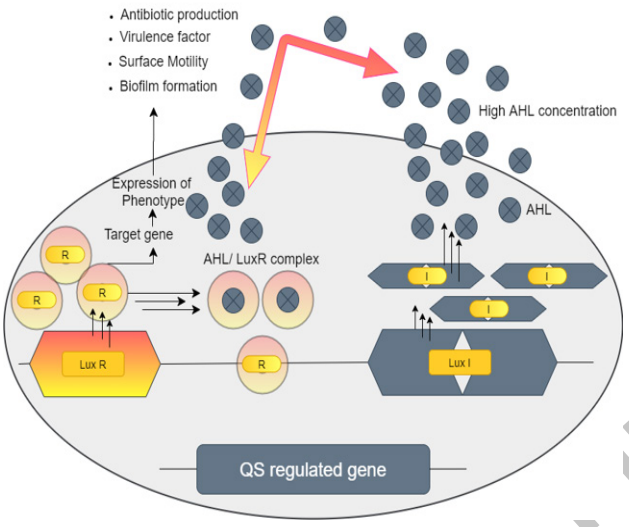

Fig. 2. LuxR/I Quorum-sensing (QS) system in Gram-negative bacteria. In threshold cell density, AHL based QS signaling molecules attach to LuxR. AHL/LuxR complex initiates transcription of target gene to carry out phenotype expression.

Quorum sensing in Gram-positive bacterial species

QS mechanism found in Gram-positive bacterial species is dissimilar with the QS mechanism found in Gram-negative bacterial species. In Gram-positive bacteria, species-specific QS is mostly mediated through small oligopeptides and are called as autoinducing peptides (AIPs) characterized with 5 to 34 amino acids in length (Chan *et al*., 2004). Principally, histidine kinases (two-component type membrane bound) are used as sensors and conversation is achieved by phosphorylation cascade (Christopher and Bassler, 2005). Three families of AIPs have been identified based on their structural uniqueness, i.e., oligopeptide lantibiotics, 16-membered thiolactone peptides and tryptophan peptides, respectively (Okada *et al*., 2005). Generally, peptides cannot diffuse through membrane, hence oligopeptide exporters are responsible to release signaling molecules. Mostly, QS-peptide molecules are usually cleaved into small peptide units from large precursors and further characterized with different rings of lanthionine, thiolactone, isoprenyl and lactone rings (Christopher and Bassler, 2005). AIPs based QS mechanism is found in large numbers of Gram-positive bacteria, i.e., *Staphylococcus aureus*, *Bacillus ceruss*, *Bacillus subtilis* and *Enterococcus faecalis*, *Lactobacillus plantarum* and *Listeria monocytogenes* (Autret *et al*., 2003; Sturme *et al*., 2005).

AI-2 (Autoinducer-II) based quorum-sensing mechanism

AI-2 based QS mechanism consists of diffusible and interconvertible signaling molecules such as furanosyl borate diester (Williams *et al*., 2007). Interestingly, both Gram-negative and Gram-positive bacterial species exhibit AI-2 QS mechanism (Hermann, 2007). AI-2 QS signaling molecules are induced by LuxS-type enzyme that breaks down S-ribosyl-L-homocysteine and results in production of AI-2 precursor (e.g., 4,5-dihydroxy-2,3-pentanedione), and homocysteine with the ability to cyclize spontaneously with the production of furanone derivatives (Miller *et al*., 2004). Generally, *LuxS* gene responsible for AI-2 has been detected in large numbers of bacterial species (i.e., more than sixty bacterial species) belong to different classes such as beta (β), gamma (γ), sigma (δ) and epsilon (ε) proteobacteria, spirochetes, firmicutes, actinobacteria and also genera belong to the groups of green-sulphur, Cytophaga and Deinococcus bacteria (Vendeville *et al*., 2005; Williams *et al*., 2007).

AI-2 based QS mechanism has been reported widely in several *Vibrio* species specially in *V. cholerae* and *V. harveyi,* and the role of this QS system in these bacterial species has been shown to be involved in regulation the traits of bioluminescence and pathogenicity (Lenz *et al*., 2004).

QS based regulation of bacterial phenotypic characteristics

Generally, bacterial species release several kinds of QS signaling molecules and once a threshold concentration of these signalings (correlating to specific cell density) is achieved then initiate transcription of QS regulated genes. Large numbers of bacterial communities inhabiting natural environment use QS mechanisms to regulate a variety of phenotypes such as extracellular hydrolytic enzyme production, toxin secretion, biofilm formation and motility (Whitehead *et al*., 2001; Gonzalez and Keshavan, 2006). These QS regulated phenotypes are crucial for establishment of a pathogenic, symbiotic or beneficial relationship with a host (Myszka and Czaczyk, 2012).

