

Review Article**Quorum sensing – Meetings in the Microbial World**

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ABSTRACT

The oral microbiota consists of many varied and distinct micro-organisms that effectively strive together through continuous adaptation and interaction with other organisms in their local environment for nutrition and their survival. The biological phenomenon by which they establish this inter-species communication is called as quorum sensing. It controls many traits of the micro-organisms like the mating, their virulence factors and action against antibiotics also. Numerous human, plant and animal diseases are mediated by quorum sensing. This review thus deals with the cell-to-cell communication mechanisms among the different gram positive, gram negative bacteria and the fungi. Additionally, various means of inhibiting quorum sensing among these pathogens have also been described but new approaches to treat periodontal disease using quorum sensing inhibition needs to be explored.

Keywords: Quorum sensing, Auto inducers, Bioluminescence, Quorum quenching**INTRODUCTION**

In the recent times, the concept that the only aim of the existence of the simple prokaryotic organisms is to divide and produce more of their kind has undergone a tremendous change. It is now evident that a variety of developmental processes and other behavioural forms generally associated with multi-cellular organisms are critical elements in the biology of microorganisms as well. Numerous studies have provided enthralling data regarding an “organised social behaviour” that acts as an efficient communication system which helps in co-ordinating among many individuals within a population. Though earlier, it was considered as a rare phenomenon found only among the marine life, currently it has been established that a number of microorganisms have this ability to perceive and respond to the presence of the neighbouring populations. This phenomenon is termed as “Quorum Sensing” [1].

What is Quorum Sensing?

It is a biological process by which bacteria are able to communicate, which modulates the expression of genes that are involved in the processes related to the survival, biofilm formation, virulence and pathogenicity [2, 3]. It is actually a bacterial cell-cell communication process that involves the production, detection and response to extracellular signalling molecules called as auto inducers (AIs). These AIs are small diffusible molecules that are synthesized intracellularly and released into the surrounding environment. At low cell density (LCD), these AIs diffuse away. At high cell density, due to

the increased release of AIs, there is an increased extracellular concentration of the AIs [4]. Finally, when the threshold concentration is reached, the population is considered to be “quorate”. This causes the binding of the AI to the cognate receptors present within the bacteria. This then further triggers a signal transduction cascade that results in wide-spread changes in the gene expression [5]. Individual bacterial function is generally unproductive and hence due to quorum sensing there is enhanced mechanism of communication within a group and therefore, the process becomes effective.

How was it discovered?

Quorum sensing was originally discovered from the luminescent bacterium *Vibrio fischeri*. These bacteria exist as either free living or as symbionts with other organisms or animals like with some fishes (such as the Japanese pinecone fish *Monocentris japonica*) and squid species (such as *Euprymna scolopes*) [6]. The bioluminescent phenotype is exploited by the squid in order to perform a behavioural phenomenon called counter-illumination. At night, the squid disguises itself from predators that reside below it by preventing a visible shadow formation under moonlight and it does this by controlling the intensity of light that it projects downwards. The host in turn provides the nutrients for the bacterium. But it was found that liquid culture of this species produced light only when they are in high density/ concentration. The reason behind this is that when a *V. fischeri* cell is alone; the auto inducer acyl homoserine lactone (3 Oxo C6 HSL, an AHL) is at a low concentration, so no productive

effect but, when they are at high cell concentrations, the level of the autoinducer becomes adequate to induce transcription of the genes that produce the enzyme luciferase. This enzyme production causes the bioluminescence [7].

Further studies on *V. fischeri* have shown that the growth of the bacterium is very fast and that it directly enters the exponential phase, but it also showed that the luminescence increased only at about mid-log phase of its growth [8]. The sudden increase in luminescence was accredited to the transcriptional regulation of the enzyme, luciferase. Thus, this whole track is based on the assessment of the population density by means of release of autoinducers by the bacteria [8].

Bioluminescence

The bioluminescence gene cluster of *V. fischeri* consists of eight *lux* genes (*luxA–E*, *luxG*, *luxI* and *luxR*). They are arranged in two bi-directionally transcribed operons separated by about 218 bp [9]. This structure is called as the *lux* regulon. While the regulators of bioluminescence are the products of *luxI* and *luxR* genes, [10] the *luxA* and *luxB* genes encode subunits of the heterodimeric luciferase enzyme. This enzyme causes the oxidation of the reduced flavin mononucleotide to produce a long-chain fatty acid, water and flavin mononucleotide. This reaction causes the emission of blue-green light along with the oxidation reaction and therefore is termed as bioluminescence. Different luminescent bacteria may show different luminescence spectrum and colour of the emitted light due to the shift in wavelength caused because of the sensitizer proteins [11].

