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Review

DSF-family quorum sensing signal-mediated intraspecies, interspecies, and inter-kingdom communication

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While most bacteria are unicellular microbes they communicate with each other and with their environments to adapt their behaviors. Quorum sensing (QS) is one of the best-studied cell–cell communication modes. QS signaling is not restricted to bacterial cell-to-cell communication – it also allows communication between bacteria and their eukaryotic hosts. The diffusible signal factor (DSF) family represents an intriguing type of QS signal with multiple roles found in diverse Gram-negative bacteria. Over the last decade, extensive progress has been made in understanding DSF-mediated communication among bacteria, fungi, insects, plants, and zebrafish. This review provides an update on these new developments with the aim of building a more comprehensive picture of DSF-mediated intraspecies, interspecies, and inter-kingdom communication.

Highlights

DSFs are *cis*-2-unsaturated fatty acids containing fatty acid carbon chains of various lengths and *cis* double-bond configurations. DSF-producing bacteria are widely distributed in nature.

Based on their genomic origins, DSF-mediated intraspecies communication pathways are generally affiliated to three different groups, respectively represented by *Xanthomonas campestris* pv. *campestris* (*Xcc*), *Burkholderia cenocepacia* (*Bcc*), and *Pseudomonas aeruginosa*.

DSF-mediated interspecies communication occurs between DSF-producing bacteria and *B. cenocepacia, Stenotrophomonas maltophilia*, *Bacillus*, *Francisella novicida*, *Salmonella*, and *Bdellovibrio bacteriovorus*.

DSF-mediated inter-kingdom communication occurs between DSF-producing bacteria and *Candida albicans*.

DSF signaling occurs in plant development and defense, *Xylella fastidiosa* colonization of insects, and the zebrafish inflammatory response.

DSF represents an intriguing type of quorum sensing signal

Microbes are ubiquitous and populate plants and animals, as well as soil, water, and air. They form diverse communities in a multitude of environments. It is becoming increasingly apparent that microbes are not isolated in specific communities but rather interact and communicate with each other and with their surroundings [1]. One of the key challenges in microbiology is to dissect their diversity, metabolic activities, and interactions within their community and between communities and associated environments. **Quorum sensing** (**QS**) (see Glossary), a process whereby bacteria monitor their population density and regulate gene expression using chemical signals, is the best-studied type of bacterial communication [2]. QS is widespread in eubacteria, archaea, and fungi. Research in recent decades has identified a rich inventory of QS signals and QS-regulated beneficial or competitive interactions [2]. Recent evidence shows that QS signaling is not limited to bacterial cell–cell communication but also allows communication between bacteria and their eukaryotic hosts [3–5]. Understanding the mechanisms and outcomes of QS signal-mediated communication has important implications for appreciating host–pathogen interactions and, ultimately, may provide new targets and strategies for antimicrobial therapies that block or interfere with these communication networks.

The **diffusible signal factor** (**DSF**) family represents an intriguing type of QS signal found in diverse Gram-negative bacteria. Over the last decade, extensive progress has been made in understanding DSF-mediated **intraspecies communication** and **interspecies communication** [6,7]. Recently, **inter-kingdom communication** between DSF-producing bacteria and insects, plants, and zebrafish has been reported [8–10]. This review provides an update on these new developments with the aim of building a more comprehensive picture of DSF-mediated intraspecies, interspecies, and inter-kingdom communication. More detailed descriptions of the chemical

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structures, biological functions, biosynthesis, and signaling pathways of the DSF family signals can be found in previous reviews [6,7,11–13]. For convenience, DSF will be used as a collective term for the DSF-family signals unless otherwise specified.

DSF-producing bacteria and structural diversity of the DSF family

The first discovered DSF was the *cis*-11-methyl-dodecenoic acid (XcDSF) in the phytopathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*). Subsequently, several analogs, *cis*-2-dodecenoic acid (BDSF), *cis, cis*-11-methyldodeca-2,5-dienoic acid (CDSF), *cis*-10-methyl-2-dodecenoic acid (IDSF), 13-methyltetradecanoic acid (LeDSF), *cis*-2-tetradecenoic acid (XfDSF1), *cis-2*-hexadecenoic acid (XfDSF2), and *cis*-2-decenoic acid (PDSF), have been identified. Except for LeDSF, all DSFs are *cis*-2-unsaturated fatty acids containing fatty acid carbon chains of various lengths and *cis* double-bond configurations (Figure 1). Until now, DSF members have been detected in a range of Gram-negative bacterial species belonging to the γ-proteobacteria and β-proteobacteria groups, including *Xanthomonas* spp., *Xylella fastidiosa*, *Stenotrophomonas maltophilia*, *Lysobacter enzymogenes* and *L. brunescens*, *Leptospirillum*