In natural environments, the coordinated mechanism for microbial cells is highly essential to regulate the most important phenotypes for the adaptation of new trends of growth, which may help in protection from

hazardous environments. The regulation of a wide variety of phenotypes in several Gram-negative bacterial species based on LuxI/R QS mechanism has been reported such as bioluminescence in *V. fischeri*, biofilm and exo-protease in *Aeromonas hydrophila,* swarming, exoenzyme and biofilm in *Burkholderia cenocepacia,* exopolysaccharides in *Pantoea stewartia,* plasmid conjugation in *Agrobacterium tumefaciens* and pigment production in *Chromobacterium violaceum* (Jatt *et al*., 2015).

QUORUM QUENCHING (QQ)

Quorum sensing is a conversation mechanism reported in several bacterial species in order to regulate various biological functions including pathogenicity. Unlike QS mechanism, quorum quenching is a mechanism of preventing QS system by disrupting QS signaling molecules (Dessaux *et al*., 2011). QQ bacteria have been isolated from various environments. Several abundant and strong QQ agents have been recognized from various synthetic and natural chemical products. Many types of bacterial species particularly isolated from natural marine environments have been shown to release inhibitors and use these small QS inhibitors to gain benefits in competitive environment (Tang and Zhang, 2014). Several QS inhibitor molecules have been identified to disrupt the signaling generation and leading to the process QS inhibition. AHL based QS mechanism can be inhibited simply by exploiting the unique specificity of LuxR-type proteins and can also be inhibited by interrupting the biosynthetic pathways that leads to AHL synthesis. Mainly, analogues to precursors of AHL molecule can be employed to control the autoinducer synthesis. Dong *et al*. (2000) reported an enzyme (AiiA) from *Bacillus*, capable of degrading AHL molecules. It has been recorded that AiiA enzyme in *Erwinia carotovora* has drastically inhibited production of AHL QS signaling molecules and significantly reduced pectolytic enzyme synthesis (Dong *et al*., 2000).

MARINE SNOW

Marine snow term indicates large marine aggregates in ocean environment that play a critical role in transporting components from upper to deeper layers. These marine aggregates are the sites of elevated levels of biological activities and involved in energy flow through marine ecosystems (Jatt *et al*., 2015). Bacterial communities associated with marine snow particles play a vital role in breaking down of these particles and remineralization of carbonic materials. Marine snow aggregates range from few µm to several centimeters in size. Marine aggregates are enriched in nutrients that enhance the colonization by microbial communities (Artolozaga *et al*., 1997; Hmelo *et al*., 2011). These large particles are characterized with bacterial numbers and biomass in greater concentration than those present in the surrounding sea water (Simmon *et al*., 1990; Alldredge, 2000; Kiorboe, 2000).

Marine snow aggregates are found ubiquitous from the surface to the deep oceans and the highest levels have been reported in euphotic zone with maximum production (Lampitt, 2001). Predominantly, the major characteristics of these marine snow particles which render this aggregated material very crucial are high sinking rates, microenvironment, enhanced biogeochemical rates and sites of food source for living organisms surrounding these aggregates. Marine snow aggregates have been found in all over the world's oceans in every part of the water column. These are not uniformly dispersed either in space or time; however, elevated rates of these marine particles have been reported in upper water column (Jatt *et al*., 2015). These aggregates are comprised of 60% of particulate organic carbon (POC), which decreases with increasing depth (Martin *et al*., 2011). Some potential mechanisms are described as the responsible for reduction with depth of particulate organic carbon flux such as solubilization to dissolved organic matter, consumption by zooplankton, abiotic stress-induced fragmentation and the most important one is microbial decomposition (Goldthwait *et al*., 2004).

Composition of natural marine snow particles

Natural marine snow particles are composed of both organic and inorganic detritus and living organisms including phytoplankton, zooplankton, and bacteria. Formation of marine snow in marine environment occurs by aggregating small marine particles with the help of several processes. The major processes that play a role in marine snow aggregation are physical coagulation and zooplankton mediated aggregation (Kiorboe, 2001). Moreover, formation of particulate organic matter (POM) takes place by aggregation of colloidal organic material from dissolved organic matter (DOM) (Kepkay, 1994). Presence of transparent exopolymer particles (TEP) in increased levels has been reported within POM pool in coastal as well as open oceanic environment (Alldredge *et al*., 1993). TEP influences aggregation process in different ways and may involve in rapidly development of marine snow aggregates (Alldredge *et al*., 1993). Zooplanktons aggregated with marine snow are considered as food source for various types of planktonic organism including fish, copepods and euphausiids (Lampitt, 2001).

Marine snow particles provide a substrate for microbial attachment, while the production and release of refractory organic material by bacterial communities

associated with these marine snow aggregates results in formation of the fibrillar matrix, aggregating together of particles, ultimately leading to the formation of large particles (Alldridge and Silver, 1988; Jatt *et al*., 2015).

