MINIREVIEW

Secondary metabolites and other small molecules as intercellular pathogenic signals

Nicholas Dufour[1] & Reeta Prusty Rao[2]

[1]McGovern Institute for Brain Research (MIT), Cambridge, MA, USA; and [2]Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA, USA

Correspondence: Reeta Prusty Rao, Biology and Biotechnology, Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA 01609, USA. Tel.: +1 508 831 6713; fax: +1 508 831 5936; e-mail: rpr@wpi.edu

Received 20 July 2010; revised 29 September 2010; accepted 30 September 2010.
Final version published online December 2010.

DOI:10.1111/j.1574-6968.2010.02154.x

Editor: Derek Sullivan

Keywords
pathogenesis; secondary metabolite; small-molecule signaling; cell–cell communication; intercellular signal; antimicrobial therapies.

Abstract

Microorganisms often use small chemicals or secondary metabolites as informational cues to regulate gene expression. It is hypothesized that microorganisms exploit these signals to gain a competitive advantage. Here, we present examples of pathogens that use this strategy to exclude other microorganisms from the site of infection. An emerging theme is that inhibiting these systems presents a novel approach to antimicrobial therapies.

Introduction

Beginning with experiments using the *Lac* operon in *Escherichia coli* (Jacob & Monod, 1961), our understanding of the use of small molecules as regulatory instruments has expanded greatly. We now know that small molecules also play a large role in shuttling information between cells. In prokaryotes, cell–cell small-molecule signaling regulates numerous phenomena, including biofilm formation (Parsek & Greenberg, 2005) and virulence factor production (Higgins *et al.*, 2007). More recently, eukaryotes have been shown to respond to small-molecule cues (Chen *et al.*, 2004; Hogan *et al.*, 2004; Prusty *et al.*, 2004; Chen & Fink, 2006). Because of the vastness of the field (for other reviews, see Miller & Bassler, 2001; Bassler, 2002; Taga & Bassler, 2003; Camilli & Bassler, 2006; Hogan, 2006; Nickerson *et al.*, 2006; Rasko & Sperandio, 2010), this review will focus on several prominent examples of small-molecule signaling in microorganisms of relevance to human health, highlighting an emerging theme of competitive exclusion, where small-molecule signals from one species inhibit growth of another competing species.

Current antimicrobials rely on drugs that either kill pathogenic cells directly or inhibit their growth. These drugs are effective but pose several potential issues. For instance, broad-spectrum antibiotic treatment can disrupt microbial gut flora and can leave one more susceptible to certain types of infection (Carman *et al.*, 2004). Further, general disruption of gut flora is directly implicated in antibiotic-associated diarrhea (Beaugerie & Petit, 2004), although the precise effect of this disruption is disputed. A greater cause for concern is the rise of antifungal-resistant pathogenic fungi (Kontoyiannis & Lewis, 2002) and antibacterial-resistant bacteria. By targeting small-molecule signals specific to a species, through the use of an inhibitory molecule, it is possible to prevent the disruption of natural gut flora. Further, by targeting small-molecule cues responsible for infection (for instance, regulation of virulence factor expression), but not necessary for growth, the strong selective pressure favoring resistance is potentially ameliorated (Otto *et al.*, 1999; Muh *et al.*, 2006). On a cautionary note, a thorough understanding of the particular microorganism's virulence strategies is crucial to the development of effective therapies. For example, it is possible that these drugs may trigger the unanticipated production of metabolites with detrimental consequences to the host. A broad-scale clinical study will ultimately determine the efficacy of such novel therapies with respect to toxicity and effect on the resident microbiota.

FEMS MICROBIOLOGY LETTERS

Small-molecule signals

Homoserine lactones (HL)

HLs are diffusible molecules synthesized from *S*-adenosylmethionine by many gram-negative bacteria (Schaefer *et al.*, 1996) to monitor population density. A pathogen that actively uses HL signaling is *Psuedomonas aeruginosa* (Lazdunski *et al.*, 1995). *Psuedomonas aeruginosa* is an opportunistic pathogen that accounts for a considerable portion of hospital-acquired infections and is also a common source of infection for sufferers of cystic fibrosis (CF). *Psuedomonas aeruginosa* uses two HL signaling systems, which combined regulate over 300 genes (Schuster & Peter Greenberg, 2006), many of which are implicated in virulence factor production.

HL signaling results in extensive changes in gene expression affecting secondary metabolism, sporulation, the elaboration of virulence factors and the formation of biofilms (Schuster & Peter Greenberg, 2006). Because HL concentration in the extracellular medium increases with population density, the system allows bacteria to coordinate population-wide gene expression simultaneously. Studies have shown that another related HL produced by *P. aeruginosa* was able to abrogate *Candida albicans* filamentation (Hogan & Kolter, 2002; Hogan *et al.*, 2004), a virulence trait. This study provides a striking example of competitive exclusion because restricting the ability of *C. albicans* to transition between morphotypes (an important virulence trait) presumably gives *P. aeruginosa* a competitive advantage.

