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Quorum sensing: A less known mode of communication among fungi

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ABSTRACT

Quorum sensing (QS), a density-dependent signaling mechanism of microbial cells, involves an exchange and sense of low molecular weight signaling compounds called autoinducers. With the increase in population density, the autoinducers accumulate in the extracellular environment and once their concentration reaches a threshold, many genes are either expressed or repressed. This cell density-dependent signaling mechanism enables single cells to behave as multicellular organisms and regulates different microbial behaviors like morphogenesis, pathogenesis, competence, biofilm formation, bioluminescence, etc guided by environmental cues. Initially, QS was regarded to be a specialized system of certain bacteria. The discovery of filamentation control in pathogenic polymorphic fungus *Candida albicans* by farnesol revealed the phenomenon of QS in fungi as well. Pathogenic microorganisms primarily regulate the expression of virulence genes using QS systems. The indirect role of QS in the emergence of multiple drug resistance (MDR) in microbial pathogens necessitates the finding of alternative antimicrobial therapies that target QS and inhibit the same. A related phenomenon of quorum sensing inhibition (QSI) performed by small inhibitor molecules called quorum sensing inhibitors (QSIs) has an ability for efficient reduction of gene expression regulated by quorum sensing. In the present review, recent advancements in the study of different fungal quorum sensing molecules (QSMs) and quorum sensing inhibitors (QSIs) of fungal origin along with their mechanism of action and/or role/s are discussed.

1. Introduction

Quorum sensing, a mechanism of microbial communication wherein accumulation of signaling molecules enables a cell to sense a cell density. It regulates several ecologically and medically important traits in microorganisms such as competence & bioluminescence, biofilm formation, secretion of virulence factors, sporulation & antibiotic production. Quorum sensing phenomenon which relies on the interaction between small diffusible signal molecules with transcriptional activator proteins, couples the gene expression with cell density (Mallick and Bennett, 2013; Avbelj et al., 2016; Wongsuk et al., 2016). Quorum sensing is a well-known and widespread mechanism of cell–cell communication in bacteria, wherein they communicate through signaling molecules called autoinducers, and contribute to the regulation of the gene expression (Wongsuk et al., 2016). Initially, QS was regarded to be a specialized system of *Vibrio fischeri* wherein *LuxI/LuxR* transcriptional activator and autoinducer system mediate cell density-dependent control of *Lux* gene expression important for the production of luminescence. Later some other homologous systems in other proteobacterial species with diverse biological roles were revealed experimentally. For example, in *Agrobacterium tumefaciens*, *luxI/R* homologs (*traI/R*), controls the conjugal transfer of plasmid between bacteria (Lang and Faure, 2014). Similarly, in *Pseudomonas aeruginosa*, expression of many virulence factors is controlled by two circuits acting in parallel (Rasamiravaka and El Jaziri, 2016; Kariminik et al., 2017). Besides their role in signaling, quorum sensing molecules (QSMs) do perform other activities, like, mediation of interspecies interactions within microbial populations. For example, chemotaxis in marine diatoms toward N-acyl homoserine lactone (AHL) signals (Williams, 2007) and the regulation of gene expression in *Burkholderia cepacia* by AHLs produced by another bacterium (Lewenza et al., 2002). A number of cell density-dependent cellular processes regulated by such factors have been reported in fungal classes as well. Studies have revealed that in fungi, like bacteria, many population-level behaviors like pathogenesis/virulence and biofilm formation are regulated by quorum sensing (Tarkka et al., 2009). Small signaling molecules which concentrate in the extracellular environment, mediate QS both in fungi as well as in bacteria. The signaling export mechanism responsible for the accumulation of these molecules in the medium occurs via passive diffusion across the membrane, involving efflux pumps and specific transporters (Hogan, 2006). Once a sufficient concentration of signaling molecules is reached, it results in the activation of a cognate response regulator

