**THE ROLE OF QUORUM SENSING IN MICROBIAL BIOFILM FORMATION**

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**ABSTRACT**

Microbial cells do not live in isolation in their environment, but rather they communicate with each other using chemical signals. This sophisticated mode of cell-to-cell signaling, known as quorum sensing, was first discovered in bacteria, and coordinates the behaviour of microbial population behaviour in a cell-density-dependent manner. More recently, these mechanisms have been described in eukaryotes, particularly in fungi, where they regulate processes such as biofilm formation, pathogenesis, morphological differentiation, and secondary metabolite production. Quorum sensing is an important mechanism used by bacteria to exchange information among themselves and as well helps in regulating the expression of related genes and other physiological processes. Bacteria are able to perceive and respond to self-produced signal molecules and regulate their behavior in response to their population size. The main role of QS is the regulation of vital processes in the cells such as virulence factor production or biofilm formation. In biofilm formation, microorganisms use QS systems to regulate their population density and as well communicate directly with other microbes, or indirectly mediate the production of molecules that affect the survival of their neighbouring populations. QS play a vital role in biofilm formation during replication in biofilm development as well as biofilm dispersal as a control to the size of microbial population.

**KEYWORDS:** Quorum sensing, Biofilm formation,extrapolymeric substances, N-acyl-homoserine lactones, autoinducing peptides

**INTRODUCTION**

The study of microbial development revealed that microorganisms are able to interact and differentiate in a complex way. Biofilm, simply explained as the formation of microbial communities that attach to surfaces and embedded in a self-produced extracellular matrix has been studied to be an excellent model system for the study of microbial development (Solano *et al.,* 2014). Biofilm occurs on both natural and artificial environment and adhere strongly to surfaces with the help of exopolymeric substances, which ensures a complex interaction among microbial cells and protect them against environmental stress and antimicrobials. In addition, many bacteria are able to interact among one another and carry out different complex social behaviors (Carradori *et al.,* 2020). Biofilms are formed through a multistep process that includes cell attachment to surface, cell maturation, and dispersal. The prokaryotic genome expresses the formation of biofilm, which help in providing a more secure environment for the microbial community. This biofilm provide a structural support and protects the microbial cells from any form of environmental stress as well as cidal effects of biocide. There are varying processes involved in the formation of biofilm, which include: organic molecule adsorption by microbial cells from the fluid phase, transport of microbial cells to the surface through chemotaxis and sedimentation, reversible followed by irreversible adhession of cells to substratum, microbial metabolisms, growth and replication, secretion of extrapolymeric substances (EPS), depletion of nutrients from the environment followed by cell lysis and death (often characterized by biofilm sloughing or detachment) (Lazar, 2011).

Biofilm could be homogeneous (of a particular species) or heterogeneous (more than one species) microbial cells attached of abiotic or biotic surfaces. Nonetheless, the most predominant biofilm community in most environment are made up of more than one microbial species synergistically working together as one entity. On the contrary, biofilm made up of one microbial cells are often found in medical implants where they cause infections. Although there are other single microbial species that forms biofilm, however, the study of biofilm has seen *Pseudomonas aeruginosa* being a model used in understanding how other microbial biofilm form and interact with their environment. Environmental factors such as availability of nutrient velocity of bulk fluid among many others influences the development and quality of biofilms. Availability of nutrient ensures the continuity of microbial cells in a biofilm otherwise, they detach themselves and assume a planktonic way of life and move on in search for greener pastures (Carlier *et al*., 2015).

Without any external help, a planktonic cell can grow, multiply, sense and adjust to environment changes. They can however, intercommunicate with species of other organisms in a form of cooperation to accomplish biofilm formation, bioluminescence production and secretion of exoenzyme among many other cooperative activities. Microbial cells have been demonstrated to carry out cell-to-cell mode of communication between same and different species in a mechanism known as quorum sensing (QS). QS is defined as a means of intercellular communication in microorganisms and as well helps regulate the expression of genes through autoinducers (AI). AI are extracellular molecules utilized by microorganisms in QS. This molecules are density dependent and often react when it reaches a particularly threshold (known as quorum) to either surpress of activate genes responsible for a particular phenotypic traits (AIs) (Carlier *et al*., 2015; Zhang *et al*., 2019). AIs play an important role in several physiological processes in microorganisms, which include motility, formation of biofilms, and production of antibiotics and secretion of virulence factor by bacteria (Hmelo, 2017, Zhang *et al*., 2019). The two well reported social behaviors of microbial population are biofilms formation and quorum sensing. Some species of bacteria form surface bound communities known as biofilms. Microbial biofilm is a community of microbial cells (predominantly bacteria) attached to a surface and embedded in matrix composed of extracellular polymeric substance produced by the cells (Lazar, 2011). These two social behaviors and their relationship have been reported by Irie and Parsek (2008) that biofilms formation result from gene expression and regulated by quorum sensing.

**BIOFILM FORMATION**

The emerging branch of microbiology that deals with the study of the social aspects of bacterial life, that include biofilm formation, is referred to as socio-microbiology (Parsek and Greenberg, 2005). Naturally, most bacterial cells are believe to form biofilms and these bacterial cells are usually different from their planktonic form and showcase certain differences in gene expression (Toyofuku *et al.,* 2016). Biofilm is formed through some steps such as cell attachment, biofilm maturation and biofilm dispersion (Figure 1**)**.

**Cell Attachment:** One of the most important processes in biofilm formation is surface attachment because it’s a point of transition from planktonic mode to the biofilm life. First, cells undergo reversible attachment to surface (where they either attach to surface and involve in biofilm formation or separate themselves and assume their planktonic lifestyle) using cell appendages like flagella, pili, and fimbriae. Cells undergo irreversible attachment after reversible attachment, through the extracellular secretion of surface proteins such as LapA and SadB as well as EPS help the cells to adhere firmly to the surface. Differences in the ability to attach play a vital role species differentiation, thus stressing how important surface attachment is in the formation of biofilm (Petrova and Sauer, 2012; Toyofuku *et al.,* 2016).