HLs play a central role in regulating and coordinating infection. As a result, considerable research has been directed at identifying inhibitor HL systems. For example, a tetrazole with a ^{12}C alkyl tail (Muh *et al.*, 2006) was recently identified as an effective inhibitor ($IC_{50} = 30$ nM) of *P. aeruginosa*. Importantly, this molecule may not interfere with the growth of *P. aeruginosa*. This means that while highly effective at disrupting the machinery used to coordinate infection, the compound does not create a strong selective pressure to develop resistance unlike current therapeutics. This is another emerging common theme among chemical inhibitors of small-molecule signals.

Vibrio cholerae is the etiological agent of the debilitating human disease cholera. While *V. cholerae* uses the autoinducer-2 (AI-2, described in more detail later in the review) like many other bacterial species, in addition, it also uses a unique autoinducer, cholerae autoinducer 1 (CAI-1), an α-hydroxyketone. CAI-1 serves to terminate host colonization, halting biofilm formation and virulence factor expression (Higgins *et al.*, 2007). This observation is consistent with *V. cholerae*'s transmission route, where bacteria leave the host simultaneously during the onset of the diarrhea that characterizes the illness. Thus, host colonization and biofilm formation continue until the population reaches a sufficient density, at which point the bacteria reverse the colonization process to spread to other hosts. Exploiting the small-molecule signaling involved in *V. cholerae* infection is quite simple, as introducing high concentrations of the HL autoinducer will terminate host colonization, thus ending the infection.

Modified oligopeptides

Gram-positive bacteria use post-translationally modified oligopeptide pheromones to transmit information (Kleerebezem *et al.*, 1997). Because oligopeptides are impermeable to biological membranes, dedicated proteins (ABC transporters) are used to secrete the oligopeptides into the growth environment where they function as input for two-component transduction systems. Once they interact with a membrane-bound receptor, information is transmitted via a series of phosphorylation events that ultimately coordinate gene expression.

Staphylococcus aureus is a gram-positive human pathogen, which causes a variety of conditions ranging from relatively harmless conditions, such as styes, to those that constitute a medical emergency, such as toxic shock syndrome, which occurs when the bacteria enters the body through a cut, sore, catheter, or breathing tube. Recent emergence of *S. aureus* strains that are resistant to methicillin, the antibiotic of choice for staph infections, has become a significant health problem. *Staphylococcus aureus* exhibits a highly complex adaptive behavior, with gene regulation that is population density, time, and environment specific. A part of this behavior is regulated by at least four two-component systems (Novick, 2003), one of which, termed the *agr* system, uses a modified octapeptide in signaling (Ji *et al.*, 1995). Since its identification, several genes homologous to those involved in *agr* signaling have been identified in pathogens including *Listeria monocytogenes* (Autret *et al.*, 2003), *Staphylococcus saprophyticus* (Sakinc *et al.*, 2006) and *Clostridium perfringens* (Ohtani *et al.*, 2009).

Like the HLs, the octapeptides also exhibit competitive exclusion by inhibiting signaling in foreign strains (Ji *et al.*, 1997). The precise reasoning for this is not well understood; however, it is hypothesized to be a mechanism by which strains can exclude each other from infection sites. Further, it has been shown that the octapeptide signal from *Staphylococcus epidermidis* inhibits virulence factor expression in *S. aureus* (Otto *et al.*, 1999) without affecting growth. Therefore, the use of 'inhibitory' oligopeptides as treatment for certain gram-positive bacterial infections is a promising route, offering a directed therapeutic with, presumably, small chances of the target bacteria evolving resistance.

A novel mechanism

Pseudomonas quinolone signal (PQS) was recently discovered as a novel, signaling molecule. It was surprising to find

PQS, an inhibitor of DNA gyrase and topoisomerase (Pesci *et al.*, 1999; McKnight, 2000), as a potential small-molecule signal due to its hydrophobicity. It has now been shown to have a role in cell-to-cell communication (Déziel *et al.*, 2004) and is secreted in concentrated form via vesicular transport (Mashburn & Whiteley, 2005). This makes the signaling mechanism of *P. aeruginosa* unique in that it does not rely on diffusion-mediated communication of the small molecule, which remains concentrated within the exported vesicle. PQS is now regarded as being crucial for signaling between cells existing in biofilms formed in the lungs of CF patients. Like HL, PQS induces its own expression as well as the expression of secretion vesicles required for PQS export. Further, PQS has antibacterial qualities, and may be used by *P. aeruginosa* to destroy rival bacterial cells by delivering PQS *en masse* via vesicular transport. It is hypothesized that this type of signaling is also required to carefully control growth of populations in delicate niches such as the lungs. This notion is supported by the fact that PQS and its precursor, hydroxy-2-heptylquinoline, are produced in the lungs of CF individuals with *P. aeruginosa* infections (Machan *et al.*, 1992), implying that it may have clinical relevance in treating such infections.

