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New Developments in Microbial Interspecies Signaling

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Summary

There is a growing appreciation that in addition to well-documented intraspecies quorum sensing systems [1], small molecules act as signals between microbes of different species [2]. This review will focus on how bacterial small molecules modulate these interspecies interactions. We will particularly emphasize complex relationships such as those between microbes and insects, interactions resulting in non-antagonistic outcomes (i.e. developmental and morphological processes), how co-culture can lead to the discovery of new small molecules, and the use of known compounds to evoke unexpected responses and mediate crosstalk between microbes.

Introduction

Historically, interspecies interactions have focused on growth-inhibitory interactions, yet a variety of phenotypic outcomes other than antibiosis are possible, including alterations in developmental processes such as sporulation and biofilm formation or production of secondary metabolites (Fig 1). Over the years, studies of antibiosis have undoubtedly led to a deeper understanding of how microbes relate to a major component of their natural environments—their fellow microbes—as well as to the discovery of clinically-useful compounds. Examining interspecies interactions using a broader framework that encompasses both alternative signals and more diverse responses will accordingly continue to advance these vital fields.

The last few years have seen a surge of studies [2] covering all aspects of these possible interactions (Fig. 1). Detecting phenotypic or developmental biomodulation between two organisms can indicate when they are communicating via small molecules, and thus can denote the presence of overlooked compounds. In other cases, signaling has been shown to occur via “repurposed” compounds—known molecules that are functioning in an unexpected manner. One exciting potential result of interspecies interactions is the induction of novel secondary metabolite production by the responding organism. Thus, examination of microbial relationships can lead to the discovery of new molecules—in some cases as the small molecule mediating the interaction, and in others as the consequent *result* of two microbes interacting.

The scope of this article will be limited primarily to microbial interactions, although a few studies are referenced that highlight the complex relationship that microbes have with multicellular eukaryotes, and all demonstrate how little we understand of the complicated interplay occurring between microbes and the potential chemical eavesdropping occurring between them (Table 1).

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I. Alliances and Antagonisms

A. Microbial-Eukaryotic

Here we focus on insects that have evolved specific associations with fungi and bacteria, a biological context that has selected the evolution of myriad antagonistic and beneficial interactions that highlight microorganisms' ability to exert exquisite biological specificity in mediating their interactions.

Attine ants grow fungal cultivars as food, and have been shown to have co-evolved with both their food cultivar and actinomycetes that help protect their food from being infected by a parasitic *Escovopsis* fungus [3]. There is specificity in both the attraction and repulsion between these two sets of fungi and these conflicting forces explain why in natural environments particular *Escovopsis* fungi infect only a restricted set of the food fungi [4]. Although the active compounds driving these responses are not yet known, *Escovopsis* spp. are attracted to and grow especially on cultivars that are hosts to that parasite, and the food cultivars produce compounds that actively inhibit the growth of other *Escovopsis* strains [4].

The southern pine beetle, *Dendeoctonus frontalis*, exemplifies another example of the intriguing symbioses between the insect, fungal, and bacterial worlds. The beetle is symbiotically associated with an *Entomocorticium* sp. fungus that helps nourish the beetles' larvae, but an antagonistic fungus, *Ophiostoma minus*, can outcompete this beneficial symbiont to the detriment of the beetle larvae [5]. An actinomycete bacterium mediates the retention of the beneficial fungus by producing mycangimycin, a novel linear polyene peroxide antifungal that selectively inhibits only the antagonistic fungus and not the symbiotic one [5]. This discovery shows that examining insect symbioses can reveal not only new biology, but also interspecies signaling molecules, some of which will be chemically novel.

The importance of how the larger microbial context can influence biological activity was highlighted in a study that overturned a long-asserted understanding regarding the mechanism of the anti-insecticidal activity of *Bacillus thuringiensis*. The presence of the insect mid-gut microbiota (in particular an *Enterobacter* sp.) was shown to be required for anti-insecticidal activity, and the *B. thuringiensis* toxin alone—in the absence of enteric bacteria—was insufficient to kill insects [6].

The biology of the fungus *Fusarium* also underscores the significance of microbial context. Some strains act as plant pathogens, while others are protective agents against pathogenic *Fusarium* strains [7]. The non-pathogenic *Fusarium* are associated with a consortium of endosymbiotic bacteria that alter fungal gene expression and eliminate their ability to invade plants [7]. The protective capacity of these non-pathogenic strains is explained because *Fusarium* associated with its endosymbionts—but not the endosymbionts alone—produce volatile sesquiterpenes that repress virulence genes in pathogenic *Fusarium* strains [8].

