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Deborah A. Hogan
Dartmouth College

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MINIREVIEW

Talking to Themselves: Autoregulation and Quorum Sensing in Fungi

Deborah A. Hogan*

Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, New Hampshire 03755

Extracellular autoinducing compounds in the supernatants of microbial cultures were first recognized for their roles in the induction of genetic competence in gram-positive bacteria (17, 94) and in the regulation of light production in marine vibrios (60). In 1994, this form of population-level regulation in microbes was dubbed "quorum sensing" since it enabled bacterial cells to chemically measure the density of the surrounding population (18). Subsequently, many examples of cell density-dependent regulation by extracellular factors have been found in diverse microorganisms. The widespread incidence of diverse quorum-sensing systems strongly suggests that regulation in accordance with cell density is important for the success of microbes in many environments (see references 25 and 95 for reviews).

Cell density-dependent regulatory networks in microorganisms generally control processes that involve cell-cell interactions, such as group motility (10, 37) and the formation of multicellular structures (67, 93, 95). In a wide array of environmental and medically relevant bacteria, the development, maintenance, and dispersion of multicellular, surface-associated biofilms are in part controlled by quorum-sensing regulatory pathways (for reviews, see references 67 and 93). The uptake of extracellular DNA is often regulated in accordance with cell density presumably to enhance the chances of taking up DNA from closely related strains. For some bacteria, a link between the competence and biofilm formation has been established (69, 93). Both pathogens and symbionts that live in association with plant or animal hosts often use quorum sensing to regulate factors involved in microbe-host interactions (20, 86, 96). Quorum-sensing regulation may allow host-associated microbes to delay detection until an effective population has formed in the appropriate niche within the host.

Recently, it has become apparent that fungi, like bacteria, also use quorum regulation to affect population-level behaviors such as biofilm formation and pathogenesis. Considering the extent to which quorum-sensing regulation controls important processes in many distantly related bacterial genera, it is not surprising that cell density-dependent regulation also appears to be prevalent in diverse fungal species. Because fungal quorum sensing has been most extensively studied in *Candida albicans*, a dimorphic opportunistic pathogen, the majority of this review focuses on the details of quorum-sensing regulation in this fungus. A discussion of other fungi that appear to use quorum-sensing regulation and the identities of other known fungal signaling molecules are presented, and potential connections between mating in fungi and cell density-dependent regulation are explored. The review will conclude with points relating to the ecology of quorum sensing and a discussion of quorum-sensing networks as potential targets for antifungal therapies.

* Mailing address: Department of Microbiology and Immunology, HB7550, Dartmouth Medical School, Hanover NH 03755. Phone: (603) 650-1252. Fax: (603) 650-1318. E-mail: dhogan@dartmouth.edu.

BASIC ELEMENTS OF QUORUM-SENSING REGULATION

In both bacteria and fungi, quorum sensing is mediated by small diffusible signaling molecules that accumulate in the extracellular environment. The signal molecules themselves are generally, though not always, specific to a species or strain, and there is a high degree of structural diversity among the signaling molecules produced by different microbes (Fig. 1) (16, 25, 95). The mechanism by which a signal accumulates in the medium depends on the system; signal export has been shown to involve passive diffusion across the membrane (68), the action of efflux pumps (68), and specific transporters (32). When a signal accumulates to a sufficiently high concentration, the cognate response regulator is activated within the local population of cells, leading to coordinated gene expression. While the details of signal detection in fungi have yet to be elucidated, a number of different strategies for signal detection in bacteria have been described, including cytoplasmic, signal-binding transcription factors and cell-surface localized receptors. A number of recent reviews provide an overview of the literature describing the different elements involved in cell density-dependent regulation in bacteria (2, 66, 95).

C. ALBICANS AND AUTOREGULATION

C. albicans, a dimorphic fungus that is well studied for its importance as an opportunistic human pathogen, was among the first fungi reported to have a quorum-sensing system. *C. albicans* can transition between growth as a budding yeast and a polarized filament, and this ability appears to be essential for causing disseminated disease (48, 80). It has long been documented that *C. albicans* hyphal formation is suppressed at high cell densities and by supernatants from stationary-phase *C. albicans* cultures (22, 47; reviewed in reference 63), suggesting that hyphal formation was controlled, at least in part, by a soluble factor. The repression of *C. albicans* filamentation also occurred in response to supernatants from a strain of *Candida tropicalis* but not in when exposed to supernatants from cultures of other fungal species (*Candida laurentii* and *Candida parapsilosis*) (22).