Degradation of marine snow aggregates

Several processes are involved in high rates of dissolved organic carbon of marine snow aggregates; however, there are two major processes believed to be involved in degradation of marine snow aggregates such as physical process and biological process. Physical degradation of marine snow particles is due to shear stress that breaks down the large marine aggregates into small particles, while, biological degradation of marine snow aggregates is due to a very high flora and fauna associated with these aggregates and responsible for enhanced heterotrophic activities (Kiorboe, 2001). Moreover, the hydrolytic activities by bacterial extracellular enzymes are known as the vital source that degrade and solubilize the components of particulate organic matter aggregates. It has been reported that the hydrolytic activities of bacterial populations associated with marine snow aggregates are substantially higher (up to 10^5 times) as compared to free living microbial communities (Ivancic *et al*., 2018; Jatt, 2021).

EXTRACELLULAR HYDROLYTIC ENZYMES

Bacterial cells use specific QS systems that perform coordination to express certain genes encoding behaviors that support the cells in extracellular hydrolytic enzymes production, luminescence and formation of biofilms specifically at high cellular density and communicate within and between species. Extracellular or exoenzymes are the enzymes that are secreted by cells and work outside of these cells. Extracellular enzymes are mainly hydrolases, which breakdown bonds of C-O and C-N used in connection of monomers, i.e., glycosidases, peptidases or esterases (Cunha *et al*., 2010). Extracellular hydrolytic enzymes are the essential components of biological processes and may play a crucial role in degradation of large organic matter. Extracellular enzymatic activity (EEA) in bacteria is regulated by various factors including QS signaling molecules. Since, particulate organic matter and dissolved organic matter components are dominated by high molecular-weight substances and thus complex substrates are first degraded externally into small molecules. This type of degradation of complex substrates is achieved by extracellular hydrolytic enzymes (i.e., protease, lipase, amylase, phosphatase, cellulase etc.), produced by several types of bacterial species inhabiting various ecological niches. Extracellular enzymes secreted by marine bacteria are of great importance in degradation of particulate organic matter in ocean environments (Baltar *et al*., 2010).

Extracellular hydrolytic enzymes have also been potentially used in biomedical sciences, food industries and different chemical industries. Mainly, proteases, glucosidases, chitinases, lipases and phosphatases are the hydrolytic extracellular enzymes widely studied in marine water (Cunha *et al*., 2010). Bacteria associated with marine snow are of highly important in hydrolysis of marine snow particles by producing wide range of extracellular enzymes (Su *et al*., 2019). In general, marine snow associated bacteria exhibit higher levels of exoenzymes, hence it is speculated that free-living organisms may benefit from their overproduction of hydrolytic enzymes (Hmelo *et al*., 2011; Jatt, 2021).

The role of AHL based QS system in regulation of extracellular hydrolytic enzymes in marine snow

Bacterial species characterized with QS system have been reported in wide range of marine environments such as marine snow, dinoflagellates, sponges, and corals (Gram *et al*., 2002; Mohamed *et al*., 2008; Tait *et al*., 2010). The marine environments generate unique challenges to bacterial communities characterized with QS system, i.e., signaling molecules can be lost very rapidly by the diffusion in aqueous environment, pH of sea water that is slightly basic and thus degradation of signal molecules by base-catalyzed hydrolysis occurs (Yates *et al*., 2002). The activation of gene expression in bacterial communities by AHL based QS system is dependent on the threshold concentration of AHL producing bacterial species and also depends on the ability of extracellular environment to retard diffusion of signaling molecules away from related bacterial cells. Intriguingly, this can be retained within the micro-environments such as marine snow aggregates that will slow down the hydrolysis of signaling molecules by diffusion and abiotic base-catalyzed hydrolysis. Moreover, marine snow isolates can be able to evolve tactics to compete with challenges generated by chemical environment, i.e., these bacterial species use long chain AHL molecules or with oxo-substitution on 3-carbon at acyl chain of AHL signals. Essentially, long acyl chain AHL signals have been found more stable in the sea water and are hydrolyzed more slowly by base-catalyzed hydrolysis as compared to AHL molecules with short acyl chains (Hmelo *et al*., 2011).

Generally, detection of a broad range of AHL based QS signals in marine snow directly and in associated bacteria is vigorous and may influence on coordination of expression of certain genes encoding behaviors that