Secondary metabolite signals in fungi

Candida albicans is a widespread opportunistic pathogen that causes high rates of mortality during systemic infections. *Candida albicans* also causes superficial mucosal infections, which can be intractable in immunocompromised individuals such as AIDS patients (Koh *et al.*, 2008). Its universal presence as part of the human gut flora makes *C. albicans* the most common cause of human fungal infections in general. The ability of *C. albicans* to freely transition between the yeast and hyphal forms has been shown to be critical for virulence (Lo *et al.*, 1997). *Candida albicans* exhibits a complex quorum-sensing system utilizing the two secondary metabolites, tyrosol and farnesol, which have opposing effects. Farnesol inhibits transition from the yeast morphotype to hyphal cells (Hornby *et al.*, 2001; Nickerson *et al.*, 2006); however, it cannot completely abolish hyphal development, indicating that additional unknown inhibitory molecules with similar function must exist. Tyrosol stimulates a more rapid transition from yeast form cells to hyphal cells under favorable conditions (Chen *et al.*, 2004). Discovery of these secondary metabolite signals stems primarily from the observation that inoculation of stationary phase yeast cells into fresh medium at the optimal growth temperature (37 °C) induced hyphal formation. Fresh medium releases the yeast cells from the influence of secondary metabolite signals such as farnesol, present in the conditioned media, by diluting it.

Recent studies in the filamentous fungus *Aspergillus nidulans* (Semighini *et al.*, 2006) and the plant pathogenic fungus *Fusarium graminearum* (Semighini *et al.*, 2008) indicate that externally added farnesol triggers morphological features characteristic of apoptosis mediated by reactive oxygen species (ROS). Conversely, farnesol appears to protect *Candida* from oxidative stress (Deveau *et al.*, 2010). Farnesol also induces accumulation of intracellular ROS in *Candida*; however, this does not appear to be the mechanism of oxidative stress protection as attenuation of farnesol-mediated ROS build-up by antioxidants α-tocopherol and ascorbic acid failed to reduce oxidative stress resistance. It is hypothesized that farnesol's mechanism of action in affecting cellular morphology is via the Ras1-adenylate cyclase-signaling pathway. The mechanism of action in producing oxidative stress resistance and morphogenetic transitions appears to be closely related, as strains lacking Ras1 and Cyr1 cease to demonstrate the same resistance as wild type when exposed to hydrogen peroxide when preincubated with farnesol. The mechanism of action probably does not depend on the Hog1 pathway, as *hog1* mutants fared no differently from the wild type when farnesol-mediated oxidative stress resistance was measured (Menon *et al.*, 2006). The fact that farnesol induces such resistance indicates that it plays a role during infections, as ROS has been shown to play a central role in host defense against fungal pathogenesis (Jain *et al.*, 2009). Furthermore, the induction of oxidative stress by macrophages is part of the defense repertoire against pathogens (Lorenz & Fink, 2001, 2002) and resisting such stresses is critical for survival of *Candida* within macrophages. Thus, it is hypothesized that *C. albicans*, via farnesol-mediated resistance, may survive action by macrophages and neutrophils (Fan *et al.*, 2007). If *Candida* survives the host ROS, it can differentiate into a hyphal form (which farnesol inhibits) and subsequently invade and lyse the host cell to escape. Inhibition of farnesol, and therefore the oxidative resistance it produces, promises new development strategies for antifungal drugs.

Aromatic alcohols

Opposing the action of farnesol is the aromatic alcohol tyrosol, a catabolic product of the amino acid tyrosine. In diluted cultures, tyrosol concentration is reduced and *C. albicans* experiences an exceptionally long lag phase before re-entering exponential growth (Chen *et al.*, 2004). This long lag phase is abolished by the addition of tyrosol to the culture medium. The dilution of exponential-phase culture may destabilize transcripts necessary for cell division; therefore, it is hypothesized that tyrosol stabilizes them, enabling exponential growth to proceed. Because tyrosol is released into the culture medium by *C. albicans* and has a concentration-dependent behavior, it is an autostimulatory small molecule; however, unlike those observed in bacteria, it does not appear to explicitly upregulate its own production (Chen *et al.*, 2004).

Although *Saccharomyces cerevisiae* is not a threatening pathogen, it has been used as a model for fungal pathogenesis (McCusker, 2006). *Saccharomyces cerevisiae* uses at least two aromatic alcohols, phenylethanol and tryptophol (Chen & Fink, 2006), as environmental cues, whose effect is also dependent on population density. The ambient concentration of these aromatic alcohols, in turn, regulates morphogenesis by encouraging a transition from the unicellular morphotype to a 'multicellular' filamentous one. The biosynthetic pathway for the two alcohols is activated upon nitrogen starvation and repressed in rich medium. Thus, filamentous growth is partially regulated by the amount of available nitrogen. Phenylethanol and tryptophol are also autoinducers, which transmit information about both the population density and the amount of available nitrogen. Interestingly, the signaling capacity of these alcohols appears to be species specific, as the same response is not observed in pathogenic yeasts, such as *Candida albicans* (Chen & Fink, 2006).