B. Microbial-Microbial

Two recent studies follow-up on phenomena described years ago. *Pseudomonas aeruginosa* and *Staphylococcus aureus*, two human-associated bacteria, have been known since the 1950s to have a paradoxical relationship in which 4-hydroxy-2-heptylquinoline-*N*-oxide (HQNO) produced by *P. aeruginosa* suppresses the respiration of *S. aureus*, but also increases its resistance to antibiotics in co-culture. It was shown that HQNO selects for small colony variants —a form of *S. aureus* that conveys antibiotic resistance [9]. HQNO was only detected in the sputum of cystic fibrosis patients infected with *P. aeruginosa*, highlighting the potential relevance of the interaction of these pathogens in clinical cases [9].

Since the 1970s, *Streptococcus sanguinis*, a commensal oral bacterium, and *Streptococcus mutans*, a common resident of the mouth that contributes to dental caries, have been known to be antagonistic towards one another, but no chemical mediators were known. It was demonstrated that *S. sanguinis* makes hydrogen peroxide (H_2O_2) and *S. mutans* produces mutacins (bacteriocins), each at levels sufficient to effectively inhibit each other [10]. Interestingly, the production of these inhibitory compounds is *decreased* in co-culture versus mono-culture via an unknown mechanism [10].

Streptococcus oligofermentas is another oral bacterium that inhibits *S. mutans* by producing H_2O_2 either from peptone or lactic acid [11,12]. In this case there is an additional twist: *S. mutans* produces the lactic acid substrate, which typically acts as an inhibitory compound towards other microbes [11]. This illustrates how a microbial competitor may turn a potentially antagonistic signal produced by *S. mutans* against itself in its environmental setting.

II. New Roles for Known Molecules

A. Peptidoglycan

Peptidoglycan and cell wall fragments are increasingly being recognized as important signaling molecules [13], and recent studies continue to elucidate additional roles for these microbial products.

1. Growth—In a microbial interaction of unknown specificity, both *Bacillus cereus* and its purified peptidoglycan stimulated the growth of rhizosphere bacteria from the *Cytophaga-Flavobacterium* group in a medium containing root exudates in a manner that did not depend on either *B. cereus* sporulation or growth inhibition [14]. The rhizosphere bacteria produce a cell-wall degrading enzyme that presumably permits them to mobilize *B. cereus* peptidoglycan fragments as a carbon source for their growth, although the details of what the degradative enzyme is, or whether it is preferentially expressed in the presence of root exudate as the data suggests are not clear [14].

2. Development—Recent studies examine how peptidoglycan acts as a signal influencing microbial development. A beautiful combination of chemistry and biochemistry was used to identify peptidoglycan fragments in human serum that are powerful inducers of hyphae in *Candida albicans* [15]. In a splendid example delineating how a responding cell detects and transduces a signal, purified muramyl dipeptides were shown to enter fungal cells and bind to a specific adenylate cyclase that induced cAMP production and hyphal growth [15]. While initially this study seems to involve an exclusively eukaryotic interaction, an intriguing aspect is that mammals cannot produce muramic acid, suggesting that the high quantities of these compounds found in human serum are originally produced by bacterial intestinal microbiota [15].

Another fascinating study investigated how disaccharide tripeptides stimulated germination in *Bacillus subtilis* at concentrations equivalent to that available from a single cell [16]. A peptidoglycan-specific receptor was identified that was a eukaryotic-like serine/threonine membrane kinase and was localized in the inner membrane of the spore [16]. In addition to phosphorylating a ribosomal GTPase potentially responsible for the downstream effects of peptidoglycan-fragment binding, this receptor kinase is specific for disaccharide tripeptides containing diaminopimelic acid and does not bind to those containing lysine [16]. This is germane because the composition of different species' peptidoglycan differs and thus only disaccharide tripeptides from certain bacteria stimulate *B. subtilis* to germinate [16]. Finally, natural products such as staurosporine (produced by *Streptomyces* spp.) were shown to control germination even in the absence of a peptidoglycan cue by directly interacting with this receptor kinase, hinting at potentially relevant interspecies signaling [16].

The predator *Myxococcus xanthus* responds to prey signals by altering both its chemotactic and developmental patterns. Groups of *M. xanthus* cells form ripples as a predatory behavior in response to direct contact with *B. subtilis*, *Escherichia coli*, or *Saccharomyces cerevisiae* prey [17]. Peptidoglycan and some of its components were previously known as rippling signals [18], but other cell components such as DNA and insoluble cell fractions from organisms that do not contain peptidoglycan also stimulate rippling behavior [17]. These macromolecules only stimulated rippling upon physical contact with *M. xanthus*, and their breakdown into their component parts diminished their activity [17]. The availability of prey was also shown to spatially direct *M. xanthus*' development into fruiting bodies in a manner that is not simply due to changes in nutrient levels [19]. Thus, the presence of prey governs both chemotaxis and development in this organism.