support the cells to promote the extracellular hydrolytic enzymes production involved in marine snow degradation process. Several extracellular hydrolytic enzymes such as elastase, chitinase, cellulase, lipase, phosphatase and gelatinase are believed to be regulated by AHL based QS system (Jatt *et al*., 2015). Extracellular hydrolytic enzymes are regulated based on environmental factors, ecosystem, and enzyme-substrate interactions. Enzymatic activities are mainly controlled by enzyme-substrate interactions, i.e., adsorption, inhibition, stabilization and humification (Jatt *et al*., 2018). Moreover, growth phase is considered as a major interesting factor for regulation of extracellular enzymes. Related to this, there is a QS system that deals with the coordinated functions at high cell density. The maximum enzyme production has been reported at late logarithmic to early stationary phase for proteases and lipases (Jatt *et al*., 2015). This suggests extracellular enzymes production at high cell density and thus QS might be involved in regulation of these enzymes. The regulation of extracellular enzymes by QS system has been reported in several bacterial species such as cell wall hydrolyzing enzymes in *Erwinia carotovora*, metalloprotease gene expression in *Vibrio vulnificus*, metalloprotease synthesis in *Vibrio anguillarum*, pectate lyases in *Erwinia chrysanthemi*, and protease synthesis in *Burkholderia cetaceans*, extracellular protease synthesis in *Serratia liquefaciens* and exoprotease in *Aeromonas salmonicida* (Reverchon *et al*., 1998; Shao and Hor, 2001). Some studies have directly shown addition of exogenous QS autoinducers such as AHLs and AI-2 signal molecules to marine snow aggregates and resulted a profound effect on EEA in these natural marine particles (Hmelo *et al*., 2011; Krupke *et al*., 2016). Moreover, Su *et al*. (2019), reported AHL based QS in marine snow bacterium *Ruegeria mobilis* Rm01 and identified two AHL synthase genes such as *psal* and *psbl* by genomic analysis. Similarly, addition of exogenous AHL QS signaling molecules was shown to influence enzymatic activities particularly increased production of extracellular lipase activity in bacterium Rm01 associated with marine snow particles (Su *et al*., 2019). Mainly, lipids, glycerols and fatty acids are highly important components in oceanic environment and the cytoplasmic membranes of dead organisms are a major source of these lipo-particles along with other biological materials found in marine environmental area.

QS regulation of EEA has also been reported in marine snow isolates of *Trichodesmium consortia* and *Ruegeria* bacterial species (Van-Mooy *et al*., 2012; Su *et al*., 2019). In addition to EEA, QS system has also been involved in regulation of several other phenotypic traits particularly in particle attached ecological niche such as biofilm formation and flagella mediated motility in *Ruegeria* species, biosynthesis of indigoidine antimicrobial agent in *Phaeobacter* species and production of TDA (tropodithietic acid) in bacterium *Phaeobacter inhibens* DSM17395 (Zan *et al*., 2012; Cude *et al*., 2015). Several types of bacterial species, e.g., *Pseudomonas aeruginosa*, *Vibrio cholerae* and *Burkholderia pseudomallei* use QS system to compete and resist QQ producing bacterial communities with a pronounced effect on microbial ecology (Garcia-Contreras *et al*., 2015). Intriguingly, bacterium *Deinococcus radiodurans* has been reported to regulate a specific gene involved in regulation of oxidative stress resistance by coordinated effect of both QS and QQ mechanism (Lin *et al*., 2016). QS signal molecules found directly in natural marine snow particles are due to microbial species characterized with different QS systems associated with these large particles. Generally, QS has a great concern with biofilm formation in carbon rich marine aggregates and accelerates the production of extracellular enzymes (Table I). Likewise, QS has been considered as a major constituent involved in regulation of biofilms and enhanced EEA in marine snow aggregates. One of the studies has shown that AHL based QS signaling molecules significantly influenced extracellular aminopeptidase, phosphatase, and lipase enzymatic activities in marine snow aggregates collected from the region of Atlantic and Pacific Ocean (Krupke *et al*., 2016). Moreover, further incubation of marine snow aggregates with exogenous AHLs increased and decreased the rate of hydrolysis of marine particles and thus indicated a link between QS system and hydrolysis of marine particles (Krupke *et al*., 2016).

Table I. AHL based QS signal molecules produced by marine snow associated bacteria and their influence on extracellular enzymatic activities (EEA).

Marine snow associated bacteria	Major AHLs	Phenotypes	References
Citrobacter freundii B1	C6-HSL, C8-HSL, C10-HSL, C12-HSL, C14-HSL	Cellulase	Jatt, 2021
Ruegeria mobilis RMO1	3OC10-HSL, C10-HSL, C12-HSL	Lipase	Su *et al*., 2019
Pantoea ananatis B9	C4-HSL, 3OC6-HSL, C6-HSL, C10-HSL, C12-HSL, C14-HSL	Phosphatase	Jatt *et al*., 2015
Roseobacter strain HP12	C6-HSL, C8-HSL	ND	Gram *et al*., 2002

ND*, Not detected.