Cross-species communication

Beyond the canonical AHL/modified oligopeptide systems found in bacteria, and aromatic alcohols in fungi, there are a number of other signaling systems that are less easily grouped. One interesting commonality between these systems is their ability to function across species barriers, which may be viewed as microorganisms 'eavesdropping' on each other, expressing the receptors for certain small-molecule signals but not the molecular machinery to produce it (Walters & Sperandio, 2006). For example, *S. aureus* and *C. albicans* have been shown to act synergistically in a biofilm where *S. aureus* can penetrate through host epithelial layers by 'hitchhiking' on candidal hyphae (Peters *et al.*, 2010; Shirtliff, 2009). Another recent study showed that bacterial peptidoglycan-like molecules promote filamentation in *C. albicans* (Xu *et al.*, 2008). Other examples of interspecies communication involving HLs exist (Riedel *et al.*, 2001; Lewenza *et al.*, 2002; Venturi *et al.*, 2004), although the degree to which this is due to incidental homology between HSL receptor molecules (LuxR) is unknown. Clearly, such complex interactions between diverse pathogens have significant clinical implications. Understanding the underlying signaling mechanisms can lead to the development of novel therapeutic strategies for polymicrobial diseases. A few examples of cross-species signals are discussed below.

AI-2 was first discovered in the marine bacterium *Vibrio harveyi*, working as a second cell-density-sensing system in addition to the already known *luxL/luxM* system in the regulation of bioluminescence (Bassler *et al.*, 1994). AI-2-like molecules, derived from the 4,5-dihydroxy-2,3 pentanedione, have since been identified in a number of bacteria including *Salmonella typhimurium* and *E. coli* (Surette *et al.*, 1999; Chen *et al.*, 2002; Xavier & Bassler, 2005). One study estimates that the AI-2 synthase is present in nearly half of all bacterial genomes analyzed (Xavier & Bassler, 2003). More interestingly, bacterial species lacking the capacity to produce AI-2 have been shown to respond to it (Duan *et al.*, 2003). Further, AI-2 remains the only signaling molecule that enables interspecies communication between gram-positive and gram-negative bacteria (Schauder & Bassler, 2001). The apparent prevalence of AI-2 and its ability to carry information between species suggests that it might be a 'universal language' among bacteria.

Another novel diffusible signaling factor (DSF) was discovered among the genus of plant pathogens *Xanthomonas* (Barber *et al.*, 1997). Both aspects of the DSF system, synthesis and detection, are contingent upon a single gene cluster, *rpf*, which is widely conserved among the xanthomonads. The molecules involved, the DSF family, are all varied but structurally related to the canonical unsaturated fatty acid *cis*-11-methyl-2-dodecenoic acid (Wang *et al.*, 2004), first discovered in *Xanthomonas campestris* pv. *campestris*. DSF and related molecules play a role in the formation of biofilms (Dow *et al.*, 2003), nutrient uptake (Huang & Wong, 2007) and pathogenic behavior such as the production of exoenzymes (Slater *et al.*, 2000). DSF has been found to exert influence on and be produced by bacterial species outside of the xanthomonads. For example, in *P. aeruginosa*, DSF causes a change in biofilm architecture when grown in coculture with *Stenotrophomonas maltophilia*, but only when *S. maltophilia* possesses the genes necessary to produce DSF (Ryan *et al.*, 2008). Recently, a molecule secreted by *Burkholderia cenocepacia* (BDSF, subsequently identified as *cis*-2-dodecenoic acid) was shown to restore wild-type biofilm formation characteristics on DSF-deficient *X. campestris* pv. *campestris* (Boon *et al.*, 2008). Interestingly, BDSF is structurally similar to farnesol, a fungal signaling molecule, and behaves in a manner similar to farnesol, inhibiting germ tube formation (Boon *et al.*, 2008).

A secondary metabolite, indole-3-acetic acid (IAA), has recently been shown to function as a signal in *S. cerevisiae* and *C. albicans* (Rao *et al.*, 2010). IAA inhibits growth at high concentrations and induces filamentation and substrate adhesion at low concentrations (Prusty *et al.*, 2004), two morphogenetic changes relevant for pathogenesis of dimorphic fungi (Fig. 1). At least two pathways for IAA synthesis have been identified in *S. cerevisiae*, and loss of one of these pathways alters the dimorphic transition in yeast. IAA is best known as the plant growth hormone auxin, affecting various aspects of plant growth and development (Normanly & Bartel, 1999; Woodward & Bartel, 2005). IAA is present at plant wound sites where an invading fungus may capitalize on this signal by upregulating its pathogenic processes. Interestingly, IAA is also present in the human