3. Secondary Metabolite Production—Finally, the peptidoglycan component N-acetylglucosamine blocks development and antibiotic production of *Streptomyces coelicolor* on nutrient-rich media through the transcriptional regulator DasR, while both processes are stimulated on nutrient-poor media [20]. An intriguing aspect of this work is that transcription stimulation of an otherwise cryptic biosynthetic gene cluster was observed [20].

B. Antibiotics

1. At Subinhibitory Concentrations—Antibiotics are likely the best known bacterial secondary metabolites. Now there is a growing appreciation that they can act as signaling molecules in their own right and mediate outcomes other than death [21]. Unfortunately, many recent papers exploring this hypothesis have used derivatives of natural antibiotics or synthetic ones, making it difficult to argue that the observed effects are representative of natural microbial interactions. That said, subinhibitory levels of various antibiotics were seen to variously upregulate expression of SOS-response and methyl-mismatch repair genes [22], decrease biofilm mass [23], and alter virulence factor expression in different bacteria [24,25]. It is challenging to consolidate these disparate results into a straightforward model of how antibiotics at subinhibitory concentrations affect bacteria, but they encourage us to consider the potentially pleiotropic and complicated responses of bacteria to small molecule secondary metabolites.

2. Altering Morphology—Phenazines—redox-active, pigmented antibiotics—have recently been shown to have a role controlling colony morphology in *P. aeruginosa*, and a similar phenotype was seen in *S. coelicolor* in response to its own pigmented antibiotics [26]. This effect was mediated in both organisms through a related transcriptional regulator, SoxR, and demonstrates how secondary metabolites can alter development in unexpected ways. In the future, it will be exciting to determine whether there is any crosstalk between these different signaling systems in these organisms.

C. Signaling System Crosstalk

1. Autoinducer-2—The well-characterized quorum-sensing molecule autoinducer-2 (AI-2) dictates the ability of the oral microbes *Actinomyces naeslundii* and *Streptococcus oralis* to form dual-species biofilms in flowing saliva under experimental conditions analogous to their natural setting [27]. Only very low concentrations of AI-2 were optimal for biofilm formation, reminding us of the precision with which organisms sense signals and that in the natural environment the concentrations of small molecules mediating interactions may be well below those observed in typical laboratory growth conditions [27].

2. Fatty Alcohols and Acids—Exploration of related signaling systems in different organisms has revealed biologically-relevant crosstalk (Fig. 2). In particular, intraspecies small molecules signals may modulate the microbial development of different species. The

sesquiterpene farnesol is an intraspecies signal for *C. albicans* that inhibits filamentation [28] and the diffusible signal factor (DSF) from *Xanthomonas campestris*—cis-11-methyl-2-dodecenoic acid—controls its biofilm formation and virulence capacities [29-31]. DSF can act as an interspecies signal as well, mimicking the effect of farnesol on *C. albicans* [32]. A novel signaling molecule from *Burkholderia cenocepacia*, BDSF—cis-2-dodecenoic acid—has been identified that restores biofilm production in *X. campestris* DSF-deficient mutants and inhibits *C. albicans* hyphal growth either as a pure compound or in co-culture [33]. Thus BDSF functions similarly to DSF with regards to *X. campestris*, and similarly to both DSF and farnesol by inhibiting hyphae formation in *C. albicans*; however, farnesol does not appear to be effective at stimulating DSF-controlled genes in *X. campestris* [32].

Farnesol does affect *P. aeruginosa*, however [34]. Farnesol alters the activity of a transcriptional regulator in *P. aeruginosa* when applied as a purified compound or during *C. albicans* co-culture, decreasing production of the secondary metabolites pyocyanin and PQS [34] and decreasing swarming [35]. Considering the ability of farnesol and DSF to mediate crosstalk between fungi and bacteria, one wonders whether DSF also has an effect on *P. aeruginosa* [34]. DSF from *Stenotrophomonas maltophilia* [36] indeed affects *P. aeruginosa*, stimulating the development of filamentous biofilms and increasing its resistance to the antibiotic polymyxin [37]. The sensor kinase in *P. aeruginosa* responsible for mediating this response was identified, and has homologs in many pseudomonads, suggesting that interspecies communication between the pseudomonads and xanthomonads may be common [37]. The sensor kinase receptor was also shown to be specific to DSF, not responding to either dodecanoic acid or farnesoic acid [37]. Thus, although both DSF and farnesol affect *C. albicans* in similar ways, they may instead function in parallel signaling pathways in *P. aeruginosa*. Indeed, this is one of the more intriguing aspects of interspecies signaling crosstalk—how related signals are recognized by different organisms, and whether their downstream signaling systems are analogous or different. DSF triggering different downstream effects in two related *Xanthomonas* spp. [38] and homologous two-component systems in *X. campestris* and *Xylella fastidiosa* resulting in different virulence regulation [39] are examples that illustrate this diversity of effects.