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within the local cell population, resulting in a synchronized gene expression (Hogan, 2006). Various inter as well as intraspecific communications, their diversity, nature, and mode of action of communication has been studied widely in fungi (Hornby et al., 2001). Fifteen years ago, a remarkable discovery of filamentation control in pathogenic polymorphic fungus *C. albicans* by farnesol revealed the phenomenon of QS in fungi. Due to the accumulation of sesquiterpene alcohol farnesol, dense cultures of human opportunistic pathogenic fungus *C. albicans* has been reported to display a reduced tendency for the yeast-to-hyphal switch, inferring the role of farnesol in inhibiting hyphae formation (Hornby et al., 2001; Ramage et al., 2002). In *C. albicans* physiology, farnesol plays multiple roles as signaling molecule besides having detrimental effects on host cells and other microbes (Albuquerque and Casadevall, 2012). Besides farnesol, another aromatic alcohol tyrosol, which controls growth, morphogenesis and biofilm formation also acts as a quorum sensing molecule (QSM) in *C. albicans* (Albuquerque and Casadevall, 2012). Aromatic alcohols, 1-phenylethanol, and tryptophol regulate morphogenesis during nitrogen starvation and act as QSMs in *Saccharomyces cerevisiae* (Albuquerque and Casadevall, 2012). Although QS research in fungi is still in its infancy, population density behaviors resembling QS have been documented in several fungal species (Albuquerque and Casadevall, 2012; Wongsuk et al., 2016). In the last decade, research in the field of QS focused much on probing for the mechanism that inhibits QS signaling in microbes. To attain an understanding of QS inhibition, a generalized review of its regulation mechanism is important. This regulation of quorum sensing phenomenon involves the synthesis of signal molecules which are then secreted by the synthesizing cells either by active transport or diffusion. Since fungi co-habit different groups of organisms- plants, animals or microbes, they need to interact with all these different groups of organisms. Some fungi are also extremophiles i.e., occupy extreme niches (Kogej et al., 2006). Organisms living in close association with different organisms have evolved different mechanisms to cohabit different types of organisms by producing different enzymes, chemicals or metabolites. For example, fungi interacting closely with bacteria in soil, combat bacterial populations for space, nutrition or pathogenicity by producing secondary metabolites (mycotoxins) and QSIs (enzymes and other chemicals). These metabolites and other QSIs, inhibit bacterial populations around them present in their habitat (Pitt, 2000; Frisvad et al., 2008). In the subsequent sections, we will discuss some of these aspects of communication in fungi brought about by the phenomena of quorum sensing, its molecular mechanism and some quorum sensing inhibitor molecules of fungal origin.

2. Quorum sensing in fungi

The accumulation of small diffusible molecules in the extracellular environment mediates quorum sensing in fungi. The signaling molecules are not generally strain specific and a huge diversity of those molecules has been reported in fungi. The discovery of quorum sensing molecule (QSM) farnesol in the pathogenic fungi *C. albicans* was a remarkable breakthrough of QS in eukaryotes. Existence of QS systems in fungi have revealed that lipids (oxylipins), peptides (pheromones), alcohols (tyrosol, farnesol, tryptophol, and 1-phenylethanol), acetaldehydes, besides some volatile compounds are actively involved in fungal QS (Fig. 1, Table 1), regulating the diverse key functions like pathogenesis, morphogenesis, filamentation (Fig. 2) etc (Cottier and Mühlschlegel, 2012; Albuquerque and Casadevall, 2012; Polke et al., 2015; Hirota et al., 2016).