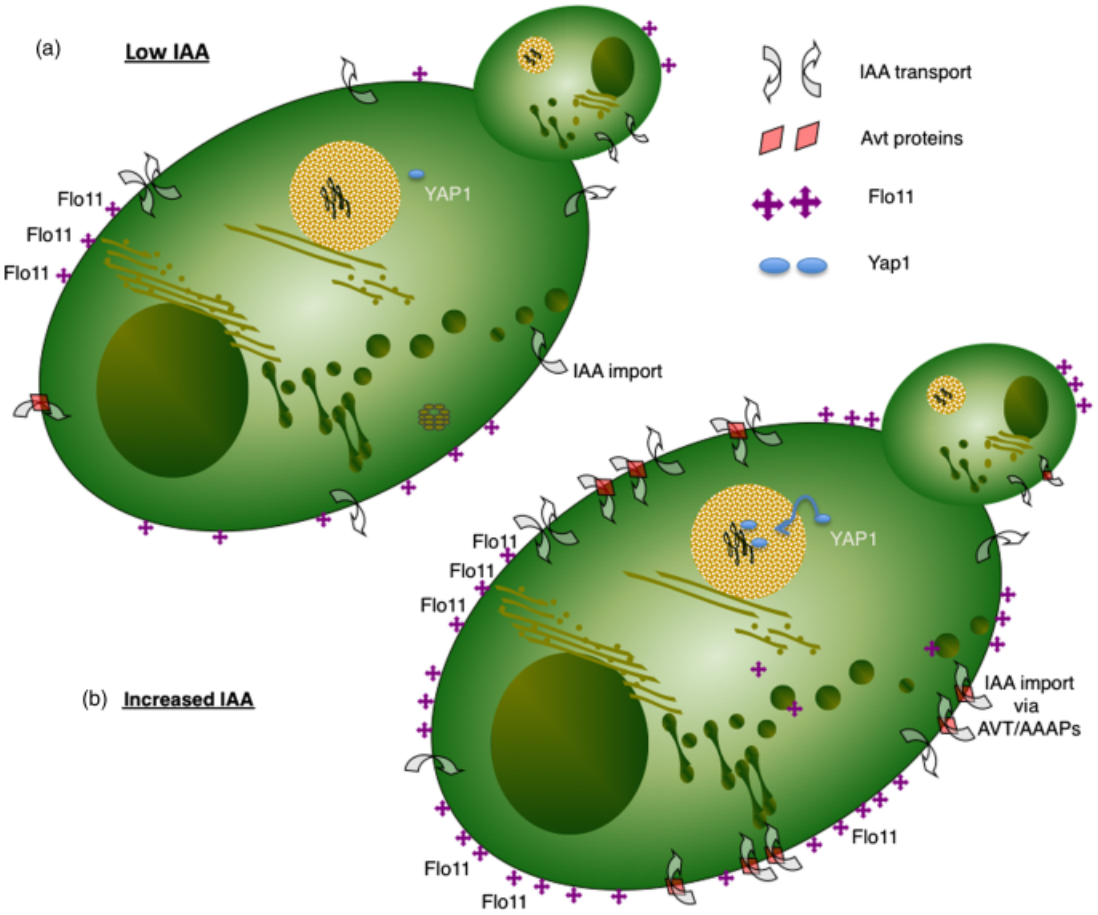

Fig. 1. The working model for indole-3-acetic acid (IAA) signaling in yeast. (a) At low concentration of extracellular IAA, amino acid auxin permeases (AAAP, or AVT in yeast)-mediated IAA import is maintained at a basal level, Yap1 transcription factor is localized in the cytoplasm and the cell surface glycoprotein, Flo11, is maintained at housekeeping levels. (b) At high concentrations of extracellular IAA, which may either be secreted by yeast or derived from a plant host, Yap1 transcription factor is translocated to the nucleus where it induces expression of AAAPs. AAAP (AVT)-mediated IAA import is elevated. The cell surface glycoprotein, Flo11, expression is induced, which leads to increased surface adhesion and filamentation.

urogenital tract where it is excreted as a catabolite of 5-hydroxytryptamine (serotonin) (Kurtoglu *et al.*, 1997). IAA induces filamentation in the human pathogen *C. albicans*, suggesting an involvement in candidiasis (Rao *et al.*, 2010). These studies suggest that IAA may function as a secondary metabolite signal that regulates virulence in fungi.