The first step in bioluminescence is interaction between OOHL and the transcriptional regulator protein, LuxR. When present in low densities, *V. fischeri* cells express *luxI* at a basal level, so the concentration of OOHL in the medium remains low. However, as the population density increases, the concentration of OOHL in the environmental medium also increases. Once the threshold concentration of OOHL is achieved OOHL diffuses back into the cell and binds to LuxR [12]. LuxR and CAP are the two proteins that regulate the expression of *luxR*. Induction of transcription from *luxI/CDABEG* operon increases the cellular levels of mRNA transcripts required both for bioluminescence and OOHL synthesis. This process is referred to as auto induction. Rise in the concentration of the OOHL molecules causes its increased diffusion into the cells which causes the activation of more LuxR protein within the *V.*

fischeri population. Thus, auto induction ensures that bioluminescence and signalling molecule production continues. However, when OOHL is abundant, activated LuxR represses the transcription of *luxR* and this is called as auto-repression, the mechanism of which is unknown [13]. The three components that are necessary to sense cell density include: (i) a signal (a LuxI homologue), (ii) a means of recognizing the signal (a LuxR homologue), and (iii) accumulation of the signal.

Phenotypes employing quorum-sensing systems

Many diverse microorganisms, both Gram-negative and Gram-positive use quorum-sensing systems to regulate their various biological behaviours ranging from mating to virulence against the host, antibiotics and production of other metabolites, and many more [14]. The common feature between these miscellaneous phenotypes is the success of a microbial function that is based on appropriate population size and cross-communication to discover its own community or even differentiate within the community.

Quorum sensing in gram-positive bacteria

Quorum sensing in Gram-positive bacteria relies on principles that are common to all quorum sensing circuits: production, detection, and response to AIs. In many Gram positive bacteria, the AIs are oligopeptide AIPs. They are detected by the membrane-bound two component signal transduction systems [15]. Quorum sensing controls virulence factor production in Gram-positive human pathogens including *S. aureus*, *Listeria monocytogenes*, *Enterococcus faecalis* and *Clostridium perfringens*. The well-studied system in this group of pathogens is the *S. aureus* Agr system [16].

Studies have proved that *S. aureus* is the leading cause of hospital related infections. Its virulence factors include expression of a range of adhesion molecules, toxins, and compounds that affect the immune system. Quorum sensing regulates expression of genes encoding these virulence factors. Another key component of the *S. aureus* virulence program is the biofilm development. Some Gram-positive bacteria use a quorum sensing system in which AIPs, following their release, are imported back into the cell and detected by cytoplasmic transcription factors. In these systems, the pro-AIP is secreted and they are processed to form the mature AIP by the action of extracellular proteases. After it is transported back into the cell,

the AIP binds to the transcription factor and alters its activity [17]. Such quorum sensing systems are found during sporulation, competence, and enzyme production in *B. subtilis* and plasmid transfer in *E. faecalis*.

Quorum sensing in gram negative bacteria

Gram-negative bacteria typically use LuxI/LuxR type quorum sensing systems. These are similar to the first described quorum sensing system from the bioluminescent marine symbiotic bacterium *Vibrio fischeri* [18]. LuxI/LuxR homologous has been identified in more than 100 Gram-negative bacterial species [19]. In these systems, the LuxI homolog is an AI synthase that catalyzes a reaction between S-adenosylmethionine (SAM) and an acyl carrier protein (ACP) to produce acyl homoserine lactone (AHL) AI [20]. It is freely diffusible and hence only when its concentration reaches the threshold, the AIs bind to cognate cytoplasmic LuxR-like transcription factors or else, LuxR-type proteins are rapidly degraded, likely to prevent bacteria from "short-circuiting" their quorum sensing systems.

Several Gram-negative pathogens regulate the virulence factor production using LuxI/LuxR type quorum sensing circuits. The examples include *P. aeruginosa*, *Serratia marcescens*, *Brucella melitensis*, *Chromobacterium violaceum*.

Quorum sensing in fungi

Quorum sensing in eukaryotic organisms was unknown until the discovery of farnesol as a quorum sensing molecule in the pathogenic fungus *Candida albicans* [21]. But still the signalling cascades that control the gene expression under quorum sensing regulation in *C. albicans* remain poorly understood. The physiological effects of farnesol comprises of its role in *C. albicans* morphology regulation, biofilm formation inhibition and *C. albicans* drug efflux modulation. In 2009, Langford and collaborators published a review about signalling pathways possibly implicated in farnesol-mediated quorum sensing in *C. albicans* [22]. Davis-Hanna et al in their study demonstrated that farnesol inhibits the activity of the Ras-cAMP-Efg1 signalling cascade involved in hyphal formation [23]. In the recent times, Hall et al. reported that the isoprenoid directly inhibited the activity of *C. albicans* adenyl cyclase thus demonstrating the effects of farnesol on cAMP signalling [24]. These studies show that farnesol can act through different regulatory pathways in *C. albicans* which are known to be also involved in several other physiological processes.

In addition to the *C. albicans* and *S. cerevisiae* quorum sensing activities were also described in *Histoplasma capsulatum*, *Ceratocystis ulmi*, *Neurospora crassa* and numerous other fungi. However, the molecules responsible for such activities have not been purified so far.