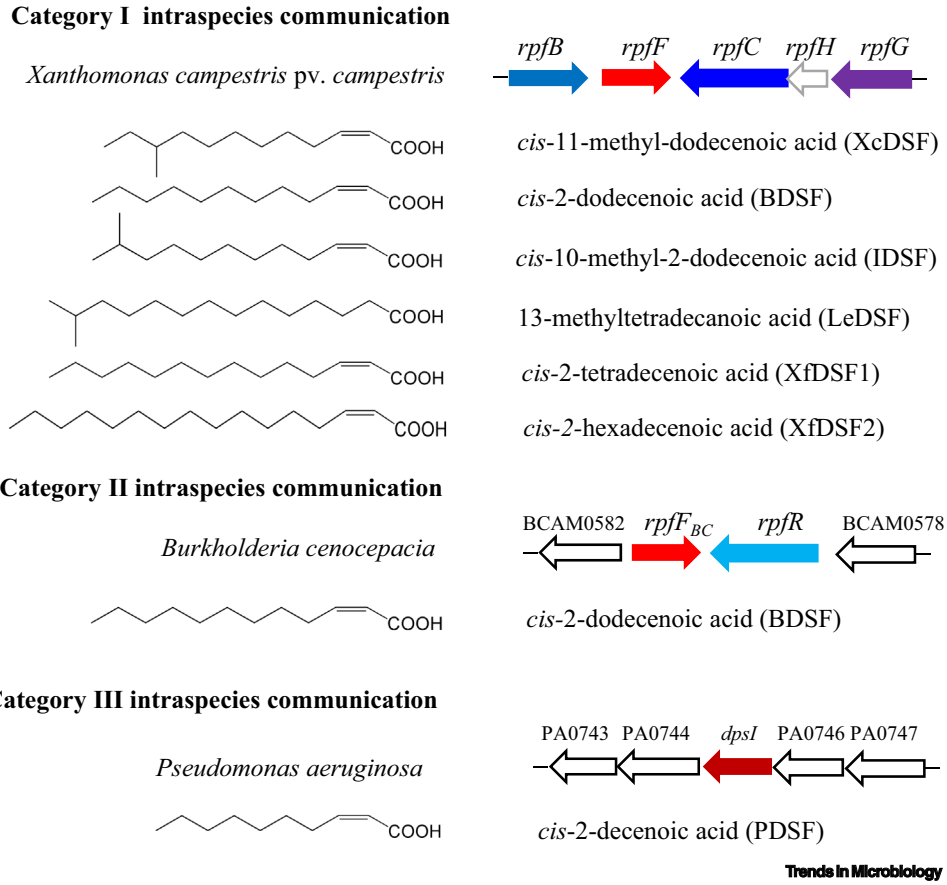

Figure 1. The three categories of diffusible signal factor (DSF)-mediated intraspecies communication systems in bacteria. XcDSF (*cis-11-ethyl-dodecenoic acid)* was the first identified DSF in *Xanthomonas campestris* pv. *campestris* (*Xcc*). BDSF is the major signal produced by *Burkholderia cenocepacia*. XcDSF and BDSF are two major DSFs produced by all *Xanthomonas* strains; XfDSF1 and XfDSF2 were identified from *Xylella fastidiosa*; LeDSF (13-methyltetradecanoic acid) was identified from *Lysobacter enzymogenes*; PDSF was identified from *Pseudomonas aeruginosa*. *rpfF*, $rpfF_{BC}$ and *dspI* are the key genes required for DSF biosynthesis. DSF is sensed by RpfC in the category I communication system while BDSF is sensed by RpfR in the category II communication system.

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ferri philum and *L. ferrooxidans*, *Burkholderia cenocepacia*, *Cronobacter turicensis*, and *Pseudomonas aeruginosa* [7,14–21]. *In silico* analysis suggests that *Methylobacillus flagellates*, *Thiobacillus denitrificans*, and bacteria in the genera *Frateuria*, *Luteibacter*, *Pseudoxanthomonas*, and *Rhodanobacter* also produce DSF.

The three different categories of DSF-mediated intraspecies communication

By definition, intraspecies communication occurs among individuals of the same species. QS signal-mediated intraspecies communication helps bacteria to sense their population size or degree of confinement, allowing them to function as a multicellular organism to execute a broad range of functions [7]. Based on their genomic origins, DSF-mediated intraspecies communication pathways are generally affiliated with three different groups, respectively represented by *Xcc*, *B. cenocepacia*, and *P. aeruginosa*.

Category I intraspecies communication

This DSF-based communication system is distinguished by the presence of a 'regulation of pathogenicity factors' (*rpf*) cluster encoding key signaling components such as DSF synthase RpfF and DSF perception system RpfC/RpfG (Figure 1). This system has been functionally verified in the phytopathogens *Xanthomonas* spp., *X. fastidiosa*, *S. maltophilia*, and strains of environmental relevance such as *Lysobacter* spp. and *Leptospirillum* spp. [14,16–21]. Bacteria using this communication system produce DSF through a classic fatty acid synthesis pathway and a final bifunctional enzyme, RpfF, having both dehydratase and thioesterase activities [22]. However, DSF biosynthesis is strictly regulated during bacterial growth. The whole QS-dependent communication comprises three stages: the pre-QS, QS, and the post-QS, in *Xcc* [7]. At the pre-QS stage, cell density-dependent DSF biosynthesis is autoinduced via a protein–protein interaction mechanism. The DSF sensor RpfC forms a complex with RpfF through its receiver domain to control DSF biosynthesis [23]. At the post-QS stage, a RpfB-dependent QS signal turnover system is induced and DSF levels return to those of the pre-QS stage [24] (Figure 2A).

DSF is perceived and transduced by a two-component system comprising the sensor kinase RpfC and the response regulator RpfG. In *Xcc*, the activated HD-GYP domain of RpfG functions as a phosphodiesterase to degrade the second messenger cyclic-di-GMP, a ligand of the global regulator cyclic AMP receptor-like protein (Clp), which, in turn, directly or indirectly regulates the production of virulence factors [25,26]. Activated RpfG was also shown to interact with two GGDEF domain-containing proteins to control *Xcc* motility [27]. In addition, Clp also negatively regulates *Xcc* aggregate gene cluster ABC (*xagABC*)-dependent biofilm formation [28] (Figure 2B). Interestingly, atypical RpfF/RpfC-regulated gene expression and phenotypes were also reported in *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain BXO43 and *X. fastidiosa* [29,30]. These findings suggest that the category I DSF signaling might be more complex than what the current models suggest.

In *Xanthomonas* plant pathogens, DSF signaling is generally associated with the regulation of biofilm, multidrug resistance, membrane integrity, and production of virulence factors [15,21,31,32]. In the soil bacterium *Lysobacter*, LeDSF regulates the production of antibiotics and pigment, and twitching motility [16,17]. DSF signaling in the acidophilic mineral-oxidizing *Leptospirilli* spp. controls bioleaching activity and niche protection [20].

Category II intraspecies communication

This communication system is characterized by the presence of the *rpfF* homolog $rpfF_{BC}$ and *rpfR*, encoding a BDSF sensor protein (Figure 1). It was reported in all strains of the *Burkholderia cepacia* complex (*Bcc*) and the *Cronobacter* genus [33,34]. BDSF is the major

Glossary

Diffusible signal factor (DSF): a family of *cis*-unsaturated fatty acid signal molecules that was first described in the plant pathogen *Xanthomonas campestris*. DSFs contain fatty acid carbon chains of various lengths and *cis* double-bond configurations. Many Gram-negative bacteria produce, secrete, and sense DSF to determine the population density and to regulate the expression of pertinent genes.
Inter-kingdom communication: the communication that occurs between prokaryotic cells and eukaryotes, such as fungi, plants, insects, and mammals, including humans.
Interspecies communication: the communication that occurs between individuals of different species.
Intraspecies communication: the communication that occurs among individuals of the same species.
Quorum sensing (QS): a type of cell–cell communication mode. Bacteria produce and detect small signal molecules with a specificity that allows them to distinguish and count their own, their cousins, and outsiders.