Conclusion

Our understanding of intercellular small-molecule signaling has expanded greatly in recent years to include a remarkable number of microorganisms. This is perhaps not surprising, as the capacity to communicate and to coordinate in response to changes in the environment is an immensely valuable ability, even for organisms as small as bacteria or single-celled fungi. An emerging theme in this field has been the hypothesis of competitive advantage among microbial populations, where molecules produced by one microorganism may be used by another to either facilitate its own infection or restrict the ability of another to establish infection. Such signaling has been the focus of intense study because of its promise as a target for the treatment of infections (analogous to static drugs rather than cidal). Since the introduction of penicillin, we have seen the rapid emergence of drug-resistant pathogens, which occurs at a rate far outstripping the development of new means of treatment. Interfering with extracellular signaling to prevent the release of virulence factors, the formation of biofilms or the morphological changes associated with pathogenesis is expected to circumvent this. Such treatments neither halt cellular division directly nor are they toxic to the cells, which means the selective pressure to evolve mechanisms of resistance is likely to be substantially reduced. With this reduced selective pressure, fewer resistant mutants may be generated, which could potentially prolong the usage of the therapeutics and increase their overall effectiveness. In

addition, targeting small-molecule signaling pathways ensures that treatments will be directed specifically at the pathogenic organism, rather than the entire microbiome. Medical science is increasingly becoming aware of the host of problems caused by host microbiome disruptions due to antibiotic treatment.

Acknowledgements

The authors appreciate the invitation to submit this review and acknowledge the insightful critiques and comments of the anonymous referees.

References

Autret N, Raynaud C, Dubail I, Berche P & Charbit A (2003) Identification of the agr locus of *Listeria monocytogenes*: role in bacterial virulence. *Infect Immun* **71**: 4463–4471.

Barber CE, Tang JL, Feng JX *et al.* (1997) A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. *Mol Microbiol* **24**: 555–566.

Bassler BL (2002) Small talk. Cell-to-cell communication in bacteria. *Cell* **109**: 421–424.

Bassler BL, Wright M & Silverman MR (1994) Multiple signalling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. *Mol Microbiol* **13**: 273–286.

Beaugerie L & Petit J-C (2004) Antibiotic-associated diarrhoea. *Best Pract Res Clin Ga* **18**: 337–352.

Boon C, Deng Y, Wang LH *et al.* (2008) A novel DSF-like signal from *Burkholderia cenocepacia* interferes with *Candida albicans* morphological transition. *ISME J* **2**: 27–36.

Camilli A & Bassler B (2006) Bacterial small-molecule signaling pathways. *Science* **311**: 1113–1116.

Carman RJ, Simon MA, Fernández H, Miller MA & Bartholomew MJ (2004) Ciprofloxacin at low levels disrupts colonization resistance of human fecal microflora growing in chemostats. *Regul Toxicol Pharm* **40**: 319–326.

Chen H & Fink GR (2006) Feedback control of morphogenesis in fungi by aromatic alcohols. *Gene Dev* **20**: 1150–1161.

Chen H, Fujita M, Feng Q, Clardy J & Fink GR (2004) Tyrosol is a quorum-sensing molecule in *Candida albicans*. *P Natl Acad Sci USA* **101**: 5048–5052.

Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL & Hughson FM (2002) Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* **415**: 545–549.

Deveau A, Piispanen AE, Jackson AA & Hogan DA (2010) Farnesol induces hydrogen peroxide resistance in *Candida albicans* yeast by inhibiting the Ras-cyclic AMP signaling pathway. *Eukaryot Cell* **9**: 569–577.

Déziel E, Lépine F, Milot S, He J, Mindrinos M, Tompkins R & Rahme L (2004) Analysis of *Pseudomonas aeruginosa* 4-hydroxy-2-alkylquinolines (HAQs) reveals a role for 4-hydroxy-2-heptylquinoline in cell-to-cell communication. *P Natl Acad Sci USA* **101**: 1339–1344.

Dow JM, Crossman L, Findlay K, He YQ, Feng JX & Tang JL (2003) Biofilm dispersal in *Xanthomonas campestris* is controlled by cell–cell signaling and is required for full virulence to plants. *P Natl Acad Sci USA* **100**: 10995–11000.

Duan K, Dammel C, Stein J, Rabin H & Surette MG (2003) Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. *Mol Microbiol* **50**: 1477–1491.

Fan J, Li Y, Vodovotz Y, Billiar TR & Wilson MA (2007) Neutrophil NAD(P)H oxidase is required for hemorrhagic shock-enhanced TLR2 up-regulation in alveolar macrophages in response to LPS. *Shock* **28**: 213–218.

Higgins D, Pomianek M, Kraml C, Taylor R, Semmelhack M & Bassler B (2007) The major *Vibrio cholerae* autoinducer and its role in virulence factor production. *Nature* **450**: 883–886.

Hogan DA (2006) Talking to themselves: autoregulation and quorum sensing in fungi. *Eukaryot Cell* **5**: 613–619.

Hogan DA & Kolter R (2002) *Pseudomonas–Candida* interactions: an ecological role for virulence factors. *Science* **296**: 2229–2232.

Hogan DA, Vik A & Kolter R (2004) A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. *Mol Microbiol* **54**: 1212–1223.

Hornby JM, Jensen EC, Lisec AD *et al.* (2001) Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. *Appl Environ Microb* **67**: 2982–2992.