Several numbers of bacterial species associated with marine aggregates are reported to produce AHL signaling molecules with elevated rates of EEA (Jatt *et al*., 2015). Previously, 43 marine associated bacterial strains were reported and four of them showed production of AHL based QS signals (Gram *et al*., 2002). Moreover, in our previous study, 53 bacterial strains associated with marine snow were isolated and ten of these isolates affiliated with different bacterial groups indicated as positive for AHL based QS signals (Jatt *et al*., 2015). Amongst the marine snow isolates, *Pantoea ananatis* B9 was shown to produce five different extracellular enzymes, i.e., alkaline phosphatase, lipase, amylase, protease and gelatinase. Interestingly, phosphatase was reported as a dominant extracellular enzyme and the biosynthesis of this enzyme was shown possibly regulated by AHL based QS system in marine snow bacterium *P. ananatis* B9 and cellulase activity in *C. freundii* B1 (Jatt *et al*., 2015; Jatt, 2021). Moreover, another study has also reported substantially higher levels of EEA in marine snow aggregates as compared to sea water and the extracellular alkaline phosphatase was shown to be critically increased followed by extracellular peptidase (Ivancic *et al*., 2018). Intriguingly, the extracellular hydrolytic enzymes are critically influenced by QS systems and are the major components to carry out nutrient transport in marine environments by hydrolysis of large marine aggregates.

CONCLUSION

This mini review focuses on AHL based QS system in marine snow and associated bacterial communities and its role in regulation of extracellular hydrolytic enzymes. AHL based QS signaling molecules influence and accelerate extracellular enzymatic activities (EEA) *in situ* natural marine snow particles. QS regulation of EEA plays a critical role in hydrolysis of large marine aggregates. Heterotrophic bacteria associated with marine aggregates secrete several kinds of extracellular hydrolytic enzymes to degrade these large marine particles into small molecules consumed by free-living microbial cells. Carbon flux associated with marine snow aggregates in ocean plays an imperative role in regulation of earth's climate. The regulation of extracellular hydrolytic enzymes by QS system particularly produced by microbes associated with natural marine snow particles might have a great influence on a wide range of biological activities by degradation of large marine aggregates with a pronounced impact particularly on carbon cycle.

ACKNOWLEDGEMENT

We are highly grateful to Prof. Dr. M. Rafiq, Institute of Biotechnology & Genetics (IBG), University of Sindh, Jamshoro, for valuable suggestions and assistance in formating this review article.

Funding

This study received no external funding.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Alldredge, A.L., 2000. Interstitial dissolved organic carbon (DOC) concentrations within sinking marine aggregates and their potential contribution to carbon flux. *Limnol. Oceanogr.*, **45**: 1245-1253. https://doi.org/10.4319/lo.2000.45.6.1245

Alldredge, A.L., Passow, U. and Logan, B.E., 1993. The abundance and significance of a class of large transparent organic particles in the Ocean. *Deep Sea Res. I Oceanogr. Res. Pap.*, **40**: 1131-1140. https://doi.org/10.1016/0967-0637(93)90129-Q

Alldredge, A.L. and Silver, M.W., 1988. Characteristics, dynamics and significance of marine snow. *Prog. Oceanogr.*, **20**: 41-82. https://doi.org/10.1016/0079-6611(88)90053-5

Arnosti, C., 2004. Speed bumps and barricades in the carbon cycle: Substrate structural effects on carbon cycling. *Mar. Chem.*, **92**: 263-273. https://doi.org/10.1016/j.marchem.2004.06.030

Artolozaga, I., Santamaria, E., Lopez, A., Ayo, B. and Iriberri, J., 1997. Succession of bacterivorous protists on laboratory-made marine snow. *J. Plankton Res.*, **19**: 1429-1440. https://doi.org/10.1093/plankt/19.10.1429

Autret, N., Raynaud, C., Dubail, I., Berche, P. and Charbit, A., 2003. Identification of the agr locus of *Listeria monocytogenes*: Role in bacterial virulence. *Infect. Immun.*, **71**: 4463-4471. https://doi.org/10.1128/IAI.71.8.4463-4471.2003

Bai, F., Han, Y., Chen, J. and Zhang, X.H., 2008. Disruption of quorum sensing in *vibrio harveyi* by the AiiA protein of *Bacillus thuringiensis*. *Aquaculture*, **274**: 36-40. https://doi.org/10.1016/j.aquaculture.2007.11.024

Baltar, F., Aristegui, J., Gasol, J., Sintes, E., Van Aken, H. and Herndl, G., 2010. High dissolved extracellular enzymatic activity in the deep central Atlantic Ocean. *Aquat. Microbiol. Ecol.*, **58**: 287-302. https://doi.org/10.3354/ame01377

Chan, W.C., Coyle, B.J. and Williams, P., 2004. Virulence regulation and quorum sensing in

staphylococcal infections: Competitive AgrC antagonists as quorum sensing inhibitors. *J. med. Chem*., **47**: 4633-4641. https://doi.org/10.1021/jm0400754

Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczer, I., Bassler, B.L. and Hughson, F.M., 2002. Structural identification of a bacterial quorum-sensing signal containing boron. *Nature*, **415**: 545-549. https://doi.org/10.1038/415545a

