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Quorum Sensing and Quorum Quenching Facebook of Microbial World

Medical Science

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ABSTRACT

Dental Plaque is an example of biofilm leading to periodontal disease and dental caries. Many Gram-positive and Gram-negative bacteria communicate using small diffusible signal molecules called autoinducers. This process, known as quorum sensing, links cell density to the expression of genes as diverse as those associated with virulence factors production of pathogens, bioluminescence, antibiotic production, sporulation of biofilm formation. The interference with these signaling systems, also known as Quorum Quenching, represents a promising strategy to tackle bacterial infections. Recent advances have revealed that bacteria are not only limited to communicate within their own species but also are capable of "listening in" and "broadcasting to" unrelated species to intercept messages and coerce cohabitants into behavioral modification for increase in cell population and density.

INTRODUCTION:

The human oral cavity is a rich source of microorganisms where a dynamic interaction exists between the host environment and the oral bacteria consortium [1].Communication is a key element in successful organizations. The bacteria on human teeth and oral mucosa have developed the means by which to communicate and thereby form successful organizations.[2] Bacteria in biofilm communicate with each other by a process called quorum sensing. This dynamic, sophisticated communication system enables bacteria to monitor each other's presence and to modulate their gene expression in response to the number of bacteria in a given area of the biofilm.[3]

Nowadays the QS mechanisms of Gram-positive and Gram-negative bacteria are being extensively studied as a new and promising target for reducing bacterial infections in humans, animals and plant.[4]

MOLECULAR GENETIC ANALYSIS OF DENTAL BIOFILM FORMATION.

Davey and Costerton present an overview of the current understanding of the genes that regulate dental biofilm formation. They describe genes that are responsible for adhesin formation, which is necessary for cell–surface or cell–cell attachment.[5] Roberts and Mullany describe the major mechanisms for the horizontal transfer of genetic information, including plasmids, conjugated transposons and bacteriophage, as well as environmental factors that may affect gene transfer.[6]

The various technologies presented (in vivo expression technologies, differential fluorosis induction , signature tagged) have significantly contributed to our understanding of bacterial virulence. However, all of these approaches suffer from one or more important drawback, which are summarized in Table 1.[7]

Table 1: Advantages and limitations of selected in vivo expressions technologies

Handfield et al.

Table 1. Advantages and limitations of selected *in vivo* expression technologies

System	Relevant characteristics	Limitations
Reporter genes (*lacZ*, GFP, *phoA*, CAT)	Screening for differentially regulated genes.	Brute force screening which requires an animal or *in vitro* model, and a genetically domesticated pathogen.
IVET (auxotrophic or antibiotic resistance)	Promoter trap or probe which selects for up-regulated genes.	Requires an animal or *in vitro* model, and a genetically domesticated pathogen.
DFI	FACS-based screening.	Requires an animal or *in vitro* model, and a genetically domesticated pathogen.
STM	Comparative hybridization.	Requires an animal or *in vitro* model, and a genetically domesticated pathogen.
Differential display	Multiplex comparative hybridization.	Requires an animal or *in vitro* model, and sufficient high quality mRNA. Assess transcriptional level of regulation only.
Microarrays	Multiplex comparative hybridization.	Requires an animal or *in vitro* model, sufficient high quality mRNA, and a sequenced genome. Technically demanding. Assess transcriptional level of regulation only.
Proteomics and 2-D gels	Screen for differentially regulated proteins.	Amendable to relatively simple *ex vivo* systems. Technically demanding. Low sensitivity.
IVIAT	Identifies immunogenic factors during actual human infection.	Requires a cultivable pathogen and serum from patients.

IVET, *in vivo* expression technology. DFI, differential fluorescence induction. STM, signature-tagged mutagenesis.

QUORUM SENSING IN DENTAL PLAQUE: How bacteria talk to each other

Traditionally, social cooperation has been considered the peserve of higher organisms. They exhibit complex cooperative behaviours, such as conjugal plasmid transfer, biofilm maturation and virulence. Many of these behavior are regulated by quorum sensing.[8]

Quorum sensing is a mechanism of gene regulation in which bacteria coordinate the expression of certain genes in response to presence and absence of small signal molecule. This mechanism was first discovered in marine bacterium vibrio fischeri. [9] Quorum sensing in bacteria ''involves the regulation of expression of specific genes through the accumulation of signaling compounds that mediate intercellular communication''. [10]