Huang TP & Wong AC (2007) A cyclic AMP receptor protein-regulated cell–cell communication system mediates expression of a FecA homologue in *Stenotrophomonas maltophilia*. *Appl Environ Microb* **73**: 5034–5040.

Jacob F & Monod J (1961) Genetic regulatory mechanisms in the synthesis of proteins. *J Mol Biol* **3**: 318–356.

Jain C, Yun M, Politz SM & Rao RP (2009) A pathogenesis assay using *Saccharomyces cerevisiae* and *Caenorhabditis elegans* reveals novel roles for yeast AP-1, Yap1, and host dual oxidase BLI-3 in fungal pathogenesis. *Eukaryot Cell* **8**: 1218–1227.

Ji G, Beavis R & Novick R (1995) Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. *P Natl Acad Sci USA* **92**: 12055–12059.

Ji G, Beavis R & Novick R (1997) Bacterial interference caused by autoinducing peptide variants. *Science* **276**: 2027–2030.

Kleerebezem M, Quadri L, Kuipers O & De Vos W (1997) Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. *Mol Microbiol* **24**: 895–904.

Koh AY, Kohler JR, Coggshall KT, Van Rooijen N & Pier GB (2008) Mucosal damage and neutropenia are required for *Candida albicans* dissemination. *PLoS Pathog* **4**: e35.

Kontoyiannis DP & Lewis RE (2002) Antifungal drug resistance of pathogenic fungi. *Lancet* **359**: 1135–1144.

Kurtoglu S, Uzum K, Hallac IK & Coskum A (1997) 5-Hydroxy-3-indole acetic acid levels in infantile colic: is serotoninergic tonus responsible for this problem? *Acta Paediatr* **86**: 764–765.

Lazdunski A, Stewart G & Williams P (1995) Multiple *N*-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. *P Natl Acad Sci USA* **92**: 9427–9431.

Lewenza S, Visser MB & Sokol PA (2002) Interspecies communication between *Burkholderia cepacia* and *Pseudomonas aeruginosa*. *Can J Microbiol* **48**: 707–716.

Lo HJ, Kohler JR, DiDomenico B, Loebenberg D, Cacciapuoti A & Fink GR (1997) Nonfilamentous *C. albicans* mutants are avirulent. *Cell* **90**: 939–949.

Lorenz MC & Fink GR (2001) The glyoxylate cycle is required for fungal virulence. *Nature* **412**: 83–86.

Lorenz MC & Fink GR (2002) Life and death in a macrophage: role of the glyoxylate cycle in virulence. *Eukaryot Cell* **1**: 657–662.

Machan Z, Taylor G, Pitt T, Cole P & Wilson R (1992) 2-Heptyl-4-hydroxyquinoline *N*-oxide, an antistaphylococcal agent produced by *Pseudomonas aeruginosa*. *J Antimicrob Chemoth* **30**: 615–623.

Mashburn L & Whiteley M (2005) Membrane vesicles traffic signals and facilitate group activities in a prokaryote. *Nature* **437**: 422–425.

McCusker JH (2006) *Saccharomyces cerevisiae: an emerging and model pathogenic fungus*. ASM Press, Washington, DC.

McKnight SL, Iglewski BH & Pesci EC (2000) The *Pseudomonas* quinolone signal regulates rhl quorum sensing in *Pseudomonas aeruginosa*. *J Bacteriol* **182**: 2702–2708.

Menon V, Li D, Chauhan N, Rajnarayanan R, Dubrovska A, West AH & Calderone R (2006) Functional studies of the Ssk1p response regulator protein of *Candida albicans* as determined by phenotypic analysis of receiver domain point mutants. *Mol Microbiol* **62**: 997–1013.

Miller MB & Bassler BL (2001) Quorum sensing in bacteria. *Annu Rev Microbiol* **55**: 165–199.

Muh U, Schuster M, Heim R, Singh A, Olson E & Greenberg E (2006) Novel *Pseudomonas aeruginosa* quorum-sensing inhibitors identified in an ultra-high-throughput screen. *Antimicrob Agents Ch* **50**: 3674–3679.

Nickerson KW, Atkin AL & Hornby JM (2006) Quorum sensing in dimorphic fungi: farnesol and beyond. *Appl Environ Microb* **72**: 3805–3813.

Normanly J & Bartel B (1999) Redundancy as a way of life – IAA metabolism. *Curr Opin Plant Biol* **2**: 207–213.

Novick R (2003) Autoinduction and signal transduction in the regulation of staphylococcal virulence. *Mol Microbiol* **48**: 1429–1449.

Ohtani K, Yuan Y, Hassan S, Wang R, Wang Y & Shimizu T (2009) Virulence gene regulation by the agr system in *Clostridium perfringens*. *J Bacteriol* **191**: 3919–3927.