**Biofilm Maturation:** After bacterial cells have successfully attached firmly to surfaces, they grow and undergo cell division and begin to assembly to form microcolonies. These micro-colonies continue to proliferate and produce EPS. EPS comprises of lipids, nucleic acids, proteins, biopolymers, polysaccharides and water and is responsible for cell’s surface adhesion, binding cells together, as well as maintaining the biofilm in a three-dimensional architecture. EPS also protect the bacterial cells in the biofilm against stress from the environment such as the immune response of host, metallic cations, oxidation and effects of biocides. The EPS of biofilms also help in housing metabolic products, extracellular enzymes and signalling molecules (Flemming and Wingender, 2010; Drescher *et al.,* 2014).

**Biofilm Dispersion:** The final stage in the developmental cycle of biofilm formation in other to initiate another one is the dispersal of biofilm cells. During dispersal, cells from a matured biofilm detach and return to planktonic life where they look for other niche they can colonize the surface. Dispersal can be active or passive and depend on two factors such as degradation of EPS by waste metabolites and physical factors (i.e. shearing force of bulk liquid medium) respectively (Toyofuku *et al.,* 2016). Active dispersal may result from alteration in environmental conditions such as starvation, change in temperature, oxygen fluctuations, and accumulation of metabolic waste (Hong *et al.,* 2010). There are enzymes that can also cause degradation of biofilms such as alginate lyase in *P. aeruginosa*, which helps in the detachment of *P. aeruginosa* from an EPS matrix (Ansari *et al.,* 2017).

**FACTORS THAT INFLUENCE BIOFILM FORMATION**

The social aspect of microbial life is being affected largely by the environment. Environmental factors determine whether cells form biofilm or are dispersed and leave a biofilm (Ansari *et al.,* 2017). Other factors such as effects of types of substratum, hydrodynamics, conditioning films forming on the substratum, properties of the cell, microbial products, Production of extracellular polymeric substances and extracellular DNAare known to influence the processes of biofilm formation from surface colonization to growth, survival and activities of microorganisms in the biofilm as discussed as follows:

**Substratum Effects**

Researchers have reported that the physicochemical parameters of surfaces such as roughness, polarity and hydrophobicity of surfaces play a vital role in the rate and depth of microbial attachment to surfaces. The moment any microbial cell is attached to a surface the chemistry on the interface of attachment changes. Thus, influencing adhesion of cell and may confer cell survival. Colonization of surfaces by microorganisms is influenced by roughness of surface, that is; the depth of microbial colonization increase as surface roughness increases. This is due to the fact that increases of roughness on surfaces result in increase in the surface area available for microbial action and a decrease in shear force. Again, microorganisms adhere more rapidly to nonpolar and hydrophobic surfaces like plastic and Teflon as compared to glass, metals and other hydrophilic materials (Donlan, 2002; Choudhary *et al.,* 2020). Microorganisms that attach to metal surfaces mainly depend on some parameters such as the growth medium, characteristic of substratum and cell surface Some of those metals include stainless steel, aluminum and copper. However, some metals are toxic to bacteria, like lead acetate is attributed to the high lead content in disinfectants and antiseptics (Donlan, 2002; Ansari *et al.,* 2017).

**Hydrodynamics**

Hydrodynamic layer is affected by the type of interaction between microbial cells and the substratum. The zone found adjacent to liquid/substratum interface where velocity flow of liquid is negligible is known as the hydrodynamic boundary layer. The thickness of this layer is inversely proportional to the linear velocity. The characteristic velocity of the liquid controls the interaction between the submerged surface and the microbial cells. As such, at a very low linear velocity, microbial cells need to maneuver pass the hydrodynamic layer. The cell sizes of microbial cells and possession of motility structures help in controlling the interaction with the surface layer. An increase in velocity leads to a decrease in the hydrodynamic layer, exposing the cells to an increasingly larger mixing and turbulence. Therefore, a more rapid association between microbial cells and surfaces is ensured due to linear velocities. However, this could be hampered when velocity of bulk liquid increases resulting in enough shear forces capable of causing detachment of adhered microbial cells (Donlan, 2002; Choudhar *et al.,* 2020).

**Conditioning Films Forming on the Substratum**

Exposing any material to any aqueous medium often result in surface conditioning of that material by polymers from the liquid medium. This often results in chemical modification of that material, which affects to a great extend the depth and rate of microbial attachment to that particular surface. The thin films formed have been demonstrated to be organic in nature and forms when surface is exposed for some minutes and grows continuously for some hours. In the human host however, the nature of film conditioning is quite different. Example is the acquired pellicle, a proteinaceous conditioning film often found on enamels of teeth. This pellicle is made up of lipids, phosphoproteins, glycoproteins, albumin, lysozyme, and crevice fluid of gingival. Pellicle-conditioned surfaces become colonized few hours of exposure to oral cavity bacteria. Some conditioning films produced by the host like urine, tears, blood, saliva, respiratory secretions and intervascular fluid, influence bacteria attachment to biomaterials (Donlan, 2002).

**Properties of the Cell**

Hydrophobicity of the cell surface, presence cell appendages like flagella, pili, and fimbria, and EPS production affect the rate and extent of microbial cells adhesion to any surface. Hydrophobicity increase with an increase in the non-polarity of one or both the microbial cell surface and the substratum surface. Fimbriae proffer cell surface hydrophobicity because of the proportion of hydrophobic amino acid residues and play a role in cell surface hydrophobicity and attachment, by overcoming the initial electrostatic repulsion barrier that exists between the cell and substratum. Motile strains of *P. fluorescens* have been reported to attach more and against the flow faster than non-motile strains and non-motile strains do not recolonize vacant areas on the substratum as evenly as motile strains, resulting in slower biofilm formation (Donlan, 2002).

