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Microbial quorum sensing and its role in biofilm formation

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Abstract

Expression of genes within the microbial cells are regulated by the change in cellular density of microbes in their ecosystems, this mechanism is termed as microbial Quorum sensing. Almost every prokaryote synthesizes small molecules called auto-inducers, responsible for their Quorum sensing. Auto-inducers formation in Gram-negative bacteria is provoked by a chemical know as S-adenosylmethionine (SAM). Various physiological mechanisms of microbial cell systems are provoked by auto-inducer and microbial cell receptors interaction, a kind of Quorum sensing. Quorum sensing mechanism of fungi was discovered eleven years ago when farnesol panel filamentation was detected in the pathogenic metamorphic yeast called *Candida albicans*. In the previous era, it was discovered that farnesol plays a key role in the regulation of the physiology of *C. albicans* acts like a signaling chemical, and encourages destructive effects on host cells along with other microorganisms. Along with farnesol, another compound called aromatic tyrosol alcohol was also pledged to be a *C. albican* Quorum Sensing regulatory factor for growth, biofilm synthesis, and development of morphology. In *Saccharomyces cerevisiae*, phenyl ethanol and tryptophol are the two main aromatic alcohols that control QSMs regulation for morphogenesis in nitrogen deficiency circumstances. Moreover, cell density-dependent recitals that appear like Quorum Sensing have been labeled in various mycocal species. However, the study of the Quorum Sensing mechanism of fungi is yet in its commencement, its recognition has altered our sentiments about mycocal kingdom and might eventually cause the development of new fungicidal therapeutics.

Keywords: Quorum Sensing, Farnesol, Aromatic Alcohol, Pathogenic Fungi, Tyrosol, Biofilm, Prokaryotes

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1. Introduction to Quorum Sensing

Expression of genes within the microbial cells are regulated by the change in cellular density of microbes in their ecosystems, this mechanism is termed as microbial Quorum sensing [1]. Quorum Sensing is a way of communication between bacterial cells present within a biofilm through chemicals produced by those bacteria. These signaling chemicals are known as auto-inducers, and their production is directly linked with cell density in biofilm [2]. These auto-inducers act as stimulant to change gene expression with in cells of biofilm. Quorum sensing communication pathway is responsible for the regulation of various physiological activities in both Gram-positive and Gram –negative Bacteria [3]. Quorum sensing supports motility, virulence, conjugation, sporulation, competence, development of biofilm and synthesis of antibiotic of prokaryotes [4]. Lactones and acylated homoserine are the autoinducers used by Gram-negative bacteria, whereas processed oligo-peptides are autoinducers used by Gram-positive bacteria [4]. These autoinducers are synthesized by common metabolites like fatty acids; S-adenosylmethionine, and anthranilate with one signal synthetase are a battery of enzymes [5]. Quorum sensing is a means of communication within as well as between bacterial species [6]. These auto-inducers of bacteria also provoke specific responses within the host. In Quorum sensing different bacteria use different chemical molecules, and different signal mechanisms so that genes targeted by Quorum sensing also differ [7]. This changes the overall behavior of the whole community. Some scientists believe that the Quorum sensing system is the early step in the evolution from unicellular to multicellular [1].

2. Quorum sensing in bacteria and its role in biofilm formation

Almost every prokaryote synthesizes small molecules called auto-inducers, responsible for their Quorum sensing. Auto-inducers formation in Gram-negative bacteria is provoked by a chemical know as S-adenosylmethionine (SAM). Various physiological mechanisms of microbial cell systems are provoked by auto-inducer and microbial cell receptors interaction, a kind of Quorum sensing [8]. Membrane-bound histidine sensor kinase or cytoplasmic transcription factors act as receptors for auto-inducers responsible for Quo rum sensing [9]. Membrane-bound histidine sensor kinase or cytoplasmic transcription factors act as receptors for auto-inducers responsible for Quorum sensing [10]. For instance, various bacterial species that belong to the human GIT tract normal flora are capable of producing auto-inducers. They can also respond to the auto-inducers produced by other bacteria. There is enhancing proof that Quorum sensing regulates various key physiological processes in the human digestive tract and it has a strong effect on the virulence mechanism of foreign invader microbes [11]. For a long time, it was a strong belief that prokaryotes are unicellular organisms and each of them exist independently. They do not show any coordination among them. There are no multicellular behavioral activities in