Otto M, Süßmuth R, Vuong C, Jung G & Götz F (1999) Inhibition of virulence factor expression in *Staphylococcus aureus* by the *Staphylococcus epidermidis* agr pheromone and derivatives. *FEBS Lett* **450**: 257–262.

Parsek MR & Greenberg EP (2005) Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends Microbiol* **13**: 27–33.

Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, Greenberg EP & Iglewski BH (1999) Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *P Natl Acad Sci USA* **96**: 11229–11234.

Peters BM, Jabra-Rizk MA, Scheper MA, Leid JG, Costerton JW & Shirtliff ME (2010) Microbial interactions and differential protein expression in *Staphylococcus aureus–Candida albicans* dual-species biofilms. *FEMS Immunol Med Mic* **59**: 493–503.

Prusty R, Grisafi P & Fink G (2004) The plant hormone indoleacetic acid induces invasive growth in *Saccharomyces cerevisiae*. *P Natl Acad Sci USA* **101**: 4153–4157.

Rao RP, Hunter A, Kashpur O & Normanly J (2010) Aberrant synthesis of indole-3-acetic acid in *Saccharomyces cerevisiae* triggers morphogenic transition, a virulence trait of pathogenic fungi. *Genetics* **185**: 211–220.

Rasko DA & Sperandio V (2010) Anti-virulence strategies to combat bacteria-mediated disease. *Nat Rev Drug Discov* **9**: 117–128.

Riedel K, Hentzer M, Geisenberger O *et al.* (2001) *N*-acylhomoserine-lactone-mediated communication between *Pseudomonas aeruginosa* and *Burkholderia cepacia* in mixed biofilms. *Microbiology* **147**: 3249–3262.

Ryan RP, Fouhy Y, Garcia BF *et al.* (2008) Interspecies signalling via the *Stenotrophomonas maltophilia* diffusible signal factor influences biofilm formation and polymyxin tolerance in *Pseudomonas aeruginosa*. *Mol Microbiol* **68**: 75–86.

Sakinc T, Kulczak P, Henne K & Gatermann S (2006) Cloning of an agr homologue of *Staphylococcus saprophyticus*. *FEMS Microbiol Lett* **237**: 157–161.

Schaefer A, Val D, Hanzelka B, Cronan J & Greenberg E (1996) Generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. *P Natl Acad Sci USA* **93**: 9505–9509.

Schauder S & Bassler BL (2001) The languages of bacteria. *Gene Dev* **15**: 1468–1480.

Schuster M & Peter Greenberg E (2006) A network of networks: quorum-sensing gene regulation in *Pseudomonas aeruginosa*. *Int J Med Microbiol* **296**: 73–81.

Semighini CP, Hornby JM, Dumitru R, Nickerson KW & Harris SD (2006) Farnesol-induced apoptosis in *Aspergillus nidulans* reveals a possible mechanism for antagonistic interactions between fungi. *Mol Microbiol* **59**: 753–764.

Semighini CP, Murray N & Harris SD (2008) Inhibition of *Fusarium graminearum* growth and development by farnesol. *FEMS Microbiol Lett* **279**: 259–264.

Shirtliff ME, Peters BM & Jabra-Rizk MA (2009) Cross-kingdom interactions: *Candida albicans* and bacteria. *FEMS Microbiol Lett* **299**: 1–8.

Slater H, Alvarez-Morales A, Barber CE, Daniels MJ & Dow JM (2000) A two-component system involving an HD-GYP

domain protein links cell–cell signalling to pathogenicity gene expression in *Xanthomonas campestris*. *Mol Microbiol* **38**: 986–1003.

Surette MG, Miller MB & Bassler BL (1999) Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *P Natl Acad Sci USA* **96**: 1639–1644.

Taga ME & Bassler BL (2003) Chemical communication among bacteria. *P Natl Acad Sci USA* **100** (suppl 2): 14549–14554.

Venturi V, Friscina A, Bertani I, Devescovi G & Aguilar C (2004) Quorum sensing in the *Burkholderia cepacia* complex. *Res Microbiol* **155**: 238–244.

Walters M & Sperandio V (2006) Quorum sensing in *Escherichia coli* and *Salmonella*. *Int J Med Microbiol* **296**: 125–131.

Wang LH, He Y, Gao Y *et al.* (2004) A bacterial cell–cell communication signal with cross-kingdom structural analogues. *Mol Microbiol* **51**: 903–912.

Woodward AW & Bartel B (2005) Auxin: regulation, action, and interaction. *Ann Bot (London)* **95**: 707–735.

Xavier KB & Bassler BL (2003) LuxS quorum sensing: more than just a numbers game. *Curr Opin Microbiol* **6**: 191–197.

Xavier KB & Bassler BL (2005) Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. *J Bacteriol* **187**: 238–248.

Xu XL, Lee RT, Fang HM *et al.* (2008) Bacterial peptidoglycan triggers *Candida albicans* hyphal growth by directly activating the adenylyl cyclase Cyr1p. *Cell Host Microbe* **4**: 28–39.