**Microbial Products**

Biofilm formation is known to be influenced by one or more exometabolites especially in the dispersal of biofilm cells. Accumulation of microbial wastes along with other factors stimulates the active dispersal of cells. Examples of bacterial exometabolites include but not limited to siderophores, pigments, and antibiotics. Some of these microbial exometabolites may trigger the degradation of EPS. Antibiotics produced mostly by soil microorganisms, can alter the population density in the biofilm. Extracellular proteases secreted into the biofilm matrix also cause cell dispersal, through the disruption and lysis of microbial cells, thus, impairing the function of biofilm. The production and secretion of molecules responsible for QS by microorganisms also affects the rate of biofilm formation, likewise surfactin (Ansari *et al.,* 2017).

**Production of Extracellular Polymeric Substances**

EPS as earlier stated, is made up of polysaccharides, proteins, DNA, and phospholipids. The gel-like network formed by EPS act as cementing material in binding microbial cells together through hydrophobic interactions and actions of multivalent cations. Microorganisms are packed together and adhere to surfaces with the help of EPSs while granulation and flocculation protects microbial cells from external conditions and allow accumulation of nutrient within the EPS matrix. Different biofilms produce different amount of EPSs (Singh *et al.,* 2020).

**Extracellular DNA**

Extracellular DNA (eDNA) is a major component of numerous single and multispecies biofilms. It plays an important role in several steps of biofilm development such as attachment and microcolony formation. It provides the ability to protect the biofilms from abiotic stress, antibiotics, and chemicals, and as well assist as a basis of nutrients for biofilm development (Singh *et al.,* 2020).

**Environmental Factors**

The various environmental factors, which affect the formation of biofilm on both biotic and abiotic surfaces include nutrient availability, presence of oxygen, environmental pH, temperature and moisture content, salinity.

**Nutrient Availability**

Nutrient availability plays an essential role in influencing the survival, growth and metabolisms of microorganisms in any habitat. An increase in nutrient concentration is proportional to an increase in the number of attached bacterial cells (Donlan, 2002). Nutrients such as carbon source, amount of nitrate, sucrose, phosphate, and calcium enhance biofilm formation as their concentrations increase. Bacteria in biofilms may acquire nutrients by using different enzymes to break down food supplies and concentrate trace organics substances on surfaces, thus, allowing them to utilize waste products from surrounding microbes. Due to the negatively charged biofilm, most cationic nutrients drawn to the surfaces of biofilms are able to exchange ions from nutrients to microbes, thereby ensuring the availability of nutrient for microbial growth and metabolisms (Prakash *et al.,* 2003; Ansari *et al.,* 2017). Reports have been made that availability of carbohydrates such as trehalose and moanose stimulates biofilm formation of *Listeria monocytogenes* (Choudhary *et al.,* 2020).

**Presence of Oxygen**

Biofilm does not develop in absence of sufficient oxygen, which impair bacteria attachment to surfaces of substrate. Presence of oxygen has been reported to regulate formation of biofilm by *Escherichia coli*.

**Environmental pH**

Effect of environmental pH have been reported on *Vibrio cholerae.* Studies have revealed that pH 7 and 8.2 is optimum for growth and activities of *V. cholerae*. Effect of acidic pH (≤ 5) have been demonstrated to impair the biofilm forming ability of some bacterial cell due to loss of mobility. This is not the case with *Escherichia coli* and *Streptococci epidermidis*, which can grow and form biofilm in acidic environment (i.e. urethral catheters pH of urine is acidic) (Choudhary *et al.,* 2020).

**Temperature and Moisture Content**: Change in temperature drastically affects biofilm formation. Active dispersal is triggered by change in temperature and other environmental factors such as nutrient availability, oxygen deficiency, and accumulation of metabolite (McDougald *et al.,* 2012). The morphology, cell density and thickness of biofilm is affected by temperature since it goes a long way limiting the rate of EPS production. For example, the growth of spore-forming Gram positive *Clostridium perfringens* is affected by increase in temperature, which in turn affect the thickness, cell density and morphology of the biofilm. Likewise *L. monocytogenes*, which biofilm formation is impaired by increase in temperature (Choudhary *et al.,* 2020). This indicate that, the formation of biofilm is temperature dependent affecting the production of EPS (Toyofuku *et al.,* 2016).

Another factor that affects biofilm formation is water availability. The availability of water in some environment such as soil, is dependent on the features of the soil as well as the dissolved sites. At high temperature organic substances tends to be dissolved in water and increase in dissolve solutes reduces the water available for microbial activities, thus, impairing metabolism and biofilm formation (Ansari *et al., 2017*).

**Salinity**

Increase in salt concentration of a medium often translate to an equal increase in osmolytes accumulation and biofilm formation. Some halotolerant bacterial strains such as *Staphylococcus saprophyticus* and *Oceanobacillus profundus* increases accumulation of endogenous osmolytes(i.e. betaine, glycine and proline), EPS production and a subsequent increases in the rate of biofilm formation(Ansari *et al.,* 2017).

**Microbial Quorum Sensing**

Quorum sensing (QS) is a mechanism known for cell-to-cell communication among microbial species (i.e. bacteria and fungi) through the production of small signal molecules known as autoinducers (AIs). During growth, bacterial cell often secrete this AIs, which diffuses and get accumulated in the environment. Passive diffusion is the mechanism used by micribes to transoort AIs across cell membranes with the help of specific transporters and efflux pumps. The amount of AIs accumulated in the environment is being sensed by all individual cell. As such, once a certain threshhold of AIs is reached the individual cells act in synchrony to up-regulate or down-regulate gene expression responsible for a particular trait (Bandara *et al.* 2012; Padder *et al.*, 2018). This QS phenomenon was first studied using *Alivibrio fischeri, a bioluminescence bacteri, which control its light illumination through an integrated system of a synthase (Luxl), a sensor receptor (LuxR), which function in producing QS molecules (i.e. N-acyl-himoserine-lactones)* (Barriuso *et al.,* 2018; Padder *et al.*, 2018). Aside the roles played QS in signalling and chanelling QS molecules, they also function in social communication among the same and different microbial species in a given environment. Example can be seen in the chemotaxis of marine diatoms towards signals of N-acylhomoserine lactone (AHL) and Burkholderia cepacia gene regulation produced by another bacterium (Lewenza *et al.,* 2002; Williams, 2007).