prokaryotes [12]. Now microbiologists know that an unexpectedly high degree of interactive multi-cellular behavior of bacteria is responsible for Biofilm (cities of microbes) formation. Various bacteria regulate various group activities and physiological processes by producing, detecting, and responding to small molecules through a mechanism called Quorum sensing [13]. Various bacteria need a certain level of cell density within host body to express their virulence and to overcome the host immune system before starting an infectious disease [14]. This cell-cell communication (Quorum sensing) between bacterial cells plays a key role in bacterial social activities, imitation of infectious diseases, and Biofilm formation [15]. Intracellular communication within the bacterial community is regulated by production, recognition, and response to auto-inducers. Firstly, the process of Quorum Sensing was discovered within in aquatic bioluminescent bacteria *Vibrio fischeri* [8]. *V. Fischeri* develops a symbiotic relationship with various marine animals. In these relationships, *V. fischeri* provides light to these host organisms that protect them from predators, helps to attract prey, and matting [16]. In return, *V. fischeri* obtains nutrition from its host. A luciferase enzyme complex present in *V. fischeri* produces light. This bioluminescence is produced only when *V. fischeri* attains a critical level of cell density regulated by Quorum sensing [17]. Particularly the synthesis and accumulation of, and the response to a certain concentration of auto-inducers controls density-dependent light generation in *V. fischeri*, and consequence, the bacterium becomes capable of bioluminescence light emission [18]. For many centuries, sailors have observed mystery nocturnal display, where an intensive, uniform, and constant glow, called 'milk sea' emits from the sea surface. Miller and his colleagues discovered this bioluminescence emitted by *V. fischeri* in the Indian Ocean. The 'milk sea' is an outstanding display of Quorum Sensing mediated bioluminescence [19]. Quorum sensing controls various social activities and physiological processes like biofilm formation, spore synthesis, production of fruiting bodies, symbiotic relationships, gene competency, pathogenesis and programmed cell death [13]. The different signaling molecules in controlling bacterial QS are tabulated in Table 1.

Table 1. Bacterial role in biofilm formation.

Micro organisms	Main signaling molecules (Autoinducers)	QS Controlling System	Functions to be regulated	Article reference
B. subtilis	ComX, CSF, PhrA,-E,-F,-K,-H (PhrC),	ComP/ComA Rap Proteins		[20]

			Biofilm syntheis,	
Pseudomonas aeruginosa	3O-C12-HSL C4-HSL	LasI/LasR Rh1I/RhiR OscR (Orphan)	biofilm development	[21]
Staphylococcus aureus	AIP-I/AIP-II AIP-II/AIPIV	AgrC/AgeA	Biofilm production	[22]
Streptococcus mutans	CSP (ComC) XIP (ComS)	ComD/ComE ComR	Development of biofilm	[23]
Streptococcus pneamoniae	CSPs	ComD/ComE	Biofilm development and maturation,	[24]

3. Biofilms:

Biofilms are the cohesive syntrophic consortium of bacteria produced as a result of the combination of extracellular matrix of EPS (exopolysaccharide) and proteins. It also can adherence to biotic and abiotic surfaces [25]. Bacteria start the biofilm formation due to the environmental stimulants, such as nutrient and oxygen availability [26]. The intracellular adhesion *ica* operon, present in the genus of Staphylococci encodes the extracellular polymeric matrix of biofilm, referred to as polysaccharide intracellular adhesion (PIA) [27]. Biofilm protects the microorganisms from host defenses and resists the antibiotics thus impeding wound healing which may cause chronicity of wounds [28]. In recent times, the development of antimicrobial agents at a narrow or lean scale has worsened the situation and increased the necessity of research for the discovery of alternative treatments to substitute or replace antibiotics [29].

4. Stages in biofilm formation and its development:

There are four major stages in the development of biofilm e.g., cellular attachment, development of microcolonies, maturation of biofilm, and dispersion of biofilms [30] as shown in Fig.1.