In 1969, efforts to characterize the *C. albicans* quorum-sens-

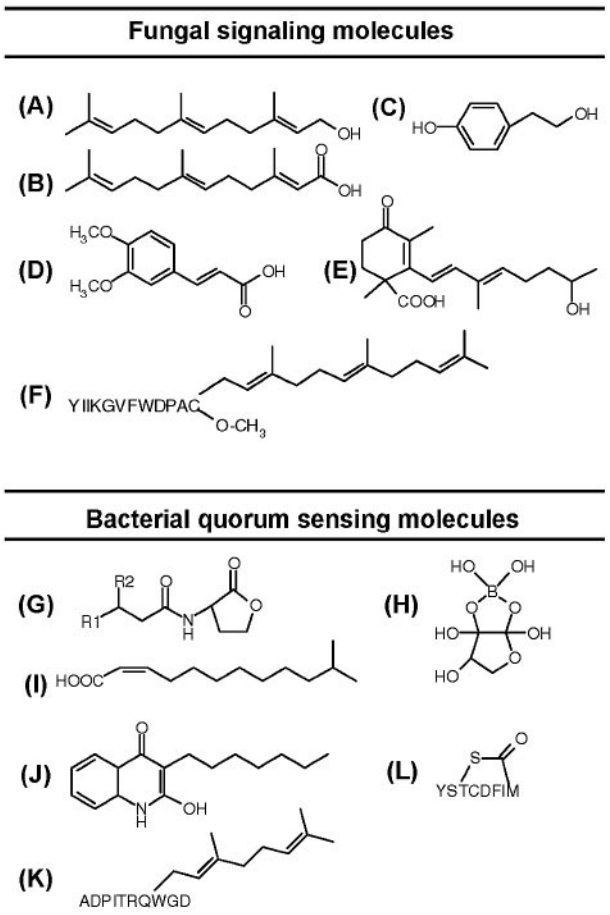

FIG. 1. Representative structures for different bacterial and fungal extracellular signaling molecules. (A) *C. albicans* farnesol, (B) *C. albicans* farnesoic acid, (C) *C. albicans* tyrosol, (D) *Uromyces phaseoli* dimethoxycinnamate, (E) trisporic acid produced by zygomycetes, (F) *Saccharomyces cerevisiae* a-factor, (G) AHL family (*Vibrio fischerii* 3-oxo-C6HSL, R1=C3H7, R2=O; *Pseudomonas aeruginosa* 3-oxo-C12HSL, R1=C9H19, R2=O; *Pseudomonas aeruginosa* C4HSL, R1=H, R2=H), (H) AI-2, furanosylborate diester, (I) *Xanthomonas campestris cis*-11-methyl-2-dodecanoic acid (DSF), (J) *P. aeruginosa* quinolone signal (PQS) 2-heptyl-3-hydroxy-4-quinolone, (K) *Bacillus subtilis* ComX peptide, (L) *Staphylococcus aureus*, cyclized oligopeptide (subgroup 1 shown).

ing molecule by Lingappa et al. led to the identification of two products, phenylethylalcohol and tryptophol, that were extracted with chloroform from supernatants from 7-day-old *C. albicans* cultures grown in Sabouraud sucrose broth (47). Although these molecules inhibit *C. albicans* growth at concentrations of 160 and 250 μM, respectively, subsequent studies showed that neither phenylethylalcohol nor tryptophol were responsible for the inhibition of germination by *C. albicans* culture supernatants (22). In 1979, Hazen and Cutler characterized *C. albicans* supernatants from 48-h cultures grown in glucose-peptone medium for their ability to inhibit the induction of hyphae formation (22). Preliminary chemical characterization indicated that the compound was inactivated at pH values at 4.0 and 9.5 and was stable at 70°C (22). The morphogenic autoregulatory substance (MARS) was later enriched by >400-fold from a dense suspension of *C. albicans* in tissue culture medium, but its chemical identity remained elusive (24). It is important to note that while the enriched MARS substance repressed germination, it did not inhibit the growth of nongerminating cells. The addition of 10 μM cobalt (Co^{2+}), but not copper (Cu^{2+}), molybdenum (Mo^{6+}), calcium (Ca^{2+}), or nickel (Ni^{2+}) at similar concentrations, appeared to promote the action of the purified inhibitory compound (23); however, the reason for this stimulation by metals is not yet known.