**QUORUM SENSING IN BACTERIA**

Acyl HomoserineLactones **(**AHL) signaling has been described in Gram negative bacteria species. AHL signals consist of a homoserinelactone moiety that is linked by an amide bond to an acyl side chain. AHL synthesis is primarily catalyzed by a single enzyme belonging to the LuxI family, while signals are catalyzed by a single enzyme belonging to the LuxI family and sensed by cytoplasmic DNA-binding regulatory proteins belonging to the LuxR family. The first described acylhomoserine lactone (AHL) QS system was in *Vibrio fischeri,* a marine species that can bioluminesce in the light organs of various marine animals, like the Hawaiian bobtail squid *Euprymnas colopes*. *V. fischeri*was found to bioluminesce at high cell densities in liquid batch culture due to an accumulation of the AHL signal, 3-oxo-hexanoyl homoserine lactone (Irie and Parsek, 2008; Zang *et al.,* 2019).

**Peptide Auto-inducers**

Many Gram-positive species have been studied to utilize peptides for QS. Streptococcal species frequently utilize competence signal peptides (CSP) among other QSMs. Accumulation of CSP induces autolysis, where chromosomal DNA is released into the environment and thereafter, taken up by neighboring cells to promote horizontal gene transfer. CSPs and other peptide-based QSMs have also been found to regulate other group-associated behaviors such as biofilm formation and bacteriocin production in different Gram-positive species (Irie and Parsek, 2008).

**Auto-inducer 2**

Auto-inducer 2(AI-2) is a QS signal produced by some Gram-positive and Gram negative bacterial species. The AI-2 structures have been found in *Vibrio harveyi* and *Salmonella enterica* serovar *typhimurium*. While the two structures are distinct, possibly allowing for species specificities, cross-species signaling appears to be prevalent. A key step in AI-2 synthesis is catalyzed by a highly conserved enzyme LuxS and the LuxSgene is found in a vast number of bacterial species, making it important in regulating different functions (Irie and Parsek, 2008).

**QUORUM SENSING IN FUNGI**

The mechanisms of QS in fungi have been reported to regulate some processes include but not limited to production of secondary metabolites, transition of morphology, sporulation and secretion of enzymes (Barriuso *et al*., 2018). Like in bacteria, fungi QS is also population dependent in causing virulence/pathogenesis as well as the regulation of biofilm formation. Various intraspecific as well as interspecific communications of QS, their nature, diversity and ways they act in phenotypic expression of a particular trait has been discussed (Tarkka *et al.,* 2009; Padder *et al.*, 2018). An important discovery was made over 15 years ago on the control of filamentation by farnesol in dimorphic *C. albicans* stressing the important role of QS in fungi. Farnesol, a sequiterpene alcohol have been demonstrated to prevent/reduce the probability of dense culture of *C. albicans* from switching to hyphal mode from yeast. Thus, Farnesol inhibits formation of hyphae in fungal (Ramage *et al.,* 2002; Shirtliff *et al.,* 2009). Aside having harmful effects on host tissues and other microbes, farnesol also plays an important role in the physiology of C. albicans since they serve as signalling molecules (Albuquerque and Casadevall, 2012; Padder *et al.*, 2018). Another QS molecule in fungi is the aromatic alcohol tyrosol, which plays important role in the regulation of biofilm morphogenesis and formation, as well as growth of *C. albicans*. In *Saccharomyces cerevisiae,* tryptophol and 1-phenylethanol regulate acts as QS molecules and regulate biofilm morphogenesis in nitrogen depleted environment (Albuquerque and Casadevall, 2012). The evidence of roles of QS in fungi being density dependent has been documented, although research of QS is still at a low level among fungal species (Albuquerque and Casadevall, 2012; Wongsuk *et al.,* 2016).

**MECHANISMS OF MICROBIAL QUORUM SENSING**

Quorum sensing mechanism, is based on microbial cell-to-cell communication mediated by self secreted small molecular compounds, known asautoinducers (AIs), which are found in Gram positive and Gram negativebacteria as well some fungal species (Irie and Parsek, 2008).

**Mechanisms of Quorum Sensing in Bacteria**

In bacteria, quorum sensing mechanism usually involve the synthesis and release of enzyme specific class of signal molecules in their environment. At a certain threshold concentration of the signal molecules, receptor proteins recognize them either directly or indirectly and coordinate bacterial group behaviors beneficial to the entire population. In Gram negative bacteria, the commonly used autoinducer is AHLs (N-acyl-homoserine lactones) for intra-species communication and over 25 different kinds of gram-negative bacteria have been reportedly regulated by AHLs (Sharma *et al*., 2020).

The mechanism involved in the regulatory role of a typical AHLs autoinducer requires the production of AHLs and binding of LuxI (AHLs signal synthase) to LuxR (AHLs signal receptor) regulatory protein within the cell. AHLs produced are permeable to the cell membrane and capable of diffusing randomly through the membrane into the environment and accumulates. When it reaches a certain threshold concentration, they diffuse through the cell membrane and bind to the amino terminus of LuxR receptor proteins in the cytoplasm to form the LuxI/LuxR protein complexes to coordinate the expressions of certain functional genes. These LuxI/LuxR protein complexes also have a feedback regulatory effect on the production of AHLs signal molecules and their receptor proteins. Aside AHLs, other autoinducers with different chemical structures have been discovered in gram-negative bacteria, which includes AI-2, PQS (*Pseudomonas* quinolone signal), indole, pyrones, DARs (Dialkylresorcinols) and CHDs (Cyclohexanediones) among others (Zang *et al*., 2019; Sharma *et al*., 2020).