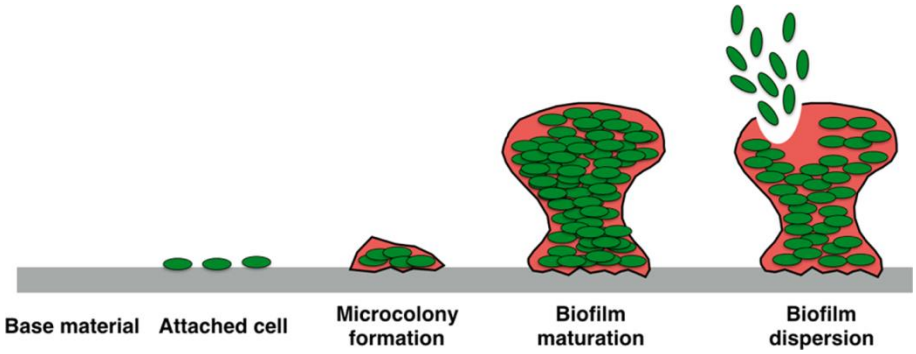

Fig.1. Stages of biofilm formation [31].

4.1 Cellular attachment:

Biofilm formation initiates with the attachment of prokaryotic cells to a surface (Fig 2). For attachment to a specific surface, bacteria have to overcome the repulsive forces generated between the negatively charged bacterial membrane and the environmental surface [32]. Hydrophilic surfaces like metal and glass have more repulsive forces as compared to hydrophobic environmental surfaces e.g. plastic. The strength of repulsive forces is inversely proportional to the strength of attachment between bacterial cells and environmental surfaces[33]. Pilli and flagella present on prokaryotic surfaces play a vital role in their attachment to the environmental surface. Attachment of bacteria to the environmental surface is reversible and bacteria can revert themselves to their planktonic lifestyle by leaving the environmental surface [34]. When prokaryotic cells adhere themselves to environmental surfaces by using their surface proteins, the attachment becomes irreversible and biofilm can bear robust chemical and physical share forces of the environment [35].

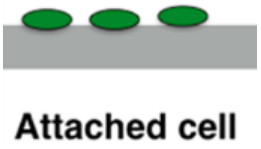

Fig 2. Step 1 Attachment of cells to substrate.

4.2 Microcolonies formation:

After this irreversible attachment of prokaryotes to the environmental surface, bacterial cells multiply and secret (EPS) extracellular proteins used to make shelter (matrix of biofilm), where living microbes attach with biofilm [36]. These EPS along with adhesion-like proteins e.g. RbmA matrix protein of biofilm formed by vibrio cholera are responsible for bacterial adhesion to biotic or abiotic environmental surfaces [37]. Cellular cohesion proteins and EPS help to bring living cells closer for the development of

microcolonies (Fig 3). Multiple types of micro-communities, coordinating with each other, are present in a biofilm. This coordination is very significant for the excretion of waste material, exchange of substrate, and the transportation of essential metabolites [38].

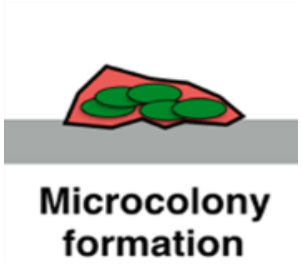

Fig 3. Step 2 Microcolony formation.

4.3 Biofilm maturation:

Microbial multiplication with constant production of extracellular proteins/exopolysaccharides leads to the development of initial biofilm, which is converted into a 3-D structure after maturation [39]. The maturation of biofilm is dependent on cell-to-cell signaling (quorum sensing) between embedded microbes of biofilm through chemical signaling molecules called auto-inducers [40]. These microbial cells receive signals to express genes of EPS. After the formation of the 3-D structure of biofilm, water channels, for the distribution of nutrients, are developed across the biofilm. A biofilm 3-D biofilm with water channels in it is called a mature biofilm [41] (Fig 4).