Finally, exciting work shows that *P. aeruginosa* produces a newly identified fatty acid, cis-2-decenoic acid, that is structurally related to DSF and BDSF (Fig. 2) [40]. Cis-2-decenoic acid is capable of completely dispersing biofilms from a diverse range of microorganisms (not only *P. aeruginosa*, but also *E. coli*, *Streptococcus pyogenes*, *B. subtilis*, and *C. albicans* among others) as well as preventing the biofilms from forming initially [40]. Although the structural similarity of these two compounds leads one to speculate that DSF is hijacking the native cis-2-decenoic acid receptor, the disparate resulting phenotypes observed suggest potentially distinct receptors with different downstream effects. These findings demonstrate that crosstalk between related signaling systems can occur (Fig. 2), and imply that we have only begun to understand how related signals and receptors are involved in interspecies communication.

III. Identifying New Compounds Using Co-culture

A. Eliciting Directly

Many bacteria have the genetic capacity to produce numerous and chemically diverse secondary metabolites but do not do so under common laboratory conditions [41]. Provoking production of such compounds is a particularly exciting potential outcome of interspecies interactions. Two recently published studies describe the stimulation of new compound production in co-cultures of marine bacteria and fungi, in striking examples that biological interactions can elicit the production of novel compounds [42,43]. The mechanisms of induction are still unclear, although in one case it is mediated by cell-cell contact [42].

The induction of a novel aminoglycoside with a new ring structure resulted from the competitive co-culture of *Rhodococcus fascians* with *Streptomyces padanus* [44]. Surprisingly, this phenomenon appears to be the result not of a diffusible small molecule, but due to the horizontal gene transfer of DNA from the actinomycete [44]. This unexpected result underscores the possible novel interaction mechanisms and phenotypic consequences that might result from interspecies interactions in the wild.

B. Using Development as Readout

Antibiosis is certainly not the only consequence of interspecies interactions, although it is often the easiest to observe. However, by looking for other types of responses in co-culture (Fig. 1) it is possible to identify small molecule signals, some of which may be structurally and biologically interesting.

This approach was validated by characterizing the development and secondary metabolite production of *B. subtilis* and *S. coelicolor* in co-culture. When grown together, *S. coelicolor*'s development of aerial hyphae and sporulation were inhibited; a similar effect was observed between a range of *Bacillus* and *Streptomycete* spp. [45]. The active compound modulating these processes was the secondary metabolite surfactin, produced by *B. subtilis*. While this compound was previously known, this result is noteworthy because it clearly demonstrated that developmental phenotypes observed in co-culture can lead to the identification of the small molecules mediating the effect and revealed a new biological role for this secondary metabolite [45].

Having characterized the co-culture phenotype of these two organisms, a *B. subtilis* transposon mutant library was used to identify developmental deviations [46]. In this way the compound bacillaene, produced by a gene cluster previously believed to be cryptic in *B. subtilis*, was shown to delay the production of a pigmented antibiotic in *S. coelicolor* and also inhibit the growth of *S. avermitilis* [46,47]. Thus, by examining the interaction of two species and observing alterations in secondary metabolite production, a cryptic signaling molecule with a new biological role was identified.

IV. Missing Pieces…

A number of studies have observed co-culture phenotypes mediated by unknown signals, while others have identified new metabolites that could act as signals. These tantalizing studies point to potential interactions that may provide a starting point for future work.

A. Growth Enhancement

It has been suggested that one reason environmental bacteria do not grow under laboratory conditions is because of a lack of signals from their microbial neighbors [48], an idea supported by the finding that a higher diversity of organisms were obtained by incubating sediment samples in quasi-natural environments than with traditional laboratory culturing techniques [49]. The signals involved in facilitating this growth are undefined, however, and may well be non-species-specific.