Mechanism of quorum sensing in Gram positive bacteria is essentially similar to that in Gram negative bacteria. The main difference is that Gram positive bacteria utilize autoinducing peptides (AIPs) as signaling molecules in exchanging information among bacterial cells. Unlike AHLs that are able to diffuse freely across cell membrane, AIPs are tansported across membrane with the help of membrane proteins such as ATP-binding cassette transporter (ABC transporter). The AIPs are sensed by this two-component signal transduction system (TCSTS) made up of transmembrane sensor kinase AgrC and response regulatory protein AgrA. When AIPs reaches the threshold concentration, they bind to receptors on the surface of the cell activating the two-component phospho-kinase system (TCS) that initiate the corresponding signal transduction and finally initiate gene transcription (Singh and Ray, 2014; Zang *et al*., 2019).

Pheromones secretion in the environment is usually detected by an extracellular and intracellular pathway. Extracellular pathway involves a two-component signal transduction system whose histidine kinase binds with the pheromones in the surface of the bacteria that subsequently leads to phosphorylation of regulators and the eventual expression of target genes. However, in the intracellular pathway, an oligopeptide transport system transports pheromones into the bacteria, which leads to the activation of receptors (transcriptional regulators), and subsequently expressing target gene. In *Bacillus* species, QS proteins bind directly to the corresponding signaling peptides, which consists of some neutral protease regulator, aspartyl phosphate phosphatases, and phospholipase C regulator. These QS systems control several microbial processes, like sporulation, virulence, biofilm formation, conjugation, and production of extracellular enzymes (Sharma *et al*., 2020).

**Mechanisms of Quorum Sensing in Fungi**

There is numerous amount of compounds that form an integrated system of QS in fungi. Since study of QS is still in its infancy, other compounds could still be discovered later. Before then, peptides (pheromones), alcohol (tryptophol, farnesol, 1-phenylethanol, tyrosol), acetaldehydes, lipids (oxylipins) and other volatile compounds have been reported to be part of molecules responsible for QS in fungi. These molecules mediate and regulate functions such as filamentation, pathogenesis, and biofilm formation among many others (Hirota *et al*., 2017; Padder *et al.*, 2018). Roles of QS in formation of biofilms have been reported in various fungal species including but not limited to genus of *Saccharomyces, Aspergillus* and *Candida* (Hornby *et al*., 2001). Generally, fungi species such as *C. solani, C. zeylanoides, C. krusei, C. stellata, C. tenuis, C. intermedia, C. utilis, C. albicans, Histoplasma capsulatum, Aspergillus niger, Aspergillus fumigates, Ustilagomaydis,* and *Ceratocystisulmi* among many others have been reported one or more of the aforementioned molecules used in fungi QS (Albuquerque and Casadevall, 2012; Padder *et al.*, 2018).

In parasitic *H. capsulatum*, α-(1, 3)-glucan control the transition from a filamentous to yeast form vice versa. *H. capsulatum* in the soil exist as free-living filamentous fungi with a saprophytic mode of nutrition. However, once it gets into the system of animals through inhalation, they switch from a filamentous cell to a yeast by forming a cell wall of α-1,3-glucan (polysaccharide) required for pathogenecity in a density dependent manner. The biosynthesis of α-1, 3-glucan has been known to be peculiar factor for *H. capsulatum* virulence. Aside playing roles virulence, α-1, 3-glucan also protects cells of *H. capsulatum* from macrophages and within the phagolysosomes as well as latency establishment intracellularly (Romani, 2011; Padder *et al.*, 2018).

Two QS signalling molecules (tyrosol and farnesol) have been reported in *C. albicans*. The farnesol molecules are sequiterpene alcohols made up of 12-carbon and three isoprene units (3,7,11-trimethyl-2,6,10-dodecatriene-1-ol). This farnesol is produced as an intermediate compound during biosynthesis of sterol (Hornby *et al*., 2001), and acts in preventing differentiation from yeast to hyphae (Hornby *et al.,* 2001), but enhances the switch from hypha to yeast form (Lindsay *et al.,* 2012). On the flip side, the lag time of cells of *C. albicans* is shorten by tyrosol accelerating the development of hyphae from germ-tube formed. Tyrosol is regarded as a minor QS molecules of morphogenesis in *C. albicans*, this is because, their effects only come into existence when molecules of farnesol are limited or absent from the environment (Padder *et al.*, 2018). QS molecules of *C. albicans* have also been reported to play active role in the regulation of structures of biofilms and their dispersal making these molecules essential in pathogenesis (Barriuso *et al*., 2018).

Fungal pheromones play important role in fungal reproduction since they serve as informative molecules in karyogamy and plasmogamy by identifying sexual partners that are compatible. Volatile compounds of fungi also affect their growth. For example, colonies of *S. cerevisiae* produce volatile ammonia, which are turbid on agar. In *Trichoderma* sp., volatile compounds have been demonstrated to induce the formation of conidia (Padder *et al.*, 2018).