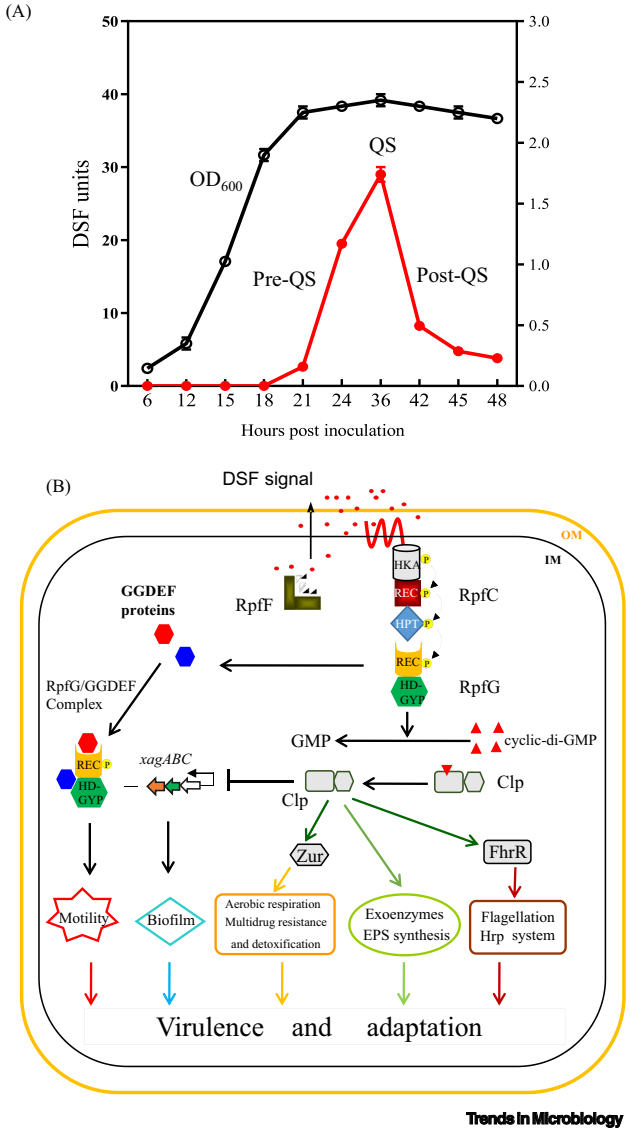

Figure 2. Schematic diagram summarizing auto-induction of diffusible signal factor (DSF) biosynthesis and DSF-mediated intraspecies signaling in the phytopathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*). (A) DSF biosynthesis is strictly regulated during *Xcc* growth. (B) RpfF is one of the key enzymes required for DSF biosynthesis. DSF is sensed and transduced by the two-component signaling system RpfC–RpfG through a phosphorelay cascade, and the downstream second-messenger cyclic di-GMP. The global regulator Clp is the effector of cyclic di-GMP, which regulates directly and indirectly the expression of genes for virulence and adaptation. Activated RpfG also binds two GGDEF domain-containing proteins to control *Xcc* motility. Abbreviations: Zur, a transcriptional regulator involving in zinc uptake regulation; FhrR, a transcriptional regulator required for flagella biosynthesis and hypersensitive response (Hrp).

signal used for category II intraspecies communication. BDSF biosynthesis is positively regulated by a two-component system, RqpS/RqpR, through modulating $rpfF_{Bc}$ transcription in *B. cenocepacia* [35].

BDSF signaling in *B. cenocepacia* has been recently reviewed [13]. RpfR is a BDSF sensor protein containing a Per/Arnt/Sim (PAS), a GGDEF, and an EAL domain. BDSF binds to the PAS domain of RpfR, causing a conformation change. This change plays a critical role in regulating the phosphodiesterase activity of RpfR, thereby influencing intracellular cyclic-di-GMP levels [36]. RpfR is also a cyclic di-GMP sensor able to bind both BDSF and cyclic di-GMP [34]. A global regulator, GtrR, interacts with RpfR to regulate the transcription of *cepI*, the acyl homoserine lactone (AHL)-type QS signal synthesis gene in *B. cenocepacia* [37]. These findings link BDSF

signaling with the AHL-dependent QS system, underlining the complexity of DSF-mediated intraspecies signaling.

Category III intraspecies communication

This category is represented by the drug-resistant bacterium *P. aeruginosa*. It produces the DSF-type molecule PDSF. PDSF biosynthesis was attributed to *dspI* (PA0745), a putative *rpfF* homolog located within the cluster PA0743–PA0747 in PAO1 [38]. However, another gene cluster, PA4978–PA4983, was also suggested to be involved in PDSF synthesis and perception [39].

PDSF was first shown to induce biofilm dispersion in the *P. aeruginosa* strains PAO1 and PA14 [38]. Microarray analysis revealed that PDSF is involved in biofilm dispersion, motility, virulence, and persistence in PAO1 [39]. In PA14, PDSF-deficiency provoked a remarkable reduction in pyoverdine production, swarming motility, biofilm dispersion, and virulence [40]. The perception and signaling transduction of PDSF remains to be elucidated. Given that PDSF-regulated functions mostly overlap with that of cyclic di-GMP signaling, it is likely that PDSF signaling is also mediated by the second messenger cyclic di-GMP in *P. aeruginosa*.

DSF-mediated interspecies communication

Microbes never live alone in natural ecosystems but rather interact physically and trophically with each other. They detect not only self-produced signals but also signals synthesized by their neighbors [41]. Given the prodigious diversity of DSF-producing bacteria in nature, it is not surprising that an enormous repertoire of DSF-mediated interspecies communication occurs.