In 2001, two independent groups reported the identity of a factor in *C. albicans* supernatants that represses hyphal development (29, 64). In Hornby et al. (29), the "quorum-sensing molecule" was identified as farnesol (Fig. 1A). Farnesol was active against a variety of *C. albicans* strains at concentrations between 1 and 50 μM (29, 58). *C. albicans* CAI-4 and four clinical isolates were found to produce farnesol at comparable levels (31). Mycelium formation was suppressed by farnesol regardless of whether proline, *N*-acetylglucosamine, or fetal bovine serum were used to stimulate the formation of hyphae (29), although higher concentrations (10 to 250 μM) are required to repress hyphal formation in serum-containing medium perhaps due to the farnesol-binding capacity of serum albumins (58). More farnesol is produced, on a per-cell basis, between 37 and 40°C compared to cultures grown at lower temperatures, and farnesol production is not dependent on the type of carbon source in the growth medium (29). A separate study by Oh et al. (64) showed that *C. albicans* strain ATCC 10231 produces farnesoic acid (Fig. 1B), a compound closely related to farnesol, and that farnesoic acid is responsible for the inhibition of hyphal growth in dense cultures. Hornby and Nickerson later confirmed that *C. albicans* strain 10231 did not produce farnesol (31). Kim et al. found that farnesol can inhibit hyphae formation at lower concentrations than farnesoic acid but that farnesoic acid has decreased toxicity at high concentrations (39). Of the seven strains tested, three laboratory strains and four clinical isolates, all but ATCC 10231 were found to produce farnesol at concentrations between 0.10 to 0.13 mg per g dry weight (31).

Several experiments suggest that farnesol is produced from farnesyl pyrophosphate, an intermediate in sterol biosynthesis. Hornby et al. (30) showed that *C. albicans* extracts possess an enzyme capable of producing farnesol from farnesyl pyrophosphate and that farnesol production increases upon zaragozic acid B-mediated inhibition of squalene synthase, an enzyme involved in ergosterol biosynthesis. Disruption of ergosterol biosynthesis by the addition of azole-derived antifungal compounds, including fluconazole, clotrimazole, ketoconazole, and miconazole, also leads to increased farnesol production by *C. albicans* cultures, perhaps by causing an accumulation of the farnesyl pyrophosphate precursor (31, 59). Consistent with the hypothesis that farnesol is derived from intermediates in the ergosterol biosynthetic pathway, farnesol is not produced when *C. albicans* is grown under anaerobic conditions (14). Dumitru et al. suggest that, since *C. albicans* is also insensitive to ergosterol-targeting antibiotics under anaerobic conditions, the lack of farnesol production may be due to a reduction or alteration of the activities associated ergosterol biosynthesis under anoxic conditions (14). Interestingly, hyphae formation is also not repressed by exogenously added farnesol under anoxic conditions (14).

The mechanism by which farnesol is sensed by *C. albicans* is not yet known. Two structure-function studies have characterized the elements of farnesol and farnesoic acid that are important for activity (39, 85). The synthesis of biologically active fluorescent analogs of farnesol was used to show that farnesol derivatives localize to the cytoplasmic and nuclear membranes (84). Kruppa et al. (40) found that *C. albicans* mutants defective in the Chk1 histidine kinase, which has similarity to *Schizosaccharomyces pombe* Mak2p and Mak3p oxidative stress response proteins, are resistant to farnesol suppression of filamentation and biofilm formation. Sato et al. (79) showed that the mitogen-activated protein (MAP) kinase cascade that governs filamentation under nutrient-limiting conditions is repressed upon addition of farnesol. The relationship, if any, between the Chk1p and the MAP kinase regulatory cascade is not yet known. In a study that examined the effects of farnesol on the formation of thick-walled, asexual chlamydospores, it was observed that farnesol addition leads to the formation of more chlamydospores but does not affect the formation of the filamentous suspensor cells (56).

In addition to its effects on hyphal development, farnesol (35 μM) confers some protection to hydrogen peroxide, and this effect does not appear to be due to the increased transcription of genes involved in the oxidative stress response, including superoxide dismutase (*SOD1* and *SOD2*) and catalase (*CAT1*) (98). The presence of farnesol does lead to the increased transcription of the genes encoding the *CDR1* and *CDR2* efflux pumps (15). These proteins may have a role in farnesol transport or may be a part of a farnesol-mediated response to dense culture conditions.