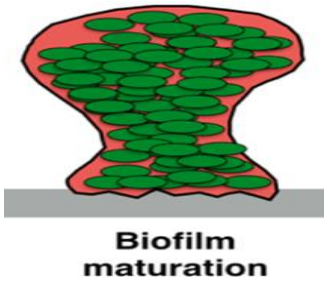

Fig 4. Step 3 Biofilm formation.

4.4 Detachment of biofilm:

The next stage after maturation in the biofilm formation process is termed dispersion (Fig 5). In this phase of biofilm development, some microbial cells detach from the biofilm surface and by swimming through water reach another place where they attach to a new environmental surface, and the process of new biofilm formation starts [42]. Dispersion of microbial cells from biofilm may be active or passive. External mechanical forces like fluid share, abrasion, or solid share are responsible for the passive dispersion of biofilm [43]. Active dispersion of biofilm depends upon the upregulation and downregulation

of microbial genes. Environmental stimuli like oxygen supply, temperature, and nutrient availability play a vital role in the active dispersion of biofilm [44]. For instance, shortage of nutrient and oxygen supply along with fluctuating temperature cause upregulation of genes responsible for flagella formation [45]. Hence, the high density of flagellar cells causes their active dispersal from the biofilm. Dispersion B enzyme production is also increased under such situations. This enzyme causes hydrolysis of polysaccharides, causing degradation of EPS. A high quantity of design B enzyme causes instability of biofilm and removal of microbial cells from it [44].

Fig 5. Step 4 Biofilm formation.

5. Factors contributing to biofilm formation:

Biofilm formation initially depends upon the structure of organisms contributing to its development. For instance, pili on the surface of prokaryotes promote their attachment and colonization with environmental surfaces[46]. Flagella on bacterial surfaces help them in motility and spreading all over the surface of the environment where biofilm is supposed to be developed. Moreover, environmental factors like oxygen content, nutrient concentration, and temperature also have a key role in biofilm formation [47].

5.1 Structural factors:

5.1.1 Exopolysaccharides (EPS):

Microbial extracellular matrix containing various polymeric substances secreted by different microorganisms plays a key role in biofilm formation. Exopolysaccharides or exopolymers (EPS) are high molecular weight substances secreted by microalgae, cyanobacteria, fungi, protists, and other prokaryotes [48]. Humic acid, polysaccharides, rare sugars, extracellular DNA, proteins, lipids, and carbohydrates are the major components of EPS. EPS production is an energy-dependent system that requires special environmental conditions [49]. For example, environmental treatments like drought can trigger the biosynthesis of EPS. EPS synthesis fluctuates by the change in water content, cellular density,

and mechanical stability of microbial cells present in biofilm [50]. Hydration of EPS is done to develop a matrix structure that holds living cells together and maintains a supply chain of nutrients within the biofilm. Due to its impermeable nature, EPS provides protection against environmental harshness and antimicrobials effect [51].

5.1.2 Pili and flagella:

Small hair-like appendages on prokaryotic surfaces termed pili, are helpful in biofilm formation. Research studies revealed that the absence of pili on the surface of bacteria causes prominent defects in the structure of biofilm synthesized by *Enterococcus faecalis* mutants [52]. Another study conducted on wild-type and mutant strains of Clostridium difficile and Streptococcus spp revealed that the absence of pili on their structure causes a reduction in the quantity of thicker masses of their biofilm [53]. Pili on the surface of *Acinetobacter baumanni* is responsible for attachment to the surface of the biotic/abiotic environment. It is stated that biofilm formation is impossible without pili because they provide twitching motility, helpful for bacteria to spread all over the surface of biofilm [54]. This mechanism has been observed in biofilm developed by *Psedudomonas aerogenosa, & A.baumanii*. Flagella is major locomotory organ of bacteria that help them in locomotion in biotic/abiotic environment for the development of biofilm [55]. The absence of flagella on the structure of Campylobacter jeijuni (non-flagellated bacteria) causes a defective biofilm formation. Flagella mediates cell-to-cell binding for initiation of biofilm formation in Pseudomonas aeruginosa in aqueous environment [56]. Locomotion by flagella plays a crucial role in prokaryotic adhesion with surfaces and the maturation of biofilm. Biofilm maturation is dependent on a process known as motility-to-sessility transition, maintained by c-di-GMP signaling molecules present in bacterial cytoplasm [57]. The concentration of c-di-GMP is in inverse relation to the bacterial flagellar activity (locomotion), and controls biofilm maturation directly. C-di-GMP signaling molecule is present in different prokaryotic species like *E.coli*, Pseudomonas aerogenos, and is helpful in biofilm maturation [58].