Communication between *P. aeruginosa*, *B. cenocepacia,* and *S. maltophilia*

P. aeruginosa, *B. cenocepacia*, and *S. maltophilia* are all multidrug-resistant pathogens. Chronic colonization and infection by *P. aeruginosa* occur in 80% of cystic fibrosis (CF) patients, who are often coinfected by *B. cenocepacia*, *S. maltophilia,* as well as the fungus *Aspergillus* sp. [42]. As described previously, these three bacteria each produce the DSF molecules, PDSF, BDSF, and XcDSF, respectively, that mediate, in order, category III, II, and I intraspecies communication (Figure 1). In *S. maltophilia–P. aeruginosa* cocultures, DSF produced by *S. maltophilia* influences the architecture of the *P. aeruginosa* biofilm. The response of *P. aeruginosa* to *S. maltophilia* DSF requires the sensor kinase PA1396 [43]. Mutation of PA1396 or exogenous addition of XcDSF to *P. aeruginosa* results in increased bacterial stress tolerance and polymyxin resistance, suggesting that DSF from *S. maltophilia* can be sensed by *P. aeruginosa* PA1396 [43]. Further, XcDSF and BDSF were both detected at physiologically relevant concentrations in sputum samples from CF patients infected with *Bcc* and *S. maltophilia* [44]. The presence of DSF was correlated with polymicrobial infections involving *Burkholderia* and/or *Stenotrophomonas* species together with *P. aeruginosa*. When *P. aeruginosa* PAO1 was inoculated intratracheally together with DSF into CF transmembrane conductance regulator (CFTR) knockout mice, *P. aeruginosa* persistence was observed in the lung [44]. Furthermore, *P. aeruginosa* could form biofilms on human CF-derived lung epithelial cells in an *in vitro* culture system. Addition of DSF to the culture system significantly enhanced polymyxin tolerance in *P. aeruginosa* biofilms.

DSF-mediated interspecies communication between *B. cenocepacia* and *P. aeruginosa* was also identified [45]. Exogenous addition of BDSF decreases the transcriptional level of the QS regulator genes *lasR*, *rhlR*, and *pqsR* in *P. aeruginosa*, leading to a reduced production of AHL and *Pseudomonas* quinolone signal (PQS) [45]. In addition, BDSF inhibits the expression of type III secretion system (T3SS) genes in *P. aeruginosa* at micromolar concentrations and reduces *P. aeruginosa* virulence in both HeLa cell and zebrafish infection models. However, BDSF

signaling in *P. aeruginosa* could not be attributed to the activity of the reported sensor PA1396, or that of the long-chain fatty acid sensor PsrA. Instead, BDSF appeared to inhibit the T3SS and QS systems through two alternative independent signaling pathways [45].

Taken together, complex DSF-mediated interspecies communication between *P. aeruginosa*, *B. cenocepacia*, and *S. maltophilia* clearly occurs. This interspecies communication may operate in CF lungs and influence the efficacy of antibiotic treatments. Further insights into DSF-mediated interspecies communication could hold significant potential for the development of measures against these infections.

Communication between *Xanthomonas* and *Bacillus*

Bacillus is a Gram-positive, rod-shaped bacterium that is quite ubiquitous in nature. This bacterium has the ability to form endospores to survive a wide range of extreme environmental conditions. These remarkable characteristics provides *Bacillus* with a competitive advantage over other species in diverse ecosystems [46]. In contrast, *Xanthomonas* species are typically plant-associated bacteria that do not commonly produce antibiotic-like molecules. Yet, a negative association was seen between the presence of *X. campestris* pv. *vitians* and *Bacillus* in leaf surface populations of lettuce in production fields [47]. How *Xanthomonas* might successfully compete with *Bacillus* was quite intriguing. Given a role of DSF in regulating bacterial biofilm formation and stress resistance, DSF may modulate *Bacillus* stress resistance, or perhaps also, antibiotic resistance. To test this hypothesis, the effects of *Xanthomonas*-derived XcDSF on antibiotic susceptibility of *Bacillus cereus*, a toxin-producing human pathogen, were investigated [48]. A dose-dependent effect of exogenous XcDSF on increased susceptibility of *B. cereus* to gentamicin or kanamycin was observed. Further testing in a HeLa cell-based *in vitro* assay revealed that a combination of 50 μM XcDSF and gentamicin was more effective in decreasing *B. cereus'* cytotoxicity towards HeLa cells than gentamicin alone. Microarray analysis revealed that XcDSF influenced the antibiotic susceptibility of *B. cereus* in multiple ways, including by reducing its drug resistance, inhibiting biofilm formation, and by attenuating bacterial persistence [48].

Other examples of interaction between *Bacillus* and *Xanthomonas* species have been observed. *B. thuringiensis* (*Bt*) has been widely used as a biopesticide for the control of insect damage to vegetables and other crops. When *Xcc* and *Bt* were cocultured, the number of viable cells of *Xcc* was significantly higher than that of *Bt*, indicating that *Xcc* was more competitive than *Bt*. In contrast, a DSF-deficient Δ*rpfF* strain of *Xcc* was much less competitive than *Bt* when cocultured [49]. Similarly, the Δ*rpfF* strain had much less ability to inhibit *Bt* endospore formation than did the wild-type *Xcc*. Inhibition of endospore formation could be restored by exogenous provision of 50 μM XcDSF. Further analysis showed that XcDSF inhibited both cell division and sporulation of *Bt* via its modulation of the cell division regulator FtsZ. Knockout of *rpfF* decreased the competitive capability of *Xcc* against *Bt* on the surface of Chinese cabbage leaves [49].

These findings may have important implication for guiding management of infections of animals and plants caused by *Bacillus* and *Xanthomonas*. *Bacillus anthracis* and *B. cereus* are important human pathogens causing anthrax and foodborne illness, respectively. DSF could be used as an inhibitor to interfere with their growth and development or as an antibiotic adjuvant to slow the development of antibiotic resistance. Conversely, for many years, *Bt*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens* have been widely used as biopesticides to prevent diverse plant diseases, including the diseases caused by different *Xanthomonas* species [50]. *Xanthomonas* DSF may antagonize the biological control ability conferred by these beneficial *Bacillus* and thus attenuate their plant-protective effects. How to overcome this DSF-mediated antagonistic interspecies communication will require further investigation.

Communication between *B. cenocepacia* and *Francisella novicida*

Francisella tularensis is a bacterial pathogen that infects a large number of animal species, causing outbreaks of tularemia in humans. *F. novicida* strain Utah112 does not cause disease in healthy humans but does so in animals. Therefore, Utah112 is used in animal models for the study of the highly infectious *F. tularensis* [51]. Many phenotypic alterations were observed when Utah112 was exposed to BDSF from *Burkholderia*. BDSF at a concentration of 5 μM inhibited biofilm formation and caused biofilm dispersion in Utah112. BDSF altered the expression of multiple genes, including those involved in biofilm formation, such as *chiA* and *chiB*-encoding chitinases [52]. BDSF also increased *relA* expression and (p)ppGpp levels, leading to decreased biofilm production in Utah112. Furthermore, BDSF altered the expression of genes involved in iron acquisition such as those for siderophore production [52].