B. Biofilm Formation

Biofilms are relevant to many human diseases and natural biofilms are frequently composed of multiple species. These features have inspired interest in interspecies interactions in biofilm formation. Many microbes form better biofilms in combination with other organisms or in the presence of their partners' diffusible compounds, as has been demonstrated for both the oral microbes *Fusobacterium nucleatum* with either *Staphylococcus epidermidis* or *Porphyromonas gingivalis* [50] as well as *S. cerevisiae* and *Lactobacillus casei* [51]. The

results were less clear-cut in work that examined biofilm formation of dual-species mixes of bacteria isolated from drinking water, in which some co-cultures exhibited enhancement and others antagonism via unknown mechanisms [52]. In work that lays the groundwork for studying a naturally-occurring, simple gut symbiosis, the spatial arrangement, dynamics, and synergistic interactions of the two organisms found in the medicinal leech gut—a *Rikenella* and *Aeromonas* sp.—were determined, and may provide insight into other more complex digestive-tract communities [53].

Many naturally-produced compounds have been shown to affect biofilm development in individual microbes, but have not been shown to exert their effect when produced by a signaling partner in a co-culture context. The polyamine norspermine and glycine betaine both increase biofilm cell density in *Vibrio cholerae* and may be produced by organisms in this bacterium's natural marine environment [54,55]. *P. aeruginosa* had an altered biofilm morphology when grown on mucin surfaces compared to other polymeric substrates, a phenotype particularly prominent on mucin from human respiratory tracts [56]. Meanwhile, nitric oxide, a known signal for microbial and eukaryotic cells, was shown to disperse cells from *P. aeruginosa* biofilms, although it is unclear which organism may exert such an influence in natural environments [57].

C. New Small Molecules

A thought-provoking paper describes a new intraspecies communication molecule in *Rhodopseudomonas palustris*, p-coumaryl-homoserine lactone, produced by a LuxI homolog [58]. This molecule is related to the well-known acyl-homoserine lactones but is produced from p-coumaric acid rather than a fatty acid. Interestingly, the p-coumaric acid must be obtained from the environment or an as-yet-unidentified plant partner [58]. This result raises the exciting possibility that other bacterial LuxI homologs produce—not canonical acyl homoserine lactones—but potentially a whole range of new signaling molecules [58]. It remains to be seen whether particular p-coumarate-producing partners have a specific interaction with *R. palustris*.

Conclusions

The last years have brought an explosion of work investigating many aspects of interspecies interactions, and revealed myriad new signal molecules, communicating partners, and phenotypic responses. In most cases we still have much to learn about some aspect of these systems, chiefly how these signals are detected and transduced within the responding cell. This area deserves attention, particularly in those systems in which crosstalk between organisms seems likely, and considering the indications that many intraspecies signaling molecules may also have roles affecting other microbes. A number of exciting new intraspecies signal systems have been uncovered in the last few years [59-62]; an exciting possibility is that these small molecule signals may also modulate the physiology of *other* microbes in as-yet-undiscovered interspecies interactions.

Finally, the genome sequences of many bacteria have revealed a huge biosynthetic capacity for producing small molecules [41]. These gene clusters frequently encode compounds that are not produced under laboratory conditions and thus have no known chemical structure or biological role. This mysterious biosynthetic potential begs the question of what the ecological function of such compounds might be—recent work indicates the possibility that they function in interspecies interactions.