Chhabra, S.R., Philipp, B., Eberl, L., Givskov, M., Williams, P. and Cā̃mara, M., 2005. Extracellular communication in bacteria. *Chem. Pherom. Semi*, **2**: 279-315. https://doi.org/10.1007/b98319

Christopher, M. and Bassler, B.L., 2005. Quorum sensing: Cell-to-cell communication in bacteria. *Annls Rev. Cell Dev. Biol.*, **21**: 319-346. https://doi.org/10.1146/annurev.cellbio.21.012704.131001

Chu, W., Zhou, S., Zhu, W. and Zhuang, X., 2014. Quorum quenching bacteria *Bacillus* sp. QSI-1 protect zebrafish (*Danio rerio*) from *Aeromonas hydrophila* infection. *Sci. Rep.*, **4**: 5446. https://doi.org/10.1038/srep05446

Cude, W.N., Prevatte, C.W., Hadden, M.K., May, A.L., Smith, R.T., Swain, C.L. and Buchan, A., 2015. *Phaeobacter* sp. strain Y4I utilizes two separate cell to cell communication systems to regulate production of the antimicrobial indigoidine. *Appl. environ. Microbiol.*, **81**: 1417–1425. https://doi.org/10.1128/AEM.02551-14

Cunha, A., Almeida, A., Coelho, F.J.R.C., Gomes, N.C.M., Oliveira, V. and Santos, A.L., 2010. Bacterial extracellular enzymatic activity in globally changing aquatic ecosystems. *F. Res. Cent.*, pp. 124-135.

Dessaux, Y., Chapelle, E. and Faure, D., 2011. *Quorum sensing and quorum quenching in soil ecosystem*. Bioco Soil Micro Springer, pp. 339-367. https://doi.org/10.1007/978-3-642-14512-4_13

Dong, Y.H., Xu, J.L., Li, X.Z. and Zhang, L.H., 2000. AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proc. natl. Acad. Sci.*, **97**: 3526-3531. https://doi.org/10.1073/pnas.97.7.3526

García-Contreras, R., Nunez-Lopez, L., Jasso-Chávez, R., Kwan, B.W., Belmont, J.A., Rangel-Vega, A. and Wood, T.K., 2015. Quorum sensing enhancement of the stress response promotes resistance to quorum quenching and prevents social cheating. *ISME J.*, **9**: 115. https://doi.org/10.1038/ismej.2014.98

Goldthwait, S., Yen, J., Brown, J. and Alldredge, A., 2004. Quantification of marine snow fragmentation by swimming euphausiids. *Limnol. Oceanogr*., 49: 940-952. https://doi.org/10.4319/lo.2004.49.4.0940

Gonzalez, J.E. and Keshavan, N.D., 2006. Messing with bacterial quorum sensing. *Microbiol. Mol. Biol. Rev.*, **70**: 859-875. https://doi.org/10.1128/MMBR.00002-06

Gram, L., Grossart, H., Schlingloff, A. and Kiorboe, T., 2002. Possible quorum sensing in marine snow bacteria: Production of acylated homoserine lactones by *Roseobacter* strains isolated from marine snow. *Appl. environ. Microbiol*., **68**: 4111-4115. https://doi.org/10.1128/AEM.68.8.4111-4116.2002

Hermann, C., 2007. Variability of host-pathogen interaction. *J. Endotoxin Res*., **13**: 199-218. https://doi.org/10.1177/0968051907082605

Hmelo, L., Mincer, T.J. and Van Mooy, B.A.S., 2011. Possible influence of bacterial quorum sensing on the hydrolysis of sinking particulate organic carbon in marine environments. *Environ. Microbiol. Rep*., **3**: 682-688. https://doi.org/10.1111/j.1758-2229.2011.00281.x

Ivančić, I., Paliaga, P., Pfannkuchen, M., Djakovac, T., Najdek, M., Steiner, P. and Herndl, G.J., 2018. Seasonal variations in extracellular enzymatic activity in marine snow-associated microbial communities and their impact on the surrounding water. *FEMS Microbiol. Ecol.*, **94**: 198. https://doi.org/10.1093/femsec/fiy198

Jatt, A.N., 2021. Influence of exogenous AHLs and quorum quenching AiiA protein on the production of cellulase enzyme in marine snow associated bacterium, Citrobacter freundii B1. *Pakistan J. Zool.*, **53***:* 827-833. https://doi.org/10.17582/journal.pjz/20200305130330

Jatt, A.N., Tang, K., Liu, J., Zhang, Z., and Xiao-Hua, Z., 2015. Quorum sensing in marine snow and its possible influence on production of extracellular hydrolytic enzymes in marine snow bacterium *Pantoea ananatis* B9. *FEMS Microbiol. Ecol.*, **91**: 1-13. https://doi.org/10.1093/femsec/fiu030

Jatt, A.N., Tunio, S.A., Memon, S.B., Qureshi, A.S. and Bhutto, M.A., 2018. API-ZYM enzymatic profile of *Shigella dysenteriae* IM isolated from drinking water. *Pakistan J. Zool.*, **50**: 977-981. https://doi.org/10.17582/journal.pjz/2018.50.3.977.981