FACTORS AFFECTING QUORUM SENSING :

Although unicellular, bacteria are highly interactive and employ a range of cell-to-cell communication or 'quorum sensing (QS)' systems for promoting collective behaviour within a population. QS is generally considered to facilitate gene expression only when the population has reached a sufficient cell density and depends on the synthesis of small molecules that diffuse in and out of bacterial cells.[11] Quorum sensing depends on cell density. Once the signaling compounds reach a threshold level, gene expression is activated. The high cell concentrations in biofilms present an ideal situation for quorum sensing, as even small microcolonies may induce gene expression since the signaling compounds may be concentrated within the microcolony and are not degraded. [10]

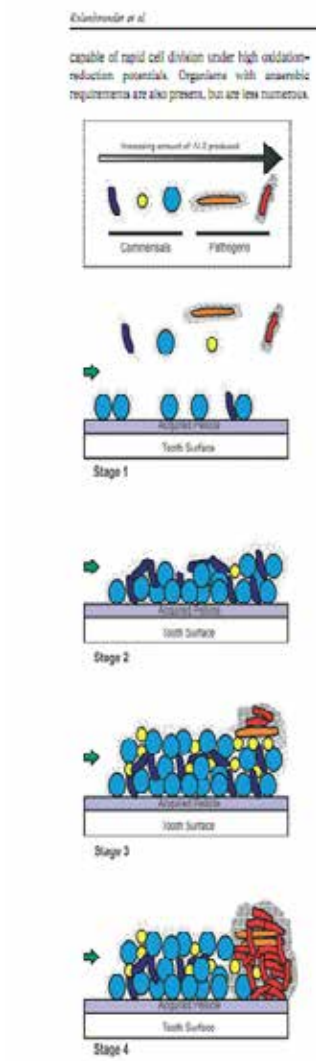

Figure 1: Diagrammatic representation of biofilm formation on the tooth surface and the potential roles of coaggregation, bridging, and autoinducer-2 in the transition from health to disease. Stages show the colonization and growth of commensal organisms (Stages 1 and 2) and the integration and invasion of pathogenic species (Stages3 and 4). Different colors represent different species. Yellow, light blue and dark blue cells are commensal species. Orange and red cells are pathogenic species. Relative amounts of autoinducer-2 are shown as sparsely placed dots (low level of autoinducer-2 produced) around commensals vs. densely packed dots (high level of autoinducer-2 produced) around pathogens . A clean tooth surface, with the associated acquired pellicle, is colonized by commensal species (Stage 1). The small green arrow indicates that these oral communities form and flourish under conditions of flow of saliva or crevicular fluid. Coaggregation interactions contribute to colonization. The commensal cells produce picomolar amounts of autoinducer-2, and this fosters mutualistic interdigitated growth. The interdigitated commensal community expands rapidly through autoinducer-2mediated mutualistic growth (Stage 2). As commensal cell numbers and diversity increase, the likelihood of pathogens integrating into the biofilm increases; integration can be through coaggregation interactions with commensals (orange cells with yellow cells) or through a bridging species (red cells coaggregating with orange cells that coaggregate with yellow cells) (Stage 3). The pathogens produce higher concentrations of autoinducer-2 than the commensals, and this high concentration retards mutualistic growth between the commensal species. With growth being impaired by high autoinducer-2 concentra-tions (Stage 4), the commensal species are unable to compete with the invading, rapidly multiplying, pathogens. The community composition shifts from that of predominantly commensals to include a community of mostly pathogens.[12]

QUORUM SENSING IN GRAM NEGATIVE BACTERIA

The vast majority of gram-negative quorum-sensing systems that have been studied thus far utilize N-acyl homoserine lactones (AHL) as signaling molecules. When in high enough concentration, these molecules can bind to and activate a transcriptional activator, or R protein, which in turn induces expression of target genes

QUORUM SENSING IN GRAM POSITIVE BACTERIA

A number of gram-positive bacteria are known to employ quorum-sensing systems. The nature of the signal molecules used in these systems differs from those of gram-negative organisms, and thus far, no gram-positive bacteria have been shown to produce AHLs. Gram-positive quorum-sensing systems typically make use of small post translationally processed peptide signal molecules. These peptide signals interact with the sensor element of a histidine kinase two-component signal transduction system.[13]

KEY REGULAORS OF QUORUM SENSING[14]