5.2 Environmental factors:

5.2.1 Nutritional availability:

Shortage of nutrients in the environment puts stress on the microbes and stimulates them to develop biofilm. Constant shortage in nutrient supply causes a hurdle in biofilm maturation [59]. Fluid channels of biofilm are responsible for material transportation within and out of biofilm, secrete toxins out of biofilm, and provide nutrition for prokaryotes present in it [60]. In highly nutritive environments bacteria are either unable to develop a biofilm or they form a loose biofilm, easily broken by shared forces of the

environment [61]. Nutrient depletion in the environment triggers matrix (EPS) production in Bacillus subtilis. EPS production initiates biofilm formation [62].

5.2.2 Oxygen supply:

A shortage of nutrients in the environment puts stress on the microbes and stimulates them to develop a biofilm. Constant shortage in nutrient supply causes a hurdle in biofilm maturation [63]. Fluid channels of biofilm are responsible for material transportation within and out of biofilm, secrete toxins out of biofilm, and provide nutrition for prokaryotes present in it [64]. In highly nutritive environments bacteria are either unable to develop a biofilm or they form a loose biofilm, easily broken by shared forces of environment [61]. Nutrient depletion in the environment triggers matrix (EPS) production in Bacillus subtilis. EPS production initiates biofilm formation [62]. Hence, continuous production of EPS strengthens the biofilm. Moreover, extremely low levels of oxygen may cause biofilm dispersal. Studies revealed that a shortage of oxygen triggers EPS and PIA polymer production from S.aureus and P.aerogenosa, and EPS production initiates biofilm development [65].

5.2.3 Temperature:

Fluctuation in environmental temperature effect to triggers biofilm formation varies for different species of prokaryotes. For example, 30ºC salmonella species are triggered to transit from the free-floating stage to biofilm [66]. Whereas *Listeria monocytogens* are triggered to biofilm formation at very low temperatures e.g. 4º to 12ºC [67]. *Vibreo cholera, P. aerogenosa, and K. pneumoniae* can develop biofilm at a temperature range of 30º to 37ºC. While temperature range for *Aeromonas hydrophila* to develop biofilm is between 20º to 25ºC [68]. Research studies showed that temperatures above 25ºC hinder biofilm formation in *Aeromonas hydrophila. Clostridium perforingens attaches to the surfaces for biofilm formation at 37ºC[69].*

5.2.4 pH:

Changes in environmental pH level can change the ability of microbes to form biofilm. A research study revealed that at 25C neutral pH is more suitable for biofilm formation for *E. coli* MG1655[70]. At 37℃ *E. coli* needs acidic pH (conditions similar to host gut) to form biofilm formation. Streptococcus agalactiae also needs acid pH at 37ºC for the development of biofilm [71].

6. Quorum sensing in Fungi and its role in biofilm formation

Notwithstanding the growing quantity of info about bacterial quorum sensing in the last eras, quorum sensing in eukaryotes was unidentified until farnesol was found as a quorum sensing in the *Candida*