**Role of Quorum Sensing in Microbial Biofilms Formation**

Biofilm formation is made up of three stepwise stages, which is initiated by surface adhesion, followed by bacterial replication and production of exopolysaccharides matrix and finally, disassembly/dispersion of cells. In finding out the role that QS play in biofilm development, the first approach is to know the step at which bacterial density reaches the threshold level that allows QS signal molecules to participate in biofilm regulation. During biofilm formation steps, quorum sensing has been observed to play an important role in biofilm development during cell replication as well as dispersion of cells. Quorum sensing is not involved in the initial attachment of cell to surface because it involves bacteria that are swimming freely in the medium and accumulation of quorum sensing signals is not involved. When the cells are well attached, they divide and form micro-colonies, which enable the population density to increase with an increasing level of quorum sensing signals that attain sufficient quantity to activate the maturation and disassembly of the biofilm in a coordinate manner (Solano *et al.*, 2014)

QS-regulated activities initiate biofilm formation from an inducing concentration of QS molecules, which may result from starvation and other types of stress associated with high cell density of microbial populations. In response to such type of stress and as a way of protecting themselves, bacteria may form biofilms, to ensure resistance to hash environmental conditions. QS may also function as a control to the size of microbial population in a biofilm, promoting dispersion of subpopulation of cells in other to escape the nutritional stress that may accompany the inducing concentrations of QS molecules. In some non-motile Gram-positive bacteria, autolysis is initiated in response to reaching a quorum (Irie and Parsek, 2008).

In cells of a particular biofilm, QS may induce behaviors during transition from a state not induced by QS to a QS induced state, which disrupt processess of biofilm development such as secretion of adhesins and EPS. Another way QS affects biofilm development is through repressing or inducing surface motility of microcommunities, which in turn affects the architecture of biofilm (Irie and Parsek, 2008). Importance of QS in biofilm dispersal cannot be overemphasized as it creates avenue for colonization of new surfaces and begin a new biofilm when nutrients are depleted and toxic waste products are accumulated in the old one. QS ensures biofilm dispersal through down regulating EPS synthesis and up regulating the synthesis of degradative enzymes that can disrupt the EPS and other bonds binding the components of biofilm together.

In *C. albicans*, QS holds a vital role in the complete biofilm cycle since secretion of tyrosol induces the formation of hyphal at the early stages of development. At the later stages, QS down-regulate the production of tyrosol while promoting the secretion of farnesol, which induces transition of hyphal cells back to yeast, thereby promoting biofilm dispersals (Alem *et al*., 2006; Padder *et al.*, 2018). This implies that, syntheses of farnesol and tyrosol are differential all through the developmental stages of biofilm in fungi and each cell respond adequately to the signal molecule accumulated at any particular stage of biofilm development (Barriuso *et al*., 2018). At high cell density during biofilms formation and growth, farnesol (an acyclic sesquiterpene alcohol in the pathogenic fungi *C. albicans*), is released into the environment, which blocks the transition from yeast to filamentous fungi, but cannot inhibit the elongation of already existing hyphae. It also inhibits germ tube formation and triggers the dissemination of yeast phase cells to inhabit new environments (Alem *et al.*, 2006). Farnesol also inhibit the formation of *C. parapsilosis* biofilms, but because this fungus does not form true hyphae, the mechanisms of action may be distinct from the inhibition produced on *C. albicans* biofilms. It alters the expression of oxidoreductases and genes involved in sterol metabolism (Albuquerque and Casadevall, 2012). In *H. capsulatum*, a pathogenic thermodimorphic fungus, α-(1, 3) –glucan quorum sensing molecules synthesized in the cell wall occur in response to cell density is responsible for pathogenecity and its absence in the yeast form result to a loss of virulence by the fungus. It is also responsible for changing from filamentous to yeast form, intracellular latency establishment, protection of yeast within phagolysosomes, and the control of proliferation of yeast in host macrophages (Albuquerque and Casadevall, 2012; Padder *et al.*, 2018).

In *P. aeruginosa* biofilm formation, QS is plays an important role in synthesis of AHL signal molecule and mediate the formation of biofilms. In some other bacteria, QS may function in the dispersal of individual organisms from the biofilm. Albuquerque and Casadevall (2012), reported that AHL signaling molecules produced by *P. aeruginosa* does not inhibit yeast form of *C. albicans.* However, in *C. albicans* hyphal form there is usually the inhibition of their cell growth due to the AHL molecules produced by *P. aeruginosa.* As such, the moment *C. albicans* notice the presence of *P. aeruginosa* through QS molecules there is usually a morphological switch to the yeast form as a survival strategy.

Quorum sensing has played a great role in inter-kingdom interactions between bacteria and fungi. For example, P. aeruginosa can inhibit biofilm formation of *Aspergillus furmigatus* through secretion of a heat-stable factor in a concentration dependent fashion (Barriuso *et al*., 2018). QS molecules of diffusible lipopeptide known as ralsolamycin, is produced by *Ralstonia solanacearum*, a soil-borne pathogenic bacteria of plant. These diffusible molecules are produced by an integrated QS system of PhcBSR in a concentration dependent manner, which enhances the hyphae of fungi to invade host tissues thereby providing a favorable niche for bacterial colonization. However, these molecules produced by *R. solanacearum* impairs fungal survival by inhibiting secretion of protective agents that helps the fungal cells against oxidative stress (Li *et al.,* 2017; Khalid *et al.,* 2018). Likewise, *Bacillus licheniformis* ComX pheromone, which inhibits *A. flavus* growth and metabolisms (Barriuso *et al*., 2018).

**Role of Quorum Sensing in Multispecies Biofilm Communities**

Biofilms found in many environments such as industrial, clinical as well as natural habitats are usually mixed microbial species of very high cell density, which result to high QS signal concentrations found in these communities. High concentrations of signal molecules produced maybe very vital for every species present in the environment. It may trigger their response to either competing for survival or in a way that is beneficial to themselves as a result of the sensing molecules they produce. However, some microbes do not produce but respond to signal molecules produced by other species as a mechanism to attain survival in the environment. Some of the interactions studied in multispecies biofilm communities may be synergistic or antagonistic interactions.