Francisella is present throughout the natural environment in water, sediments, and soil. *Burkholderia* spp. are also prevalent microbes in soil and sediments. Thus, *Francisella* in the soil may share the same environmental niche as *Burkholderia* strains and could potentially be exposed to BDSF. Further study of BDSF signaling in *Francisella* may provide clues as to how *Francisella* benefits from *Burkholderia* to survive in nature between epizootic episodes.

Communication between DSF-producing bacteria and the enteric pathogen *Salmonella*

Strains of *Salmonella* are enteric pathogens that cause gastrointestinal illness and fevers, called salmonellosis. In the intestinal milieu, the short-chain fatty acids, butyric acid and propionic acid, produced by the microbiota, are present at high concentrations, while long-chain fatty acids and oleic acids are abundant in bile [53]. These fatty acids have been shown to regulate *Salmonella* pathogenicity island 1 through the AraC-type transcriptional regulator HilD [53]. To identify compounds that could potently inhibit the expression of genes involved in fatty acid-induced invasion, a range of DSFs were tested in *Salmonella enterica* [54]. All of these DSF species were observed to repress the activity of HilD by preventing its binding to target DNA and inducing its rapid degradation by Lon protease. *cis*-2-hexadecenoic acid, XfDSF2 produced by *X. fastidiosa*, was the most potent [54]. XfDSF2 repressed the expression of the HilD-dependent transcriptional regulator *hilA* and TSS effector *sopB*. In a model mouse of colitis, application of 2.5~10 μM XfDSF2 significantly repressed the expression of invasion genes in *Salmonella* [54].

These findings raise the question of whether DSF-mediated interspecies communication between DSF-producing bacterial species and *Salmonella* naturally occurs in the gut because *cis*-2-unsaturated fatty acids are not common in dietary sources. While *X. fastidiosa* is unlikely to be found in the gut, other DSF-producing species may well be present. The DSF-producing bacterium *Burkholderia* was identified in the gut of wild and laboratory mice [55]. *S. maltophilia* was also found to be the dominant species in the gut microbiota of arsenic-treated fish [56]. The great sensitivity of *Salmonella* to DSF suggests that this enteric pathogen senses DSF signal from DSF-producing bacteria as a cue to its gut location and consequently regulates the expression of virulence-associated genes.

DSF signaling in the predatory *Bdellovibrio bacteriovorus*

B. bacteriovorus is a highly motile Gram-negative bacterium commonly present in soil, sewage, and other terrestrial and aquatic habitats. It obtains the nutrients necessary for its growth and survival by predating other Gram-negative bacteria. This unique lifestyle makes it an excellent candidate as a biocontrol agent against important plant, animal, and human pathogens [57]. Exposure of *B. bacteriovorus* 109J to XcDSF significantly delays predation and bdelloplast lysis in a dose-dependent manner. At 50 μM, XcDSF reduced strain 109J attack-phase motility by 50%. A higher dose of 200 μM leads to a significant loss in membrane integrity and the viability of strain

109J [58]. Transcriptome analysis revealed that XcDSF induced stress responses, including osmotic shock, reactive oxygen species (ROS), MarR regulon activation, and the downregulation of the flagellum assembly genes and serine protease genes in 109J [58]. Furthermore, XcDSF at a concentration of 200 μM was shown to have similar inhibitory activity towards *B. bacteriovorus* as sodium dodecyl sulfate, a highly toxic chemical surfactant, whereas it only altered gene expression of this predator at a lower and nontoxic concentration of 50 μM [58]. These findings suggest a dual role of XcDSF, acting as both a signaling molecule and a deterrent against the predatory *B. bacteriovorus*.

In this study, only XcDSF was tested and the concentrations used were much higher than those released physiologically by DSF-producing strains. Future work is needed to determine the effects of other DSF analogs, and whether DSF-mediated interspecies communication occurs between DSF-producing bacteria and *B. bacteriovorus* under natural conditions. If this hypothesis is supported, the finding that bacteria use DSF signals as a weapon to protect themselves from *B. bacteriovorus* will be a significant breakthrough. In this case, a DSF-resistant *B. bacteriovorus* mutant would be a superior biological control agent for the control of DSF-producing pathogens.

DSF-mediated inter-kingdom communication

QS signaling is not restricted to bacterial cell-to-cell communication but also allows communication between microbes and their hosts. This was best demonstrated in the context of interactions between AHL-producing bacteria and their associated plants [4]. Recently, DSF has also been demonstrated to mediate the communication between DSF-producing bacteria and the fungus *Candida albicans*, as well as plants, insects, and zebrafish (Figure 3, Key figure).

Communication between DSF-producing bacteria and *Candida albicans*

C. albicans is a component of the normal human commensal flora. However, it is also the most common fungal species causing oral and genital infections in humans. This pathogen has three distinct morphological forms: a budding yeast, pseudohyphae, and filamentous hyphae [59]. The morphological transition from yeast to the hyphal form is important for its infectiousness and is regulated by the secreted QS molecules, farnesoic acid/farnesol [60]. Given the structural similarity between DSF and farnesoic acid/farnesol, the inter-kingdom communication between DSF-producing bacteria and *C. albicans* has long been a research focus.

Burkholderia BDSF was shown to inhibit biofilm formation, virulence factor production, and morphological transition in *C. albicans* [33]. It was further revealed to reduce the ability of *C. albicans* to adhere to polystyrene surfaces by repressing the expression of adhesion genes, that is, ALS1 and EAP1 [61].

The Gram-positive bacterium *Streptococcus mutans* is a causative agent of dental caries and has been shown to secrete the DSF-like compound, *trans*-2-decenoic acid (SDSF). SDSF suppresses the transition of *C. albicans* from the yeast to hyphal morphologies at concentrations that do not affect its growth. In particular, SDSF abolishes the expression of *HWP1*, a hyphal-specific gene of *C. albicans* [62]. Streptococci and *Candida* coexist in the oral cavity. Streptococci probably use the excreted SDSF to modulate biofilm formation by *C. albicans* and thus enhance its competitive activity.