albicans that is a pathogenic yeast [72]. After the 11th year of this innovative work was printed, it enthused effort that has led to nearly 92 PubMed articles with the disputes 'farnesol' and ' *Candida* ' and various others connecting the role of this compound in different organisms. In addition to farnesol, the other identified fungal quorum sensing is all liquors extrected from benzene ring amino acids tryptophan known as tryptophol, tyrosine known as tyrosol, and phenylalanine known as phenylethanol. Tyrosol was another quorum sensing designated in *C. albicans* [73] while the other two were firstly revealed as autoantibiotics inhibitory filaments of *C. albicans* in the 1960s[74] , and advanced were found to be *Saccharomyces cerevisiae* QSMs {[75]. Biofilms are surface-related prearranged micro-organismic societies entrenched within an exopolymeric environment [76]. Eradication of biofilm Contagions is very difficult to eliminate due to antimicrobial-resistant agents of these structures and immune factors released by host cells [77]. Prokaryotic biofilm association is extremely reliant on quorum sensing, and the interface between these two mechanisms is carefully essential in prokaryotic pathogenesis [78]. Because of the significance of various *C. albicans* morphology in biofilm configuration Ramage *et al* ., assessed the role of farnesol in the development of biofilm [79]. They observed that farnesol plays a vital role in the regulation of *C. albicans* morphology and the development of biofilm. Moreover, they presented that the speed of reserve was reliant on the time taken by the cells to bind before farnesol was supplemented. When the cells began to filament, the addition of farnesol had no consequence on the expansion of biofilm configuration although cells on mature biofilms replied to the isoprenoid and consequently scattering of biofilm occurred [80]. Microarray analysis of biofilms unprotected to farnesol discovered that genes associated with antimicrobial resistance, cell wall upkeep, cell surface hydrophobicity, Fe transport factors, and heat shock proteins were prejudiced in adding to the genes linked with hyphal growth [81]. Different fungal species like *Apophysomyces elegans, Rhizopus oryzae, Aspergillus fumiga* [82] . *Rhizomucor pusillus, Blastoschizomyces capitatus, and Candida albicans* can develop biofilm. *Candida albicans* uses Tyrosol and farnesol as autoinducers and Ras1-cAMP/protein kinase (PKA) signaling pathway for quorum sensing that helps in biofilm formation [83].

6.1 Role of biofilm in fungal pathogenesis:

Just like prokaryotic biofilms, mycocal biofilms help them to develop high resistance against antifungal treatment, and disinfectants, and become evasive from the host immune system [84]. These colonies help fungi in protection, acquiring new genetic characteristics by microbial genetic recombination, metabolism, and generating economic, clinical, and therapeutic insinuations [85]. *Fusarium spp, Aspergillus spp, Candida spp and Trichosporon spp* are the most popular pathogenic fungi that form biofilms. Biofilms associated with disease are either multi-species or multi-kingdom. Fungal hyphae act

as skeletons in polymicrobial biofilms [86]. Polymicrobial biofilms are responsible for the alteration of virulence and standard therapeutics used against diseases caused by the microbes of these interkingdom communities [87]. Resistance against disinfectants and antimicrobials, and microbes' ability to develop biofilm in both biotic and abiotic environments has aided in mycocal biofilm determination in clinical setup [88]. To minimize nosocomial infections and improve patients' health safety effective decontamination of biofilms is very necessary. Various disinfectants are used to remove clinically developed biofilms but it is very hard to their efficacy against pathogenic microbes of this multi-kingdom community [89]. Persistence is the major issue faced while decontaminating polymicrobial biofilms due to biphasic death patterns. In this death pattern a large population of microbes die during decontamination by antimicrobials while a small portion of the microbial population remains alive [90]. After the end of disinfectant treatment regrow and form a biofilm that is associated with recalcitrant infections. Infections associated with biofilm are seemingly related to the high rate of mortality and morbidity in hospitals [91]. Transcriptional factor APSES (e.g., StuA) plays a vital role in virulence and morphogenesis of trichophyton rubrum. Removal of StuA from this dermatophyte damages its biofilm development[92]. Various research studies shows that interaction between prokaryotes and mycocal population (for instance interaction between staphylococcus aureus and Aspergillus fumigatus, and interaction between Mycobacterium Tuberculosis and P.brasiliensis) enhance the pathogenicity of fungal species [93]. To minimize health risks by infections developed by these polymicrobial films, their proper decontamination by disinfectants like halogens (F,Cl, I etc), alcohols, phenols, and different chemicals extracted by plants are combinations of pharmaceuticals chemicals [94].