Kepkay, P.E., 1994. Particle aggregation and the biological reactivity of colloids. *Mar. Ecol. Prog. Ser*., **109**: 293-293. https://doi.org/10.3354/meps109293

KiØrboe, T., 2000. Colonization of marine snow aggregates by invertebrate zooplankton: Abundance, scaling, and possible role. *Limnol. Oceanogr.*, **45**: 479-484. https://doi.org/10.4319/lo.2000.45.2.0479

Kiørboe, T., 2001. Formation and fate of marine snow: Small-scale processes with large-scale implications. *Sci. Mar.*, **65**: 57-71. https://doi.org/10.3989/scimar.2001.65s257

Krupke, A., Hmelo, L.R., Ossolinski, J.E., Mincer, T.J. and Van Mooy, B.A.S., 2016. Quorum sensing plays a complex role in regulating the enzyme hydrolysis activity of microbes associated with sinking particles in the ocean. *Front. Mar. Sci.,* **3**: 55. https://doi.org/10.3389/fmars.2016.00055

Lampitt, R.S., 2001. Marine snow. In. *Encyclopedia of ocean sciences.* USA Academy, San Diego. pp. 1667-1675. https://doi.org/10.1006/rwos.2001.0218

Lenz, D.H., Mok, K.C., Lilley, B.N., Kulkarni, R.V., Wingreen, N.S. and Bassler, B.L., 2004. The small RNA chaperone Hfq and nultiple small RNAs control quorum sensing in *Vibrio harveyi* and *Vibrio cholerae. Cell*, **118**: 69-82. https://doi.org/10.1016/j.cell.2004.06.009

Lim, Y.L., Ee, R., Yin, W.F. and Chan, K.G., 2014. Quorum sensing activity of *Aeromonas caviae* strain YL12, a bacterium isolated from compost. *Sensors*, **14**: 7026-7040. https://doi.org/10.3390/s140407026

Lin, L., Dai, S., Tian, B., Li, T., Yu, J., Liu, C. and Hua, Y., 2016. DqsIR quorum sensing-mediated gene regulation of the extremophilic bacterium *Deinococcus radiodurans* in response to oxidative stress. *Mol. Microbiol.*, **100**: 527–541. https://doi.org/10.1111/mmi.13331

Martin, M., Lampitt, R.S., Perry, M.J, Sanders, R., Poulton, A., Lee, C. and D'Asaro, E., 2011. Export and mesopalagic particle flux during a North Atlantic spring diatom bloom. *Deep-Sea Res.,* **58**: 338-349. https://doi.org/10.1016/j.dsr.2011.01.006

Miller, S.T., Xavier, K.B., Campagna, S.R., Taga, M.E., Semmelhack, M.F., Bassler, B.L. and Hughson, F.M., 2004. *Salmonella typhymurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. *Mol. Cell.*, **15**: 677-687. https://doi.org/10.1016/j.molcel.2004.07.020

Mohamed, N.M., Cicirelli, E.M., Khan, J.J., Chen, F., Fuqua, C. and Hill, R.T., 2008. Diversity and quorum-sensing signal production of Proteobacteria associated with marine sponges. *Environ. Microbiol.*, **10**: 75-86.

Myszka, K. and Czaczyk, K., 2012. *N*-acyl homoserine lactones (AHLs) as phenotype control factors produced by Gram-negative bacteria in natural ecosystems. *Pol. J. environ. Stud.*, **21**: 15-21.

Nealson, K.H., Platt, T. and Hastings, J.W., 1970. Cellular control of the synthesis and activity of the bacterial luminescent system. *J. Bact.*, **104**: 313-322. https://doi.org/10.1128/jb.104.1.313-322.1970

Okada, M., Sato, I., Cho, S.J., Iwata, H., Nishio, T., Dubnau, D. and Sakagami, Y., 2005. Structure of the *Bacillus subtilis* quorum-sensing peptide pheromone *ComX. Nat. Chem. Biol.*, **1**: 23-24. https://doi.org/10.1038/nchembio709

Park, S.J., Park, S.Y., Ryu, C.M., Park, S.H. and Lee, J.K., 2008. The role of AiiA, a quorum-quenching enzyme from *Bacillus thuringliensis*, on the rhizosphere competence. *J. Microbiol. Biotechnol.*, **18**: 1518-1521.