- Autoinducers
  - Acyl homoserine lactone
  - Autoinducer 2
  - Cyclic dipeptides
  - Bradyoxetin
- Autoinducer synthases
  - AHL synthases
  - AI-2 synthase
- Quorum sensing regulators
  - Lux R regulators
  - Lux P/Q regulators

AUTOINDUERS:

Bacteria communicate through the production of diffusible signal molecules termed autoinducers. The molecules are produced at basal levels and accumulate during growth. Once a critical concentration has been reached, autoinducers can activate or repress a number of target genes. [15]

Autoinducer 2 : In 2001, scaulder et. al. proposed that a small molecule called autoinducer-2 was a universal signal mediating

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message among the species in mixed species communities this idea is distinct from the regulation of gene expression mediated by autoinducer-1, a family of acyl homoserine lactones which regulate gene expression in genetically identical cells. There is no evidence of autoinducer -1 in oral bacteria.

Autoinducer-2 is an umbrella designation that covers a collection of molecules formed from the spontaneous rearrangement of 4,5-dihydroxy-2,3- pentanedione (DPD) , which is the product of LuxS. [12]

Cyclic dipeptide derivatives: a new class of autoinducers was recently identified in strains of pseudomonas based on their activity to activate AHL biosensors. Structural analysis indicated that these new signal molecules were diketopiperazines (DKPs) and cyclo (L- pro-L-Tyr) respectively [14]

Acyl homoserine lactones: The best known and understood QS mechanism is the one described for Gram-negative bacteria. This mechanism involves production, secretion and response to the concentration changes of small signal molecules belonging to the N-acyl-homoserine lactones (AHLs) family. An AHL-dependent QS mechanism was first described in the marine bacterium Vibrio fischeri (Photobacterium fischeri), where it is responsible for bioluminescence in a population density-dependent manner[4]

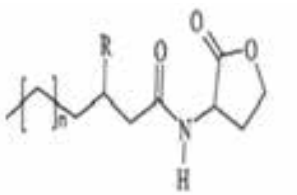


Figure 2: N- acyl homoserine lactone: the main QS signal of gram negative bacteria Lux

Lux is responsible for autoinducers 2 signal molecule production in vibrio harveyi.Luxs is an S-ribosylhomocysteinase that catalyze cleavage S-ribosylhomocysteine to produce L-homocystiene and 4,5 di hydroxyl 2-3 pentanedione.This catalysis is comman in both gram positive and gram negative bacterial pathways for autoinducer-2 synthesis.[14]

SIGNIFICANCE OF QS

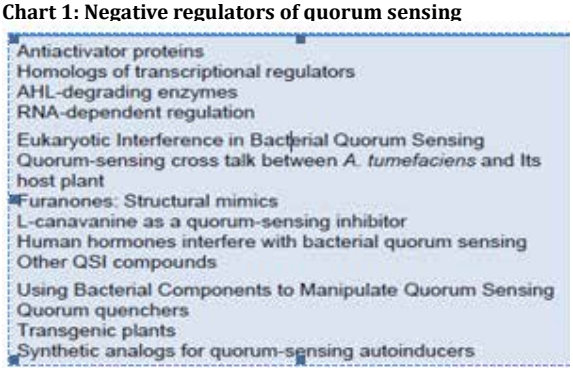
The qs system is responsible for controlling various activities, e.g. antibiotic production, and resistance, conjugation, replication, virulence determinant production ,exoenzyme synthesis, swarming,biofilm formation, plasmid transfer, motility, bioluminescence and others.[4]

QUORUM QUENCHING :

Inhibition of quorum sensing can be accomplished in several ways which include

1. enzymatic degradation of signal molecule
2.blocking signal transduction
3.blocking signal perception

Chart 1: Negative regulators of quorum sensing



INTERFERENCE IN QUORUM SENSING

As the QS mechanism, among others, controls virulence factor synthesis in many pathogen.The signal molecules are transferred into the environment and back to the cells by either passive diffusion or active ATP-dependent transport systems (low cell density). With an increasing density of bacterial population the AHLs accumulate in the environment and in the bacterial cells. When, the concentration of AHLs reaches a threshold level (quorum state), the signal molecules interact with the regulatory protein (R). The regulatory protein (R), in most cases, acts as a positive transcription regulator. The complex of R protein and AHL binds to promoters of target genes and initiates their expressions in a cell density dependent manner (high cell density). It is now generally accepted that the ability to inactivate autoinducers and suppress QS signal generation and/or response might be useful in controlling infection development and persistence of human, animal and plant bacterial pathogens Interference in the QS mechanism can be achieved in a variety of ways. First, many natural substances can disturb the signal perception by imitating AHLs structure. The AHL analogues block the AHL receptor (regulator) protein and therefore prevent activation of the target gene expression. [4]