**Antagonistic Interactions**

Competition usually occurs among microorganisms that occupy the same niche either for nutrients or other growth requirements. In a mixed species biofilm, there is high number of competing species that are fixed spatially at a close range to one another. *P. aeruginosa* is able to establish dominance over *Agrobacterium tumefacien* in any biofilm environment due to the leverage offered by it's QS molecules. When *Staphylococcus aureus* is exposed to farnesol QS molecule, produced by yeast species, they become more susceptible to antibiotics and eventually result to decreased biofilm formation. In addition to promoting biofilm dispersion, QS may sometimes mediate means by which established bacterial biofilms are prevented from predation by eukaryotes, protozoans as well as predatory bacterial species. *P. aeruginosa* is capable of degrading farnesol required by *C. albicans* to transition from yeast to parasitic filamentous form and mature biofilm formation., whereas, in the presence of AHL signal molecules produced by *P. aeruginosa, C. albicans* remain as a yeast, a form more resistant to killing by *P. aeruginosa*, and thereby enhance their survival in the environment (Irie and Parsek, 2008).

**Synergistic Interactions**

This form of interactions occurs in mixed species biofilm where a QS signal molecules produced by one species of microbes may stimulate the expression of a particular gene in another species present in the same biofilm. Interspecies QS communication has been reported among AHL-producing organisms. For example, in a dual species biofilm of *P. aeruginosa*– *Burkholderia cenocepacia,* AHLs produced by *P. aeruginosa* may promote the expression of AHL-regulated virulence gene by *B. cenocepacia.* This happens because, *B. cenocepacia*is able to sense *P. aeruginosa* AHLsignal, but *P. aeruginosa* could not perceive *B. Cenocepacia* AHL. There are other multispecies biofilm systems that have triggered some gene expressions only when mixed species are present, such as those formed by bacteria isolated from marine algae (Burmølle *et al.*, 2006; Irie and Parsek, 2008).

**CONCLUSION**

The cell density-dependent phenomenon of quorum sensing and discoveries of molecules involved in biofilm formation is an emphatic breakthrough in understanding how microbes interact effectively with each other and their environment. QS analysis on how microbes communicate in a density dependent manner between inter and intra species. The diverse study of quorum sensing and the discovery of different QSMs revealed the importance and role of QS in microorganisms as well as biofilm formation and their ability to successfully inhabit different habitats.

**REFERENCES**

Albuquerque P, Casadevall A (2012). Quorum sensing in fungi–a review. Medic. Mycolog. 50:337-345. https://doi.org/10.3109/13693786.2011.652201

Alem MA, Oteef MD, Flowers TH, Douglas LJ (2006). Production of tyrosol by Candida albicans biofilms and its role in quorum sensing and biofilm development. Eukar. Cell 5:1770-1779. https://doi.org/10.1128/ec.00219-06

Ansari FA, Jafri H, Ahmad I, Abulreesh HH (2017). Factors affecting biofilm formation in in vitro and in the rhizosphere. Biof. Plan. Soil Healt. 275-290. https://doi.org/10.1002/9781119246329.ch15

Bandara HMHN, Lam OLT, Jin LJ, Samaranayake L (2012). Microbial chemical signaling: a current perspective. Crit. Rev. Microbiol. 38:217-249. <https://doi.org/10.3109/1040841x.2011.652065>

Berlanga M, Guerrero R (2016). Living together in biofilms: the microbial cell factory and its biotechnological implications. Microb Cel. Fact*. 15:*165. <https://doi.org/10.1186/s12934-016-0569-5>

Barriuso J, Hogan DA, Keshavarz T, Martínez MJ (2018). Role of quorum sensing and chemical communication in fungal biotechnology and pathogenesis. FEMS Microbiol. Rev. 42:627-638. https://doi.org/10.1093/femsre/fuy022

Burmølle M, Webb JS, Rao D, Hansen LH, Sørensen SJ, Kjelleberg S (2006) Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. *Appl. Environ. Microbiol.* 72:3916–3923. https://doi.org/10.1128/aem.03022-05

Carlier A, Pessi G, Eberl L (2015). Microbial biofilms and quorum sensing. In Principles of plant-microbe interactions. In: B Lugtenberg: editor: Principles of Plant-Microbe Interactions. New York City: Springer Cham: 45-52. https://doi.org/10.1007/978-3-319-08575-3\_7

Carradori S, Di Giacomo N, Lobefalo M, Luisi G, Campestre C, Sisto F (2020): Biofilm and quorum sensing inhibitors: the road so far.Exp. Opin. Therap. Patent. 30:917-930. https://doi.org/10.1080/13543776.2020.1830059

Choudhary P, Singh S, Agarwal V (2020). Microbial Biofilms. In Bacterial Biofilms. IntechOpen. https://doi.org/10.5772/intechopen.90790

Donlan RM (2002). Biofilms: microbial life on surfaces. Emerg. Infec. Dis. 8:881. https://doi.org/10.3201/eid0809.020063

Drescher K , Nadell CD, Stone HA, Wingreen NS, Bassler BL (2014). Solutions to the public goods dilemma in bacterial biofilms. Curr. Biol. 24:50-55. https://doi.org/10.1016/j.cub.2013.10.030

Flemming HC, Wingender J (2010). The biofilm matrix. Nat. Rev. Microbiol*.* 8:623-633. https://doi.org/10.1038/nrmicro2415

Hirota K, Yumoto H, Sapaar B, Matsuo T, Ichikawa T, Miyake Y (2017). Pathogenic factors in Candida biofilm related infectious diseases. J. Appl. Microbiol*.* 122:321-330. https://doi.org/10.1111/jam.13330

Hmelo LR (2017). Quorum sensing in marine microbial environments. Ann. Rev. Marin. Sci*.* 9:257-281. https://doi.org/10.1146/annurev-marine-010816-060656,

Hong SH, Lee J, Wood TK (2010). Engineering global regulator Hha of *Escherichia coli* to control biofilm dispersal. Microb. Biotech. 3:717-728. https://doi.org/10.1111/j.1751-7915.2010.00220.x