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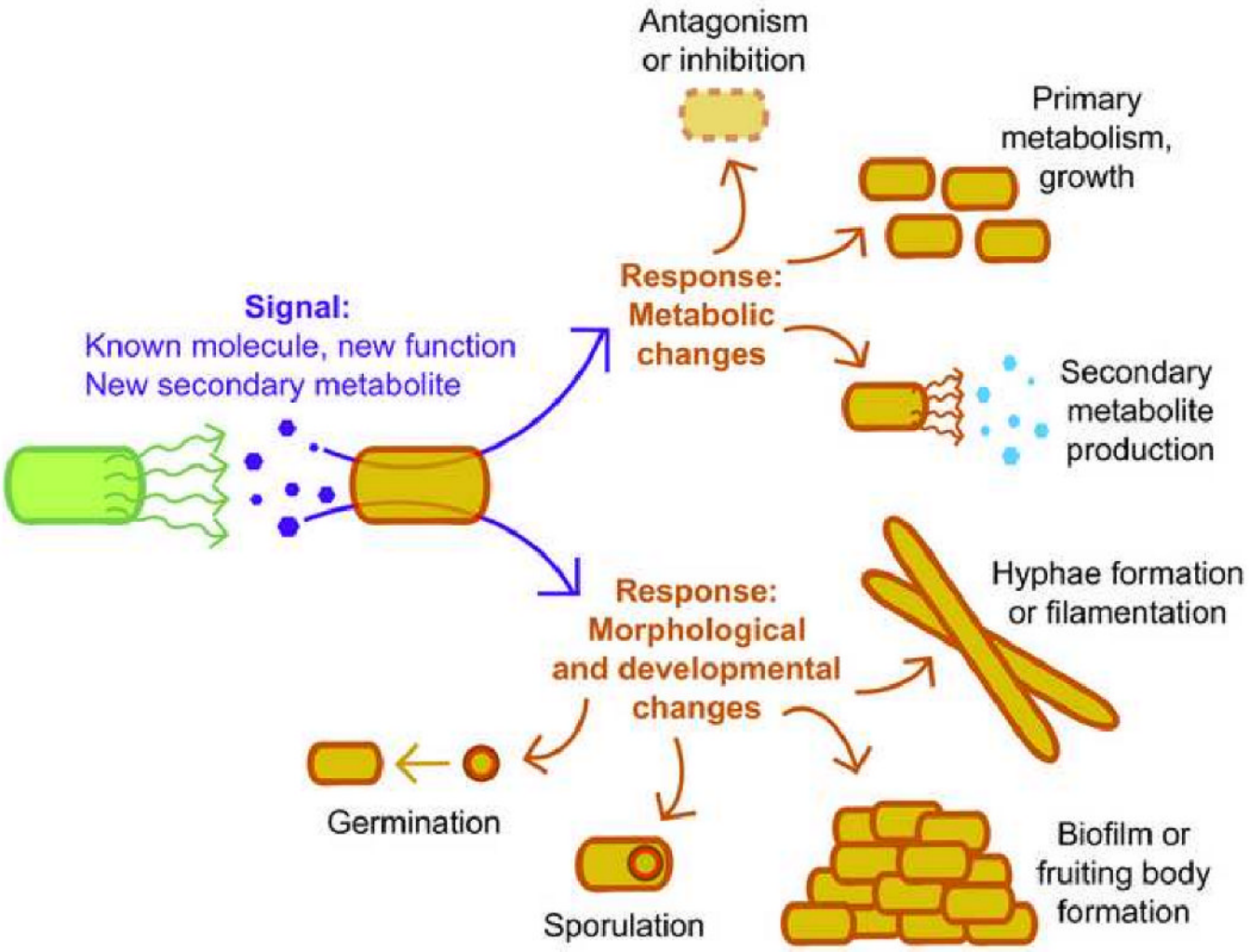

Figure 1. Schematic illustrating potential interspecies interactions

An interaction between two microbes is illustrated on the left of the figure, with the green microbe producing a signal (purple hexagons) that causes the orange microbe to respond in one of the manners illustrated on the right. The signals discussed here fall primarily into two classes: known metabolites (such as peptidoglycan, antibiotics, and intraspecies signals) that cause unexpected responses affecting other microbial speces, and novel secondary metabolites; in some cases the signals are still unknown. Upon detecting the signal, the responding organism may experience changes in metabolism (growth inhibition or stimulation, or production of new small molecules) or morphological and developmental changes (alterations in cell shape or morphology; production of biofilms or fruiting bodies; or specialized processes such as sporulation and germination). More than one response is possible to a single signal.