Many fungal signaling pathways that are the sensors in biofilm formation and cell culture density are modulated by QS in *Aspergillus*, *Candida* and *Saccharomyces* genus (Hornby et al., 2001). Quorum sensing has been reported to control budding yeast-to-polarized filamentous growth transition in the dimorphic opportunistic pathogenic fungi *C. albicans*, wherein the yeast form develops germ tube at low cell density but cannot do so at high cell density. It has been reported that at a cell density lower than 10^6 cells/ml, filamentous forms are developed in *C. albicans,* whereas at higher cell densities the fungus grows as budding yeasts (Hornby et al., 2001). The growth transition of *C. albicans* appears to be important for its pathogenicity (Saville et al., 2003; Lin et al., 2015). In general, a diversity of fungi e.g., *Histoplasma capsulatum, C. albicans*, *C. krusei*, *C. utilis*, *C. zeylanoides, C. stellata*, *C. intermedia*, *C. solani*, *C. tenuis*, *S. cerevisiae, Cryptococcus neoformans, Aspergillus fumigates, Aspergillus niger, Ceratocystis ulmi, Ustilago maydis,* besides many others have been documented to undergo quorum sensing by producing one or the other quorum sensing molecule(s) (Kruppa, 2009; Albuquerque and Casadevall 2012). In fungi, a maximum number of QSMs have been reported in *C. albicans* till date. Table 1 shows some important QS molecules among different fungal species along with their different roles.

3. Important QS molecules and their mechanism of action

Several reports of quorum sensing like phenomena have been documented in fungi in recent years, which mysteriously involve a morphological transition from filamentous mycelial form to yeast or vice-versa (Sprague and Winans, 2006). Few of the commonly known quorum sensing molecules and their role is discussed below (also see Table 1).

3.1. α-(1,3) –glucan

The first example of an evident QS mechanism in eukaryotes is the regulation of the switch between the filamentous and yeast forms in parasitic fungi *Histoplasma capsulatum* (Kügler et al., 2000). In the soil, *H. capsulatum* exists as a free-living, saprophytic filamentous fungi but once it is inhaled by an animal, its growth habit switches to a yeast form, which produces some specific cell wall polysaccharides α-(1,3)-glucan, required for its virulence, in density-dependent fashion (Sprague and Winans, 2006). α-(1,3)-glucan biosynthesis has been found to be associated with virulence and is the special trait of *H. capsulatum* yeast phase cells (Kügler et al., 2000; Rappleye et al., 2007). α-(1,3)-glucans have been reported to be involved in yeast protection within phagolysosomes (Rappleye et al., 2007), regulation of yeast proliferation within host macrophage (Kügler et al., 2000) and. the establishment of intracellular latency (Romani, 2011)

In *H. capsulatum*, this polysaccharide is absent in chemotype I, while as it is present in the cell wall of chemotype II, wherein it masks the detection of immunostimulatory β-glucans by dectin-1 (which is pattern-recognition receptor) (Brown and Gordon, 2001). Therefore in chemotype I, no such masking of β-glucans occurs and dectin-1 recognizes β-glucans, which in turn enhances phagocytosis (Marcos et al., 2016). Thus a decreased virulence of chemotype I *in vitro* compared to chemotype II has been witnessed in *H. capsulatum*. Though *in vivo*, chemotype I maintain the virulence, possibly due to the expression of *AGS1* gene, that partially, bypasses the requirement of α-(1,3)-glucan for yeast virulence (Edwards, 2011).

3.2. Farnesol (3,7,11-trimethyl-2,6,10-dodecatriene-1-ol)

The discovery of a quorum sensing molecule (QSM) farnesol (an acyclic sesquiterpene alcohol), in the pathogenic fungi *C. albicans* was a remarkable breakthrough in studies on QS in eukaryotes. It is endogenously produced by the enzymatic dephosphorylation of mevalonate pathway intermediate farnesyl diphosphate at an approximate rate of 0.12–0.133 mg/g of dry weight of cells (Hornby et al., 2001; Ramage et al., 2002). In *C. albicans*, farnesol, which is continuously released into the environment at high cell density during growth, is the best characterized QSM. It generally blocks the yeast- to- filamentous transition, but cannot inhibit the elongation of already existing hyphae (Hornby et al., 2001; Mosel et al., 2005; Navarathna et al., 2005). At a high density of interwoven filamentous cells in the late stages of biofilm,