7. Viral Quorum Sensing

Not all viruses do quorum sensing except a few viruses like phages. Phage reproduction is dependent on host bacterial cells and thus it is perilous for the bacteriophages to control the plan of replication to cell host cell densities [95]. explain the mechanism that controls the density of host cell reliant on lysis-lysogeny verdict made by Vibrio phage that is reliant on a host QMS. Bacteriophages are either temperate or obligate viruses that are intracellular parasites of bacteria [96]. While infecting bacterial cells bacteriophages undergo either a lytic or lysogenic cycle of viral replication. In the lytic cycle host cell secretes progeny virion upon bursting of the bacterial cell or integrates into the host bacterial genome [97]. Under certain conservational conditions, a prophage of integrated cells separates from the host cell genome and enters into the lytic cycle of bacterial replication. In the lysogenic life cycle bacteriophages also have a vital impact on host metabolism, fluctuation in population, ecological niche, and phage propagation [98]. Previous studies described that lytic phages also have particular mechanisms to detect

the concertation of host cells. The first-ever experimental indication of cell concentration that is controlled by prophage induction associated with QMS was studied by in groundwater and soil microbes and a model system of Escherichia coli (*E. coli*). A bacterial system was used as a model to study the molecular basis of this regulatory mechanism. The induction system that was based on homoserine lactone was SOS independent [99]. Numerous kinds of literature have also revealed that bacteriophage profusion is highly associated with the concentration of host cells in a diversity of environments, and infections by lytic phages are preferred under auspicious supporting circumstances and quick cell growth, while lysogenic phages become more communal under situations less auspicious for growth with less concentration of growing cells [100]. Though, in some cases, the high concentration of host-cell environs, like the ruminants' gut, the lysogenic cycle of viral replication may be preferred subsequent to the Piggyback-The-Winner model This seriously examines the molecular mechanism overdue the phage-host interfaces for better sympathetic of micro-organismic niche and procedures [101]. Prokaryotes can harvest, release, and sense signaling compounds ("autoinducer," AI) for cell-cell interaction to organize a vast range of behavioral activities; a mechanism called QS, which is cell density reliant on recently considered a new QS system that consists on a cytoplasmic receptor and various transcriptional factor [102]. For instance, an AI 3,5-dimethylpyrazin-2-ol (DPO) and VqmA. The authors suggested that phages can use the host QS system for lytic as well as lysogenic verdicts, this concept led to further research to verify this hypothesis. collected VqmA similar to recognizing DPO-binding proteins of viruses due to analyses done by tools of bioinformatics [103]. However, a protein of VqmAphage virophage VP882, is responsible for lysis of host cell lysis and a decrease in the concentration of host cells, in a homologus manner as mitomycin C (MMC) persuading lytic cycle of replication of virus VP882 phages [104]. It proved that the activation of VqmAphage by attaching to host-produced QS AI introduces the lytic life cycle of bacteriophages This provides a novel viewpoint on phage-host communication mechanism in which proteins of bacteriophages act as host-signaling compounds as cues for replication fate (i.e., lytic-lysogeny) verdicts[77].

8. Quorum sensing in parasites:

Many unicellulars as well as small multicellular eukaryotes shows intercellular communication to develop group behaviors as a strategy to respond to environmental stimulus for better survival in harsh environment [105]. Moreover, best developed in prokaryotes, eukaryotes have also been exhibited to co-ordinate to optimize their persistence and propagation. African Trypanosomes, an important pathogenic protozoan for both animals and humans of the sub-Sahara Desert [106]. Transmission of these unicellular parasites into the host is based on their ability to sense cellular density. These protozoans

use signal and signal transduction pathways (e.g. oligopeptide signals released by peptidase) to monitor cell density and then develop their transmission stages [107]. Trypanosoma QS signaling mechanism revealed that its "pleomorphic" (responsible for density-related growth control and development of stumpy forms) and "monomorphic" (independent of density signals via rapid propagation in animals or cultured cells) generate heat-resistant, soluble macromolecular factor named stumpy induction factor (SIF) [107]. Accumulation of SIF is associated with the density of parasites and it stimulates differentiation of slender to stumpy [108]. Some species of parasites showing the mechanism of quorum sensing include *Cryptosporidium spp. Cyclospora cayetanensis, Toxoplasma gondii* [109].

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