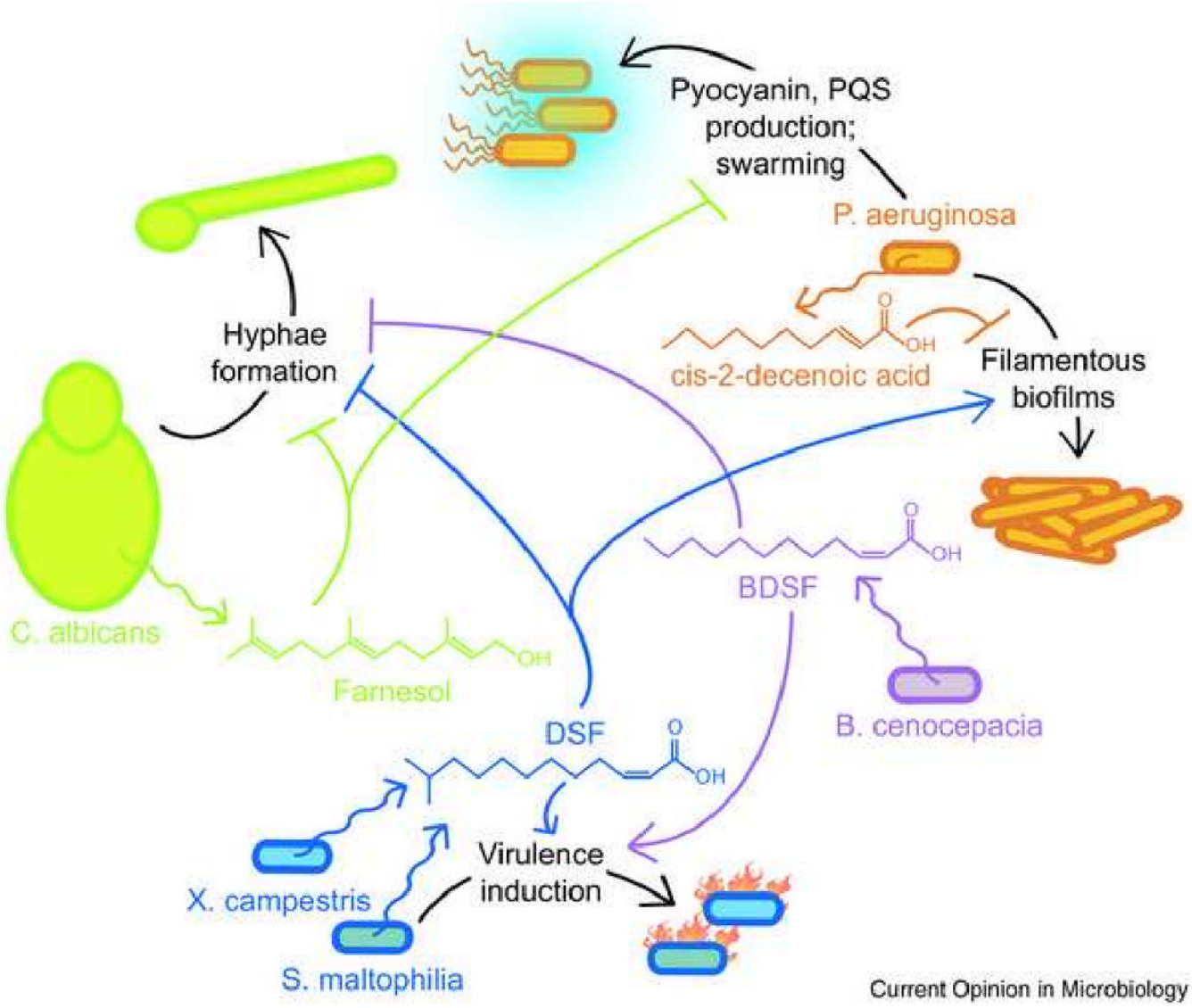

Figure 2. Schematic illustrating signal cross-talk between organisms

Some fatty acids and alcohols produced by fungi and bacteria have roles as interspecies signals. The wavy arrows indicate the production of small molecules by particular microbes; if known, the effects of these compounds on the behavior of other organisms is indicated by smooth arrow- or bar-headed lines. See text in section IIC for more details.

Table 1

Tabulated interspecies interactions discussed in this paper

This table lists the recently-described interactions discussed. The first column indicates the review section they are included in. (F) indicates a fungal participant; N/A indicates not applicable; N/D indicates not determined.

	Signal-producing organism	Small molecule signal	Responding organism	Response	Ref.
IA	**Alliances and Antagonisms: Microbial-Eukaryotic**				
	Attine ant food cultivar (F)	N/D	*Escovopsis* spp. (F)	Growth inhibition or growth attraction	[4]
	Streptomyces thermosacchari	Mycangimycin	*Entomocorticium* sp. (F)	No effect	[5]
	Streptomyces thermosacchari	Mycangimycin	*Ophiostoma minus* (F)	Growth inhibition	[5]
	Enterobacter sp.	N/D	*Bacillus thuringiensis*	Insecticidal toxin activation	[6]
	Bacillus thuringiensis AND *Enterobacter* sp.	Active inseticidal toxin	Insect larvae	Death	[6]
	Endosymbiotic bacteria	N/D	*Fusarium* sp. (F)	Virulence inhibition	[7]
	Fusarium sp. AND endosymbiotic bacteria	Volatile sesquiterpenes	*Fusarium* sp. (F)	Virulence inhibition	[8]
IB	**Alliances and Antagonisms: Microbial-Microbial**				
	Pseudomonas aeruginosa	HQNO	*Staphylococcus aureus*	Small colony variant formation	[9]
	Streptococcus sanguinis	H_2O_2	*Streptococcus mutans*	Growth inhibition	[10]
	Streptococcus mutans	Mutacins (bacteriocins)	*Streptococcus sanguinis*	Growth inhibition	[10]
	Streptococcus oligofermentas	H_2O_2	*Streptococcus mutans*	Growth inhibition	[11]
IIA	**New roles for known molecules: Peptidoglycan**				
	Bacillus cereus	Peptidoglycan: from vegetative cells	*Cytophaga-Flavobacterium* rhizosphere bacteria	Growth stimulation	[14]
	Unknown gut microbiota	Peptidoglycan: muramyl dipeptides	*Candida albicans* (F)	Hyphae stimulation	[15]
	Diaminopimelic acid-peptidoglycan producing bacteria	Peptidoglycan: disaccharide tripeptides	*Bacillus subtilis*	Germination stimulation	[16]
	Streptomyces spp.	Staurosporine	*Bacillus subtilis*	Germination stimulation	[16]
	Bacillus subtilis, Escherichia coli, Saccharomyces cerevisiae (F)	Peptidoglycan; DNA; insoluble cell fractions	*Myxococcus xanthus*	Rippling	[17]
	Availability of prey	N/D; not nutrients	*Myxococcus xanthus*	Fruiting body formation	[19]
	Unknown	N-acetylglucosamine	*Streptomyces coelicolor*	Antibiotic production altered	[20]
	Unknown	N-acetylglucosamine	*Streptomyces coelicolor*	Secondary metabolite induction	[20]
IIB	**New roles for known molecules: Antibiotics**				
	N/A	Fluoroquinolones (synthetic)	*Staphylococcus aureus*	SOS-response and methyl-mismatch repair upregulation	[22]
	N/A	Azithromycin	*Haemophilus influenzae*	Biofilm inhibition	[23]