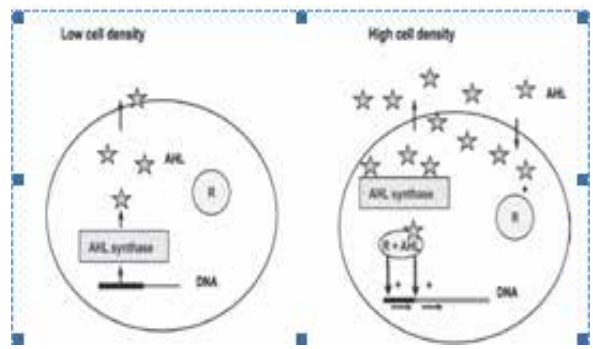


Figure 3: Overview of quorum sensing mechanism expolited by gram- negative bacteria

Bacterial cells producesignal molecules- N-acyl- homoserine lactones (AHLs) using specific enzyme- AHL synthase.The signal molecules are transferred into the environment and back to the cells by either passive diffusion or active ATP- dependent transport systems (low cell density) . With an increasing density of bacterial population the AHLs accumulate in the environment and in bacterial cells. When , the concentration of AHLs reaches a threshold level (quorum state), the signal molecules interact with the regulatory protein (R). The regulatory protein (R), in most cases , acts as a positive transcription regulator. The complex of R protein and AHL binds to promoters of target genes and initiates their expressions in a cell density- dependent matter (high cell density).

Furanones:

Halogenated furanones produced by red alga Delisea pulchra were able to thesis and extracellular plant cell wall degrading enzyme production in P. carotovorum subsp. carotovorum .inhibit AHL-dependent carbapenem antibiotic.[14]

Enzymatic degradation of AHLs

The chemical structure of AHLs suggests that the degradation of such molecules may occur in four different ways. Two of them lead to the degradation of the homoserine lactone ring mediated by lactonase or decarboxylase. Two others cause cleavage of AHL to a homoserine lactone and a free fatty acid moiety as reaction products of acylase (syn. amidase) or deaminase .

AHL lactonases

AHL lactonases hydrolyze the lactone ring in the homoserine moiety of AHLs, without affecting the rest of the signal molecule structure.

The possible ways of enzymatic degradation of AHLs Broken line mark position possible cleavages by following enzymes:

1.Lactonase; 2.decarboxylase;3,acylase;4,deaminase

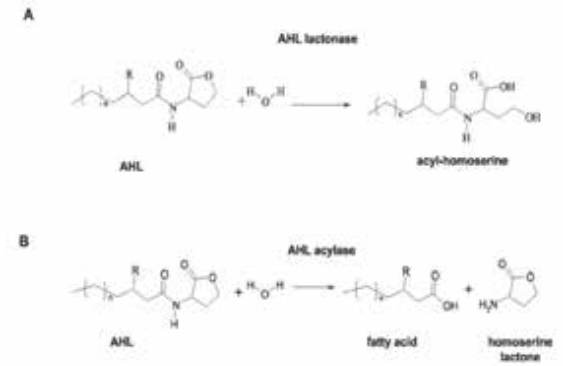


Figure 4: Mechanism Of AHL Degrading Enzymes

A.Mode Of AHL Lactonase Action

B.Mode Of AHL Acylase Action

AHL acylases

These enzymes hydrolyze the amide bond between the acyl side chain and the homoserine lactone in the AHL molecules generating the free fatty acid and the homoserine lactone .To the present, nine AHL acylases from various groups of bacteria have been reported. One of the first to be described was an enzyme from Gram-negative bacterium Variovorax paradoxus VAI-C strain .[4]

QUORUM QUENCHING IN BASIC RESEARCH AND BIOTECHNOLOGICAL APPLICATIONS:

Quorum-quenching molecules have proved to be valuable tools in addressing both the basic and the conceptional questions Since the discovery of the first quorum-quenching enzyme encoded by *aiiA* , the prokaryotic-origin AHL-lactonases and AHL-acylases have been frequently used in investigations of the role of AHL signals owing to the convenience in cloning and expression[16]