Significance of Quorum sensing

Friaset *al.* reported that organisms like *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Prevotella intermedia* produce autoinducer-2 which can induce bioluminescence in the reporter strain [25].

Studies have shown that the periodontal pathogen *Aggregatibacter*, *Actinomyces comitans*, possesses an AI-2 dependent quorum-sensing system, which regulates expression of virulence factors, biofilm formation, and iron uptake. The quorum sensing system also influences the planktonic growth of the organism under conditions of iron limitation and also up regulates leukotoxic activity by production of leukotoxin polypeptide. In another supporting study done by Shao et al. using an open flow biofilm culture system, he compared the biofilm growth of wild type, LuxS deficient and AI-2 receptor deficient strains of *Actinomyces comitans* and the results showed that the LuxS mutant formed a mature biofilm with considerably lower total biomass and biofilm depth compared with the wild type strain [26]. Cultures were grown using a LuxS-deficient strain in order to determine if the autoinducer-2 signalling was important for *P. gingivalis* biofilm growth. However, in these initial studies, inactivation of *P. gingivalis* luxS failed to show any effect on its adherence or formation of biofilms.

Measuring quorum sensing

The various methods of measuring quorum sensing include the following:

Based on the bioluminescent response of *V. harveyi* by Bassler et al [27].

Liquid chromatography based concentration and separation method with mass spectrometer determination of various AHLs in bacterial culture by Frommberger et al [28].

Colorimetric method for determining salicylic acid carboxyl methyl transferase (SAMT) activity.

Polymerase chain reaction (PCR) techniques have greatly simplified quorum data gathering and differentiation between pathogenic and non-pathogenic strains of bacteria. Hernandez and Olmos used PCR probes and the random amplified polymorphic DNA (RAPD) method for distinguishing

V. harveyi pathogenic to shrimp and they identified *V. harveyi* by the quorum sensing transcript LuxN [29]. In another PCR based method, *P. aeruginosa* mutants were screened for infectivity in a rat model using signature tagged mutagenesis (STM) and high-throughput screening [30].

Quorum quenching

The inhibition of quorum sensing is commonly referred to as "quorum quenching." It initially meant preventing quorum sensing by enzymatic hydrolysis of AHL auto inducers. However, presently the expression quorum quenching is nowadays commonly used in a more general sense to refer to any inhibition of quorum sensing due to the use of enzymatic or non-enzymatic molecules [31].

Quorum sensing can be blocked by the following means: stopping the signal molecule production, destroying the signal molecule, and by preventing the signal molecule from binding to its receptor.

Various plants, algae, fungi, etc. produce molecules which might play a role in inhibiting quorum sensing in bacteria. Few of them are as follows:[32, 33]

Horseradish-Iberin

Piper nigrum, *Piper betle* and *Gnetumgnemon*-hexane, chloroform, and methanol

Garlic-ajoene

Turmeric-curcumin

Red marine alga known as *Daleapulchra*-halogenated furanones

Grape fruit extract-furocoumarins, carotenoids, limonoids, pectin, and coumarin

Nutmeg (*Myristicacinnamomea*)-Malabaricone C

Nutmeg (*M. cinnamomea*)-Alabaricone C

Sponge *Agelasoroides*-alkaloid oroidin

Citrus flavinoids-flavoninenaringenin

Sweet basil-osmarinic acid

Garlic-disulfides and trisulphides

Clove extract-eugenol

Clove extract-hexane and methanol

Coffee extract- caffeine.

Quorum quenching in periodontics

Above discussion clearly suggests that the periodontopathogenic bacteria like *Porphyromonas gingivalis*, *Aggregatibacter Actinomycetam comitans* and *Streptococcus* species clearly demonstrate quorum sensing phenomenon through which they communicate and coordinate their pathogenic behaviour. Therefore, all the means of inhibiting quorum sensing already described may have a role to play in controlling periodontal infections. These methods if used as an adjunct along with mechanical plaque control and routine

oral hygiene practices may help us reduce periodontal disease severity.

CONCLUSION

It is only recently that the complexity and scope of quorum sensing-specific bacterial regulation has been appreciated by many in the scientific field. And it is now obvious that bacteria do exist in multifaceted communities where they are constantly communicating with each other. However, our current understanding of the extent and significance of bacterial intercellular communication is still in its early stages. While the list of bacteria that employ quorum-sensing systems is ever growing, it is dubious to be complete. It is therefore definite that further discoveries are required to know the true extent and significance of bacterial cell-cell communication in the environment. Also, based on the findings that quorum sensing is an important process in bacterial virulence, numerous therapeutic agents can be developed which can inhibit quorum sensing. This may offer an effective alternative to antibiotic mediated bactericidal or bacteriostatic approaches and may reduce the risk for development of antibiotic resistance to treat various common microbial diseases like periodontitis.

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