Farnesol differs from the MARS activity that was previously enriched from *C. albicans* supernatants in that it is stable at pH 2 and 12 (22, 29), suggesting that there may be more than one *C. albicans* quorum-sensing molecule. Several bacteria, including *Vibrio harveyi* and *Pseudomonas aeruginosa*, produce multiple extracellar signaling molecules that act either synergistically or in a hierarchy to enable populations to "fine-tune" their signaling (36, 95).

QUORUM SENSING AND BIOFILM FORMATION IN *C. ALBICANS*

A number of examples illustrate the importance of quorum-sensing regulation in the formation of microbial biofilms (67). Biofilm formation is a developmental process in which microorganisms form multicellular structures with architecture, altered gene expression patterns, enhanced resistance to stresses and, in some cases, cellular differentiation (43, 62, 93, 95). The role of quorum sensing in bacterial biofilm formation is complex and is dependent on the environmental conditions. For example, in *P. aeruginosa* biofilms, acylhomoserine lactone (AHL) signals (Fig. 1G) are important for a number of biofilm properties, including the formation channels, cell dispersal, and biocide resistance (11, 12, 44).

A role for quorum sensing in *C. albicans* biofilm formation has also been observed (see references 41 and 70 for reviews). While *C. albicans* biofilm structure and composition can change under different environmental conditions, several reports describe *C. albicans* biofilms that are comprised of a basal yeast layer, abundant hyphae, and a calcofluor-binding extracellular matrix (1, 7). Furthermore, it has been shown that the formation of hyphae is important, though not essential (19), for biofilm formation under many of different conditions (1, 7, 49, 61, 72). Ramage et al. found that addition of farnesol can effectively block *C. albicans* biofilm development (71), leading to the hypothesis that endogenous accumulation of farnesol within biofilms may serve as a means for biofilm dispersal once a critical cell density is reached (71). Microarray studies comparing biofilms treated with farnesol to untreated controls found that the addition of farnesol leads to the decreased expression of hypha-regulated genes (6). The authors suggest that the decreased expression of one such gene, *CSH*, which encodes a cell surface hydrophobicity protein, and the corresponding decrease in cell surface hydrophobicity may explain why biofilms are disrupted upon farnesol addition (6). Ramage et al. also showed that supernatants from mature biofilms inhibited planktonic *C. albicans* from forming biofilms (71), indicating that farnesol or other supernatant factors may help maintain open spaces or channels within the biofilm that are hypothesized to aid in nutrient influx and waste product efflux by preventing further colonization of the surface.

As genes and proteins that are directly regulated by exogenous farnesol are identified, researchers will be able to assess the kinetics and spatial distribution of farnesol production and response within biofilms using gene fusion techniques. Such information will provide important insight into farnesol effects on specific biofilm processes such as the production of matrix components, virulence factor expression, and elements involved in biofilm dispersal.

OTHER FUNGAL AUTOREGULATORY MOLECULES

Other fungi have been found to produce extracellular molecules that modulate cellular morphology. *Uromyces phaseoli* produces methyl 3,4-dimethoxycinnamate, a "self-inhibitor" of spore germination that is effective at nanomolar concentrations (Fig. 1D) (55). Uredospores do not germinate unless the self-inhibitor is removed by an aqueous wash, leading to the suggestion that this signal may decrease competition within the population by blocking germination until a spore has dispersed. In *Glomerella cingulata* cultures, a diffusible factor decreases mycelia formation with a concomitant increase in conidia formation at cell densities greater than 10^6 per ml (46). The chemical identity of the molecule has not been described. Similar "inoculum-size effects" on morphology were observed in cultures of *Ceratocystis* (*Ophiostoma*) *ulmi*, the causative agent of Dutch Elm disease (28). An ethyl acetate extractable factor, shown not to be farnesol, is responsible for the repression of *C. ulmi* filamentation at high cell densities.

In addition to quorum-sensing molecules that affect morphology in fungi, autoregulatory molecules with effects on growth have also been observed. *C. albicans* produces a second autoregulatory molecule, tyrosol, that accumulates in the extracellular medium of dense cultures (Fig. 1C). The lag phase after *C. albicans* cells are transferred to either hypha-inducing and noninducing media is decreased when tyrosol is present in the medium (8). Tyrosol does not appear to have a role in detoxifying oxidants in the medium (98). Microarray studies showed that tyrosol prevents the temporary decrease in expression of genes involved in DNA replication, chromosome seg-

regation, and cell cycle regulation that normally occurs during that lag phase that follows culture dilution (8). Alcohols derived from amino acid catabolism and metabolic processes may have other roles in regulating fungi since several alcohols have been shown to affect filamentous growth in *S. cerevisiae* (50).