S. maltophilia is a nosocomial pathogen of increasing importance. DSF signaling positively regulates biofilm formation, production of virulence-associated factors, and β-lactamase activity [18]. To assess possible DSF-mediated inter-kingdom communication, *C. albicans* ATCC 10231 was grown with *S. maltophilia* K279a or its DSF-deficient *rpfF* mutant. Strong antagonistic effects of

Key figure

Diffusible signal factor (DSF)-mediated interspecies and inter-kingdom communications between different DSF-producing bacteria, *Candida albicans*, plants, insects, and zebrafish

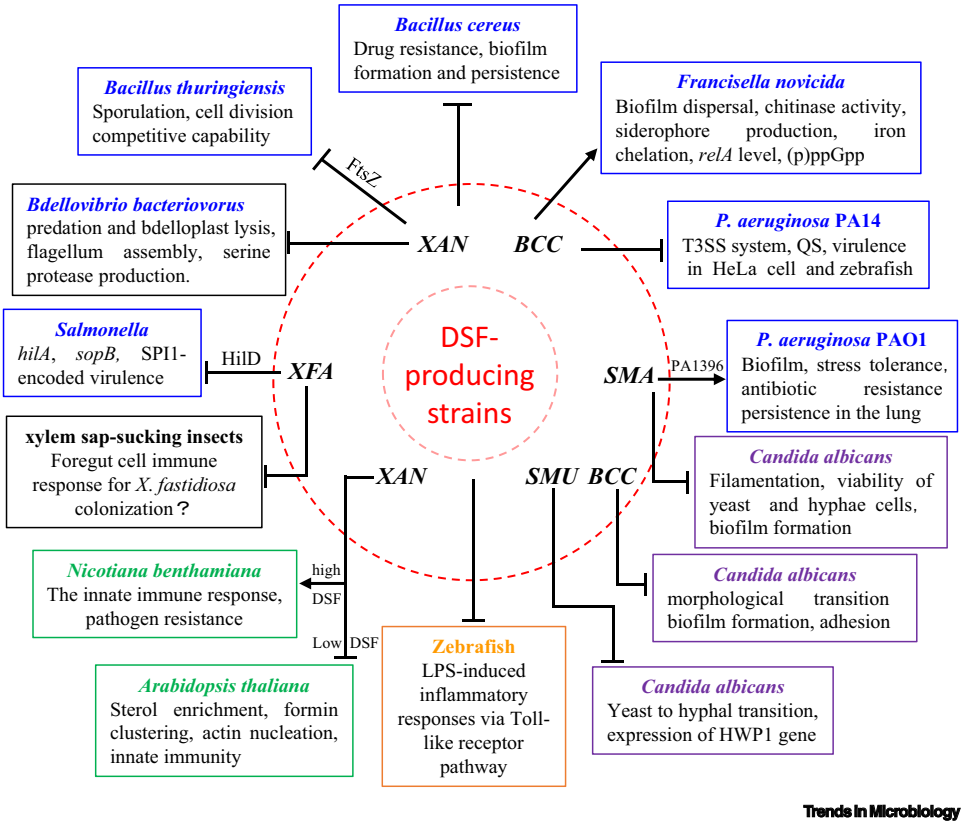

Figure 3. Arrows and blunt arrows represent positive and negative regulations, respectively. Abbreviations: BCC, *Burkholderia cenocepacia*; LPS, lipopolysaccharide SMA, *Stenotrophomonas maltophilia*; SMU, *Streptococcus mutans*; T3SS, type 3 secretion system; XAN, *Xanthomonas spp.;* XFA, *Xylella fastidiosa.*

K279a on *C. albicans* filamentation and planktonic or biofilm modes of growth were observed [63]. Strain K279a was further observed to kill both yeast and hyphae cells by affecting cell integrity. However, the K279a *rpfF* mutant lost both such activities [63]. These findings suggest that *S. maltophilia* uses DSF as an inter-kingdom signal to interfere with the yeast-to-hyphal transition and biofilm formation in *C. albicans.* Further studies are needed to investigate the role of DSF on hyphal formation and biofilm formation *in vivo*, for example, in the lungs of CF patients where *S. maltophilia* may coexist with *C. albicans*.

DSF signaling in plant development and defense

Members of the genus *Xanthomonas* are all plant pathogens, in which DSF signaling has been well characterized using laboratory-based cell culture. However, the *in planta* QS signal used during *Xanthomonas* infection has remained unclear. Using an *Xcc ΔrpfB*-Chinese cabbage infection

model and a mass spectrometry-based assay, both XcDSF and BDSF were detected in infected cabbage tissues. BDSF was found to comprise ~70% of the total DSF present in infected cabbage tissue [64]. This is the first report to directly show the DSF QS signal used during *Xcc* infection of plants.

Plants have evolved the ability to interpret signals from surrounding or invading bacteria. Infiltration of *Nicotiana benthamiana* leaves with XcDSF (250~1000 μM) induces hypersensitive reaction (HR)-like symptoms. Lower doses (100 μM) induce callose deposition, programmed cell death, accumulation of hydrogen peroxide, and expression of pathogenesis-related protein 1 (PR-1) in *N. benthamiana*, *Arabidopsis*, and rice leaves [65]. Further, pretreatment of rice leaves with 100 μM XcDSF confers resistance to *X. oryzae* pv. *oryzae* [65]. These findings suggest a DSF-mediated crosstalk between *Xanthomonas* pathogens and plants in a dose-dependent manner.

The DSF-modulation of plant immunity is highly spatiotemporally regulated and influences a diverse array of host biological systems. In the *Xcc–Arabidopsis thaliana* pathosystem, multifaceted effects of XcDSF were observed. At 25 μM, mimicking early-infection concentrations of XcDSF, this molecule hijacks *Arabidopsis* sterol biosynthesis, thereby suppressing pathogen-associated molecular pattern (PAMP)-triggered innate immune responses, including callose deposition, ROS production, and stomatal closure response [8]. Such dampening of PAMP-triggered immunity (PTI) was a consequence of a DSF-induced phytosterol enrichment, which alters the physicochemical properties of the plasma membrane. These perturbations of the host plasma membrane interfere with the endocytosis and nano-clustering of flagellin receptor FLS2 on the cell membrane, thereby desensitizing *Arabidopsis* to the presence of this pathogen PAMP (Figure 4A). Intriguingly, further evidence has suggested that the DSF–plant interaction is much more complex. XcDSF regulates the multilayer structure of the plant cell wall–plasma membrane–actin cytoskeleton (CW–PM–AC) continuum, subverting the molecular interactions and functionality of cell-surface immune-regulatory molecules, thus leading to a disruption of the cooperation between different immune signaling pathways in plants [9]. In addition to influencing plasma membrane properties and PTI, XcDSF enhances the biosynthesis of the major cell wall load-bearing structure, cellulose microfibrils. XcDSF-triggered cellulose production mechanically modulated the surface macromolecular assembly of actin nucleator formin proteins, which are physically integrated into the CW and PM, and whose surface assembly induces actin remodeling during PTI during bacterial infection [9] (Figure 4A).