CONCLUSION AND FUTURE ASPECTS :

Biofilms have major impact on human health. The oral cavity demonstrates significant interspecies cooperation between microbes in a biofilm mode of growth. Understanding bacterial community behavior will probably put new emphasis on alternative therapeutic strategies to treat diseases caused by plaque biofilm.The study of bacterial quorum sensing has suggested several idea targets for drug design.further investigation on quorum sensing systems could reveal additional mechanisms for interfering with bacterial quorum sensing.[14] The ability of bacteria to adapt to environments of high cell density or limited diffusion is fundamental to their competitive growth and survival; for some bacteria, these adaptive responses are accomplished using autoinducers. The challenge for the future will be for clinicians to apply their understanding of autoinducer-based signaling to the development of effective therapeutic agents or prevention strategies.[17]

REFERENCE

[1]. Wai-Fong Yin , Kathiravan Purmal , Shenyang Chin , Xin-Yue Chan 1 and Kok-Gan Chan ,Long Chain N-acyl Homoserine Lactone Production by Enterobacter sp. Isolated from Human Tongue Surfaces , Sensors 2012, 12, 14307-1431 | [2]. Paul E. Kolenblander; Among Oral Bacteria , Microbiology And Molecular Biology Reviews ,Sept 2002 ,P 486-505 | [3]. JoAnn R. Gurenlian, RDH, PhD ,The Role of Dental Plaque Biofilm in Oral Health,Journal of Dental Hygiene, 2007, 4-12THE A MERICAN D ENTAL H YGIENISTS'ASSOCIATION | [4]. Robert Czajkowski And Sylwia Jafra;Quenching Of Acyl Homoserine Lactone Dependent Quorum Sensing By Enzymatic Disruption Of Signal Molecules Acta Biochimica Polonica,Vol 56 No.1/2009 ,1-16 | [5].Anne D. Haffajee, Socransky Introduction to Microbial Aspects Of Periodontal Biofilm Communities ,Development And TreatmentPerio2000,Vol.42,2006,7-12 | [6].Adam P. Roberts & Peter Mullany ,Genetic Basis Of Horizontal Gene Transfer Among Oral Bacteria Periodontology 2000,Vol 42, 2006,36-46 | [7].Martin Handfield , Ann Progulske, In Vivo Induced Genes In Human Diseases. Perio 2000 ,Vol38,2005,123-134 | [8].Avantika Lal ,Quorum sensing : how bacteria talk to each other, resonance September 2009,page 866-871 | [9].Tom Defoirdt, Nico Boon, Patrick Sorgeloos, Willy Verstraete ,Peter Bossier ,Quorum sensing and quorum quenching in Vibrio harveyi : lessons learned from invivo work ,international society of microbial ecology (2008) 2,19-26 | [10]. Sigmund Socransky & Anne D. Haffajee, Dental Biofilm :Difficult Therapeutic Targets Periodontology 2000 Vol.28.2002.12-55 | [11].Paul Williams ,Quorum sensing, communication and cross-kingdom signalling in the bacterial world, Microbiology (2007), 153, 3923–3938 | [12]. Bacterial Interactions And Successions During Plaque Development,Periodontology 2000, Vol.42,2006,47-79 | [13]. Teresa R. de Kievit and Barbara H. Iglewski Bacterial Quorum Sensing in Pathogenic Relationships Infect. Immun. September 2000 vol. 68 no. 9 4839-4849 | [14]. Baswaraj Biradar, prapulla devi ,Quorum Sensing In Plaque Biofilm :Challenges And Future Aspects, Journal Of Contemporary Dental Practices, 2011, 1024-1080 | [15]. L. Caetano M. Antunes1, Rosana B. R. Ferreira, Michelle M. C. Buckner1,2 and B. Brett Finlay Quorum sensing in bacterial virulence, Microbiology August 2010 vol. 156 no. 8 2271-2282 | [16]. Yi-Hu Dong, Lian-Hui Wang, and Lian-Hui Zhang, Quorum-quenching microbial infections: mechanisms and implications Philosophical Transactions of the Royal Society B: Biological Science, 29 July 2007 vol. 362 no. 1483 1201-1211 | [17]. Costi D. Sifri, Quorum Sensing: Bacteria Talk Sense, Clin Infect Dis. (2008) 47 (8): 1070-1076. |