	Signal-producing organism	Small molecule signal	Responding organism	Response	Ref.
	N/A	Azithromycin, ceftazidime, ciprofloxacin	*Pseudomonas aeruginosa*	Virulence inhibition	[24]
	N/A	Tobramycin and tetracycline	*Pseudomonas aeruginosa*	Biofilm stimulation	[25]
	Pseudomonas aeruginosa	Phenazines	*Pseudomonas aeruginosa*	Complex colony morphology repression	[26]
IIC	**New roles for known molecules: Signaling system cross-talk**				
	Streptococcus oralis	Autoinducer-2	*Actinomyces naeslunii*	Biofilm stimulation	[27]
	Candida albicans (F)	Farnesol	*Candida albicans* (F)	Hyphae inhibition	[28]
	Xanthomonas campestris	DSF	*Xanthomonas campestris*	Biofilm alteration; Virulence stimulation	[29,30]
	Xanthomonas campestris	DSF	*Candida albicans* (F)	Hyphae inhibition	[32]
	Burkholderia cenocepacia	BDSF	*Xanthomonas campestris*	Virulence stimulation	[33]
	Burkholderia cenocepacia	BDSF	*Candida albcans* (F)	Hyphae inhibition	[33]
	Candida albicans (F)	Farnesol	*Pseudomonas aeruginosa*	Secondary metabolite and swarming inhibition	[34] [35]
	Stenotrophomonas maltophilia	DSF	*Pseudomonas aeruginosa*	Biofilm filamentation stimulation	[37]
	Escherichia coli Bacillus subtilis Candida albicans (F), other organisms	cis-2-decenoic acid	*Pseudomonas aeruginosa*	Biofilm dispersal	[40]
IIIA	**Identifying new compounds using co-culture: Eliciting directly**				
	Marine α-proteobacterium	N/D	*Libertella* sp. (F)	Secondary metabolite induction	[42]
	Salinispora arenicola	N/D	*Emericella* sp. (F)	Secondary metabolite induction	[43]
	Streptomyces padanus	Horizontal gene transfer	*Rhodococcus fascians*	Secondary metabolite induction	[44]
IIIB	**Identifying new compounds using co-culture: Using development as read-out**				
	Bacillus subtilis	Surfactin	*Streptomyces coelicolor*	Sporulation and aerial hyphae inhibition	[45]
	Bacillus subtilis	Bacillaene	*Streptomyces coelicolor*	Secondary metabolite inhibition	[46]
IVB	**Missing pieces: Biofilm formation**				
	Fusobacterium nucleatum	N/D	*Staphylococcus epidermidis*	Biofilm stimulation	[50]
	Fusobacterium nucleatum	N/D	*Porphyromonas gingivalis*	Biofilm stimulation	[50]
	Saccharomyces cerevisae (F)	N/D	*Lactobacillus casei*	Biofilm stimulation	[51]
	N/D	Norspermine	*Vibrio cholerae*	Biofilm stimulation	[54]
	N/D	Glycine betaine	*Vibrio cholerae*	Biofilm stimulation	[55]
	Human respiratory tracts	Mucin	*Pseudomonas aeruginosa*	Biofilm alteration	[56]
	N/D	Nitric oxide	*Pseudomonas aeruginosa*	Biofilm dispersal	[57]
IVC	**Missing pieces: New small molecules**				
	Unknown plant?	p-coumaryl-homoserine lactone	*Rhodopseudomonas palustris*	17-gene regulon	[58]