Although the link between phytosterol enrichment and cellulose biosynthesis, both induced by DSF, is still unclear, these findings suggest that DSF-producing phytopathogens or plant-associated bacteria use DSF signal to affect host cell biology through multilayered regulatory networks. Understanding the multifaceted interference with the plant CW–PM–AC continuum by DSF requires further dissection at different stages of the immune response and plant development.

DSF signaling in *X. fastidiosa* colonization of insects and plant infection

X. fastidiosa is a phytopathogen causing diseases in a number of important crop plants, such as Pierce's disease of grape and citrus variegated chlorosis in citrus species [66]. It is the only DSF-producing insect-borne plant pathogen yet identified and is obligately vectored by a variety of xylem sap-sucking insects. Its colonization of the foregut of these insects enables it to be transmitted to new host plants [67]. Acquisition of *X. fastidiosa* within the insect foregut depends on XfDSF-mediated intraspecies communication to positively regulate the production of the extracellular polysaccharides (EPS) and various adhesins necessary for biofilm formation and foregut surface attachment. As a result, XfDSF-deficient Δ*rpfF* cells fail to attach to the foregut during feeding on infected plants and are therefore poorly transmitted by sharpshooter leafhoppers [68,69].

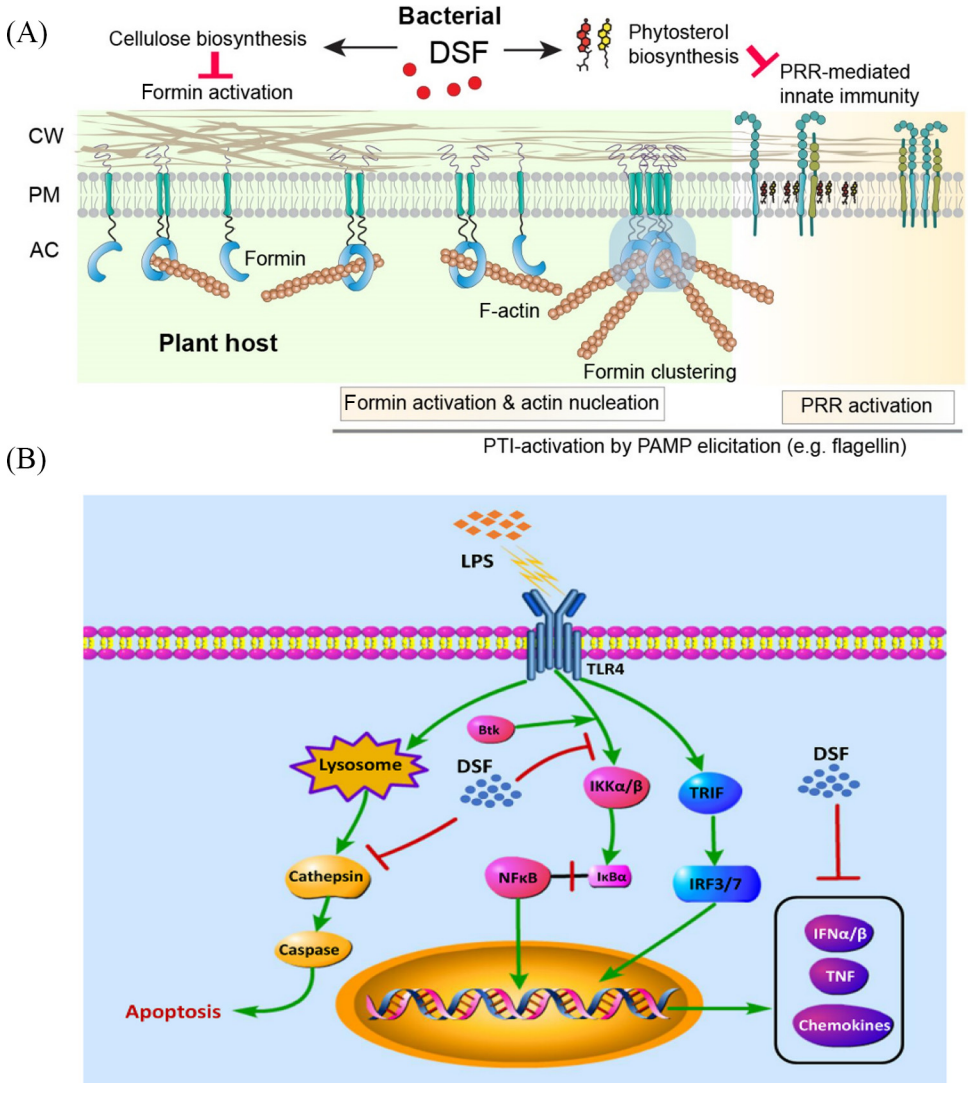

Figure 4. Diffusible signal factor (DSF) signaling in *Arabidopsis thaliana* immune response and zebrafish inflammatory response. (A) XcDSF treatment enhances phytosterol production and impairs both clathrin-mediated endocytosis (CME) of flagellin receptor (FLS2) internalization, and FLS2 nano-clustering on the plant plasma membrane (PM). All of these events desensitize *Arabidopsis* immune responses to the bacterial flagellum. XcDSF (*cis-11-methyl-dodecenoic acid)* also induces cellulose production. The enhanced cellulose production disrupts the cell wall–plasma membrane–actin cytoskeleton (CW–PM–AC) continuum, thereby preventing the intermolecular interactions between formin dimers and actin, necessary to induce actin remodeling during pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI). (B) The proposed molecular mechanism of XcDSF in the anti-inflammatory response of zebrafish. Abbreviations: PRR, pattern-recognition receptor; LPS, lipopolysaccharide; TLR, Toll-like receptor; NFκB, nuclear factor kappa-B; TNF, tumor necrosis factor. Arrows and blunt arrows represent positive and negative regulations, respectively.