Piontek, J., Handel, N., Langer, G., Wohlers, J., Riebesell, U. and Engel, A., 2009. Effects of rising temperature on the formation and microbial degradation of marine diatom aggregates. *Aquat. Microb. Ecol.*, **54**: 305-318. https://doi.org/10.3354/ame01273

Platt, T.G. and Fuqua, C., 2010. What's in a name? The semanticus of quorum sensing. *Trend. Microbiol.*, **18**: 383-387. https://doi.org/10.1016/j.tim.2010.05.003

Reverchon, S., Bouillant, M.L., Salmond, G. and Nasser, W., 1998. Integration of the quorum-sensing system in the regulatory networks controlling virulence factor synthesis in *Erwinia chrysantherni*. *Mol. Microbiol.*, **29**: 1407-1418. https://doi.org/10.1046/j.1365-2958.1998.01023.x

Shao, C.P. and Hor, L.A., 2001. Regulation of metalloprotease gene expression in *Vibrio vulnzficus* by a *Vibrio harveyi* LuxR homologue. *J. Bact.*, **183**: 1369. https://doi.org/10.1128/JB.183.4.1369-1375.2001

Simon, M., Alldredge, A.L. and Azam, F., 1990. Bacterial carbon dynamics on marine snow. *Mar. Ecol. Prog. Ser.*, **65**: 205-2011. https://doi.org/10.3354/meps065205

Sturme, M.H.J., Nakayama, J., Molenaar, D., Murakami, Y., Kunugi, R., Fujii, T., Vaughan, E.E., Kleerebezem, M. and de Vos, W.M., 2005. An agr-like two-component regulatory system in *Lactobacillus plantarum* is involved in production of a novel cyclic peptide and regulation of adherence. *J. Bact.*, **187**: 5224-5235. https://doi.org/10.1128/JB.187.15.5224-5235.2005

Su, Y., Tang, K., Liu, J., Wang, Y., Zheng, Y. and Zhang, X.H., 2019. Quorum sensing system of *Ruegeria mobilis* Rm01 controls lipase and biofilm formation. *Front. Microbiol.*, **9**: 3304. https://doi.org/10.3389/fmicb.2018.03304

Tait, K., Hutchison, Z., Thompson, F.L. and Munn, C.B., 2010. Quorum sensing signal production and inhibition by coral-associated vibrios. *Environ. Microbiol. Rep.*, **2**: 145-150. https://doi.org/10.1111/j.1758-2229.2009.00122.x

Tang, K. and Zhang, X.H., 2014. Quorum quenching agents: Resources for antivirulence therapy. *Mar. Drugs*, **12**: 3245-3282. https://doi.org/10.3390/md12063245

Van Mooy, B.A.S., Hmelo, L.R., Sofen, L.E., Campagna, S.R., May, A.L. and Dyhrman, S.T., 2012. Quorum sensing control of phosphorus acquisition in *Trichodesmium* consortia. *Int. Soc. Microb. Ecol.*, **6**: 422–429. https://doi.org/10.1038/ismej.2011.115

Vendeville, A., Winzer, K., Heurlier, K., Tang, C.M. and Hardie, K.R., 2005. Making sense of metabolism: Autoinducer-2 LuxS and pathogenic bacteria. *Nat. Rev. Microbiol.*, **3**: 383-396. https://doi.org/10.1038/nrmicro1146

Whitehead, N.A., Barnard, A.M.L., Slater, H., Simpson, N.J.L. and Salmond, G.P.C., 2001. Quorum-sensing in gram-negative bacteria. *FEMS Microbiol. Rev.*, **25**: 365-404. https://doi.org/10.1111/j.1574-6976.2001.tb00583.x

Williams, P., Winzer, K., Chan, W.C. and Camara, M., 2007. Look who's talking: Communication and quorum sensing in the bacterial world. *Biol. Sci.*, **362**: 1119-1134. https://doi.org/10.1098/rstb.2007.2039

Yates, E.A., Philipp, B., Buckley, C., Atkinson, S., Chhabra, S.R. and Sockett, R., 2002. *N*-acyl homoserinelactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infect. Immun.*, **70**: 5635-5646. https://doi.org/10.1128/IAI.70.10.5635-5646.2002

Zan, J., Cicirelli, E.M., Mohamed, N.M., Sibhatu, H., Kroll, S., Choi, O. and Fuqua, C., 2012. A complex LuxR-LuxI type quorum sensing network in a roseobacterial marine sponge symbiont activates flagellar motility and inhibits biofilm formation. *Mol. Microbiol.*, **85**: 916–933. https://doi.org/10.1111/j.1365-2958.2012.08149.x

Zheng, H., Zhong, Z., Lai, Xin., Chen, W.X., Li, S. and Zhu, J., 2006. A luxR/LuxI-type quorum-sensing system in plant bacterium, *Mesorhizobium tianshanense*, controls symbiotic nodulation. *J. Bact.*, **188**: 1943-1949. https://doi.org/10.1128/JB.188.5.1943-1949.2006

Ziervogel, K., Steen, A.D. and Arnosti, C., 2010. Changes in the spectrum and rates of extracellular enzyme activities in seawater following aggregate formation. *Biogeosciences*, **7**: 1007-1015. https://doi.org/10.5194/bg-7-1007-2010