Hornby JM, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, Nickerson KW (2001). Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Appl. Environ. Microbiol*.* 67:2982-2992. https://doi.org/10.1128/aem.67.7.2982-2992.2001

Irie Y, Parsek MR (2008). Quorum sensing and microbial biofilms. Bacter. Biof. 67-84. https://doi.org/10.1007/978-3-540-75418-3\_4

Khalid S, Baccile JA, Spraker JE, Tannous J, Imran M, Schroeder FC, Keller NP (2018). NRPS-derived isoquinolines and lipopetides mediate antagonism between plant pathogenic fungi and bacteria. ACS Chem. Biolog.13:171-179. https://doi.org/10.1021/acschembio.7b00731

Lazar V (2011). Quorum sensing in biofilms–how to destroy the bacterial citadels or their cohesion/power?. *Anaerob.* 17:280-285. https://doi.org/10.1016/j.anaerobe.2011.03.023

Lewenza S, Visser MB, Sokol PA (2002). Interspecies communication between *Burkholderia cepacia* and *Pseudomonas aeruginosa*. Canad. J. Microbiol. 48:707-716. https://doi.org/10.1139/w02-068

Li P, Yin W, Yan J, Chen Y, Fu S, Song S, Zhang LH (2017). Modulation of inter-kingdom communication by PhcBSR quorum sensing system in *Ralstonia solanacearum* phylotype I strain GMI1000. Front. Microbiol. 8:1172. https://doi.org/10.3389/fmicb.2017.01172

Lindsay AK, Deveau A, Piispanen AE, Hogan DA (2012). Farnesol and cyclic AMP signaling effects on the hypha-to-yeast transition in *Candida albicans*. *Eukary. Cel.* 11:1219-1225. https://doi.org/10.1128/ec.00144-12

McDougald D, Rice SA, Barraud N, Steinberg PD, Kjelleberg S (2012). Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. *Natur. Rev. Microbiol.* 10:39-50. https://doi.org/10.1038/nrmicro2695

Padder SA, Prasad R, Shah AH (2018). Quorum sensing: A less known mode of communication among fungi. Microbiol. Res. 210:51-58. https://doi.org/10.1016/j.micres.2018.03.007

Parsek MR, Greenberg EP (2005). Sociomicrobiology: the connections between quorum sensing and biofilms. Trend. Microbiol. 13:27-33. https://doi.org/10.1016/j.tim.2004.11.007

Petrova OE, Sauer K (2012). Sticky situations: key components that control bacterial surface attachment. J. Bacteriol. 194:2413-2425. <https://doi.org/10.1128/jb.00003-12>

Prakash B, Veeregowda BM, Krishnappa G (2003). Biofilms: a survival strategy of bacteria. Curr. Sci. 1299-1307. https://doi.org/10.1177/0020294019866854

Ramage G, Saville SP, Wickes BL, López-Ribot JL (2002). Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. Appl. Environ. Microbiol. 68:5459-5463. https://doi.org/10.1128/aem.68.11.5459-5463.2002

Romani L (2011). Immunity to fungal infections. Nat. Rev. Immunol.11.275-288. https://doi.org/10.1038/nri2939

Santos ALSD, Galdino ACM, Mello TPD, Ramos LDS, Branquinha MH, Bolognese AM, Roudbary M (2018). What are the advantages of living in a community? A microbial biofilm perspective! Memór. Instit. Oswal. Cru. 113(9). https://doi.org/10.1590/0074-02760180212

Sharma A, Singh PBK, Nandi SP (2020). Quorum sensing its role in microbial social networking. Res. Microbiol. https://doi.org/10.1016/j.resmic.2020.06.003

Shirtliff ME, Krom BP, Meijering RA, Peters BM, Zhu J, Scheper MA, Jabra-Rizk MA (2009). Farnesol-induced apoptosis in *Candida albicans*. Antimicro. Agent. Chemother., 53:2392-2401. https://doi.org/10.1128/aac.01551-08

Singh R, Ray P (2014). Quorum sensing-mediated regulation of staphylococcal virulence and antibiotic resistance. *Fut. Microbiol.* 9:669-681. https://doi.org/10.2217/fmb.14.31

Singh MP, Singh P, Li HB, Song QQ, Singh RK (2020). Microbial biofilms: development, structure, and their social assemblage for beneficial applications. In: New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Biofilms. Amsterdam: Elsevier: 125-138. https://doi.org/10.1016/b978-0-444-64279-0.00010-4

Solano C, Echeverz M, Lasa I (2014). Biofilm dispersion and quorum sensing. Curr. Opin. Microbiol. 18:96-104. https://doi.org/10.1016/j.mib.2014.02.008

Tarkka MT, Sarniguet A, Frey-Klett P (2009). Inter-kingdom encounters: recent advances in molecular bacterium–fungus interactions. Curr. Gene.55:233-243. https://doi.org/10.1007/s00294-009-0241-2

Toyofuku M, Inaba T, Kiyokawa T, Obana N, Yawata Y, Nomura N (2016). Environmental factors that shape biofilm formation. Biosci. Biotechnol. Biochem. 80:7-12. https://doi.org/10.1080/09168451.2015.1058701

Williams P (2007). Quorum sensing, communication and cross-kingdom signalling in the bacterial world. Microbiol. 153:3923-3938. https://doi.org/10.1099/mic.0.2007/012856-0

Wongsuk T, Pumeesat P, Luplertlop N (2016). Fungal quorum sensing molecules: role in fungal morphogenesis and pathogenicity. J. Bas. Microbiol.56:440-447. https://doi.org/10.1002/jobm.201500759

Zhang J, Feng, T, Wang J, Wang Y, Zhang XH (2019). The mechanisms and applications of quorum sensing (QS) and quorum quenching (QQ). J. Ocean Uni. Chi. 18:1427-1442. https://doi.org/10.1007/s11802-019-4073-5