CONNECTION BETWEEN MATING PHEROMONES AND QUORUM SENSING IN FUNGI

A recurring theme in the study of microbial populations is the coordinated regulation of DNA exchange and the expression of virulence factors. For example, the *Streptococcus pneumoniae* competence stimulating peptide that controls DNA uptake also regulates acid tolerance, a condition present within biofilm communities, and the production of virulence determinants (93). The coordinated regulation of mating and the production of virulence determinants has also been observed in a number of fungi. The initial discovery of a connection between mating and invasive growth was shown in haploid *S. cerevisiae* cells, where upstream and downstream elements of the same MAP kinase cascade were found to regulate pheromone response, agar invasion, and biofilm formation, indicating that perhaps that these pathways may have coevolved (65, 73, 75). At a basic, mechanistic level, the sensing of the mating pheromone in yeast is reminiscent of AI-2 and oligopeptide-mediated quorum-sensing pathways (25) in that the signal binds to an extracellular receptor, thereby initiating a signaling cascade that results in transcriptional activation of a specific set of genes. In *Ustilago maydis*, a-pheromones, short farnesylated peptides similar to the a-factor produced by *S. cerevisiae* (Fig. 1E), induce dikaryon formation, filamentation, and increased cell surface hydrophobicity (91) in cells of the opposite mating type. Although the mating pheromone receptor is not essential for virulence in *U. maydis*, the same regulatory network that governs mating in response to pheromone also controls genes that may be important for virulence (21). As is seen in many quorum-sensing systems, exposure to mating factor from the opposite mating type leads to the upregulation of mating factor production. It has recently been discovered that *C. albicans* has a mating system that, in many ways, parallels that observed in *S. cerevisiae* (34). In genome-wide expression analysis of *C. albicans* a/a cells exposed to α-factor (3), it was noted that the pheromone induces genes involved mating, hyphae formation, and virulence factor production, again leading to the hypothesis that coregulation of mating and virulence has some biological significance. Because temperature and growth phase impact both farnesol production (29) and switching to the mating competent opaque phase in *C. albicans* (90, 102), there may be some relationship between farnesol production and mating. At present, no such connection has been described. It is interesting that the *Bacillus subtilis* ComX peptide, which bears significant overall resemblance to yeast mating pheromones (Fig. 1K), also regulates DNA uptake (i.e., competence), stress responses, and the production of extracellular products (9).

Communication between different mating types within the zygomycete fungi, including *Mucor mucedo*, *Phycomyces blakesleeanus*, *Blakeslea trispora*, occurs via carotene-derived compounds, including trisporic acid and its biosynthetic progenitors (Fig. 1E). The trisporic acid pheromone is made through the cross-feeding of precursors between strains of opposite mating types since neither mating type has the complete biosynthetic pathway (81). Trisporic acids can have a range of effects beyond the induction of mating, including carotenoid production (42) and parasitism (82), suggesting that these molecules can act as both mating pheromones and as population-wide signals. A wide range of trisporic acids are produced by zygomycetes, and the different compounds have various abilities to induce aerial hyphae and growth arrest in fungal test strains (81).

MULTIPLE ROLES OF AUTOREGULATORY SECRETED PRODUCTS

Quorum-sensing molecules can have activities beyond their roles as signals within microbial populations. In some cases, quorum-sensing molecules mediate interspecies interactions. Examples include the chemotaxis of marine diatoms toward AHL signals produced by bacteria (35) and the regulation of genes in *Burkholderia cepacia* by AHLs produced by another bacterium (45). Bacterial quorum-sensing molecules produced by *Pseudomonas aeruginosa* and *Xanthomonas campestris* pv. *campestris* (Fig. 1G and I) have been shown to repress *C. albicans* hyphal development (27, 97) and may represent either a mechanism by which the bacteria modulate fungal behavior or the response of *C. albicans* or related fungi to the presence of antagonistic bacteria (27).

Quorum-sensing signals themselves can have antibiotic activities toward potential competitors or host organisms. The fungus *Aspergillus nidulans* undergoes apoptosis in the presence of farnesol, indicating that farnesol may have a role in competition with other fungi (83). *A. fumigatus* does not appear to produce farnesol itself (83). Farnesol, and the related compound gernanylgeraniol, also cause apoptosis in human lung adenocarcinoma cells after prolonged (72 h) incubation (57). Although farnesol does inhibit mammalian cholinephosphotransferase, an enzyme involved in phosphatidylcholine biosynthesis, it appears that this activity may be distinct from its proapoptotic effects (99). The effects of farnesol on calcium signaling and membrane fluidity in mammalian cells have also been examined (76–78). The effects of farnesol on human cells is particularly relevant in light of reports that subinhibitory concentrations of some antifungal compounds lead to increased production of farnesol by *C. albicans* (31, 59). Machida et al. reported that farnesol induces mitochondrion-generated reactive oxygen species and growth arrest in *S. cerevisiae* yeast cells after a 30-min treatment with farnesol but not after treatment with other isoprenoid compounds (52, 53) and have proposed that farnesol inhibits a phosphatidylinositol-type signaling pathway *S. cerevisiae* (54). Several studies have shown that farnesol may also participate in fungal-bacterial interactions. *Staphylococcus aureus* membrane integrity, as measured by potassium leakage, is disrupted by 50 to 200 μM farnesol, and this concentration leads to a 1,000-fold decrease in the number of viable *S. aureus* cells (33). Farnesol sensitizes both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) to a number of different antibiotics (4). Alternative functions for bacterial signaling molecules have also been documented (38, 87, 88).

QUORUM SENSING IN MICROBIAL COMMUNITIES

In vitro analyses have been essential for our understanding of the elements that comprise quorum-sensing cascades in different microorganisms, but extrapolation of these findings into natural settings is not a simple matter. First, the impact of external factors on quorum-sensing regulation has been well documented in almost all microbial systems studied. Many environmental inputs have been identified in well-studied bacterial models for quorum sensing (89), and some environmental parameters that affect quorum sensing in *C. albicans* have already been identified (14, 58). Second, quorum-sensing signals may be degraded, sequestered, or inhibited by antagonists produced by other organisms. Enzymes capable of degrading signals have been isolated from microbial communities and a number of quorum sensing signal agonists and antagonists have been identified (see reference 13 for a review). Lastly, the chemical and physical environment can affect the half-life and the dispersion of signal molecules. Some signals are sensitive to pH or oxidation (101) and thus may not persist in some environments. Biosurfactants may also affect the dispersion of signaling molecules; signaling via the *Pseudomonas* quinolone signal (PQS) (Fig. 1J) and *U. maydis* pheromones is impacted by the presence of surfactants (5, 26). The number of factors that can affect signaling within microbial populations indicates that "quorum sensing" provides microbial cells with information beyond just the local cell density. In order to understand the importance of quorum sensing within microbial communities, we will have to develop multispecies and host-associated model systems and sensitive molecular and chemical detection techniques capable of spatial and kinetic analyses of signal production and response.

Recent work has identified a furanosylborate diester quorum sensing molecule, AI-2 (Fig. 1H), that governs communication between bacterial species (for reviews, see references 2, 16, and 25). The AI-2 signaling molecule is synthesized by dozens of different gram-positive and gram-negative bacterial species. AI-2 has been shown to impact multicellular activities such as biofilm formation and virulence within single-species populations and has been proposed to play a role in gene regulation within mixed-species microbial communities (51, 92). Fungal responses to this "universal" bacterial signaling molecule have not been reported, and it is not known whether a similar type of interspecies (or interdomain) signaling exists in fungi.

CONCLUSIONS

Once the importance of quorum sensing is established in pathogenic fungi and the mechanistic details are uncovered, the value of quorum-sensing pathways as potential therapeutic targets can be assessed. As described above, a number of intraspecies and interspecies quorum quenching systems have been identified in bacteria (13), and there are likely many additional examples of bacterial and fungal activities that similarly modulate fungal signaling pathways. Some of the mechanisms for quorum-sensing interference within a population may have applications in the clinic. Because of the role of quorum-sensing regulation in biofilm formation, there is particular interest in identifying chemical agents that can control biofilm-associated infections and attenuate chronic infections that involve a biofilm-like state (74). A synthetic furanone that inhibits quorum sensing in *P. aeruginosa* by binding competitively with respect to the AHL signal itself was found to enhance the clearance of *P. aeruginosa* infections in mice (100). In the same way that pathogens appear to use quorum-sensing regulation to control the production of virulence determinants, quorum-sensing pathways may have roles within protective microbial communities, such as those that comprise the human microflora. It remains to be seen whether agents that impact quorum-sensing regulation will be useful for modulating these complex communities in beneficial ways.

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