After delivery of *X. fastidiosa* directly into the xylem vessels by insect vectors, it proliferates as a biofilm along the xylem wall, but is also capable of digesting pit membranes that separate paired xylem vessels. Such a breach allows the pathogen to move to adjacent vessels in a process that is repeated numerous times after infection, enabling it to spread widely through the plant. XfDSF-repressed traits include type IV pili that are involved in twitching motility, as well as production of

extracellular enzymes such as polyglacturonases and endoglucanases [70]. DSF also suppresses the release of outer membrane vesicles (OMVs) that serve to prevent the attachment of *X. fastidiosa* cells to xylem vessels [71,72]. OMV release and extracellular enzyme production are presumably most active in those xylem vessels in which *X. fastidiosa* is at low populations, and thus have not accumulated XfDSF. Consequently, *X. fastidiosa rpfF* mutants are more virulent to grapevines than is the wild-type strain [69]. Because accumulation of XfDSF facilitates insect transmission between plants, but inhibits its movement within plants, XfDSF serves as a switch to coordinate the plant and insect lifestyle by enabling substantial phenotypic plasticity of the cells within a plant that is driven by the local cell density within individual xylem vessel [66].

Because of the intricate XfDSF-mediated regulation of *Xylella* attachment to different hosts, targeting each of these steps could be an effective way to interfere with bacterial transmission. Experiments using transgenic grapevines harboring *X. fastidiosa rpfF*, and thus producing XfDSF, demonstrated that cells of *X. fastidiosa* were less capable of movement within plants after inoculation. The number of symptomatic leaves in these plants was therefore reduced drastically, being found only close to the site of inoculation. The rather contained colonization by *X. fastidiosa* in the presence of XfDSF, as compared with its typical systemic spread, provided protection against the disease through a process of pathogen confusion [67,73]. It is possible, however, that the augmented presence of XfDSF in the transgenic plants might also induce host defenses that could then reduce pathogen success. This deserves further investigation.

DSF signaling in the LPS-induced inflammatory response in zebrafish

Zebrafish (*Danio rerio*) is a small, freshwater fish commonly found in the tropics. It is increasingly used as a laboratory animal model in basic biology and drug development. Diverse bacterial species have been identified in the zebrafish gut, being crucial for zebrafish metabolism, intestinal development, and general evolution of the intestinal ecosystems [74]. Lipopolysaccharides (LPS) are a major component of the outer wall of Gram-negative bacteria. They can act on the host cells to induce a systemic inflammatory response syndrome [75,76].

Exposure of zebrafish embryos to LPS induces a pathological inflammatory reaction in the intestinal tract. However, if XcDSF and LPS are applied together, the presence of exogenous XcDSF (20–100 μM) inhibits a series of inflammatory reactions caused by LPS, thus reducing intestinal pathological injury and dampening excessive production of ROS, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, and reducing the accumulation of neutrophils and macrophages and their migration to inflammatory sites [10]. Furthermore, RNA-Seq analysis revealed that the anti-inflammatory activity conferred by DSF counteracted the effects of LPS by interfering with Toll-like receptor signaling and caspase cascade activation through restoring the expression of lysosomal cathepsins and apoptosis signaling [10] (Figure 4B). These findings suggest a possible role for DSF in LPS-induced inflammatory response of zebrafish and the possibility to exploit DSF as an anti-inflammatory in the food and pharmaceutical industries.

Concluding remarks

We have provided an update on the current state of knowledge for the DSF family of QS signal-mediated intraspecies, interspecies, and inter-kingdom communication. These exciting findings have broadened our understanding of the concept of sociomicrobiology and have raised several questions on the signaling networks, the underlying mechanisms and potential applications of the DSF-mediated communication systems (see Outstanding questions). First, category I and II DSF-mediated intraspecies communication have been well studied. Category III DSF-mediated intraspecies communication remains to be further elucidated. Second, given that DSF-producing bacteria are widely distributed in nature, it can be expected that future research will discover

Outstanding questions

What is the PDSF signaling network in the human opportunistic pathogen *P. aeruginosa*?

What are the DSF-mediated beneficial, competitive, antagonistic, or deceptive interactions and the underlying signaling mechanisms between DSF-producing bacteria and other microbes?

Given that most identified DSF-mediated interactions are competitive or antagonistic, could these communications be exploited for novel antimicrobial therapy?

Is there any crosstalk between bacterial DSF signals and host hormones or metabolites in microbial–host communication?

What are the receptors and signaling pathways of DSF signals in host plants, insects, animals, or humans?

Based on the fascinating finding that DSF interacts with the zebrafish immune system, can one expect a tripartite communication between beneficial commensals, pathogenic bacteria, and the immune system in humans?

How does DSF-mediated inter-kingdom communication drive the coevolution of prokaryotes and eukaryotes?

vast networks of DSF-mediated interspecies communication in different settings. Further characterization of the beneficial, competitive, antagonistic, or deceptive interactions between these bacteria and the underlying signaling mechanisms is needed. Third, with the widespread occurrence of antibiotic resistance, remedies aimed at attenuating virulence rather than the survival of pathogens would help alleviate selection pressure. Given that most identified DSF-mediated interactions are competitive or antagonistic, could these communications be exploited for novel antimicrobial therapy?

Finally, although the studies of DSF-mediated inter-kingdom communication are still in their infancy, there is already a solid core of support for future studies in this field. There are several key questions that remain to be answered. For example, is there any crosstalk between bacterial DSF signals and host hormones or metabolites in microbial–host communication? What are the receptors and signaling pathways of DSF signals in hosts? Based on the fascinating finding that DSF interacts with the zebrafish immune system, can one expect a tripartite communication between beneficial commensals, pathogenic bacteria, and the immune system in humans? Understanding the mechanisms and outcomes of inter-kingdom communication may help design novel therapeutics and strategies to combat bacterial infections and enhance host immune defenses.

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Declaration of interests

There are no interests to declare.

References

1. Thompson, L.R. *et al.* (2017) The Earth microbiome project consortium. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* 551, 457–463
2. Leoni, L. *et al.* (2017) In *Quorum Sensing Methods and Protocols* (Leoni, L. and Rampioni, G., eds), pp. 1–4, Humana Press
3. Hughes, D. *et al.* (2008) Inter-kingdom signaling: communication between bacteria and their hosts. *Nat. Rev. Microbiol.* 6, 111–120
4. Hartmann, A. *et al.* (2021) Importance of N-acyl-homoserine lactone-based quorum sensing and quorum quenching in pathogen control and plant growth promotion. *Pathogens* 10, 1561
5. Fan, Q. *et al.* (2022) Structure and signal regulation mechanism of interspecies and interkingdom quorum sensing system receptors. *J. Agric. Food Chem.* 70, 429–445
6. Deng, Y. *et al.* (2011) Listening to a new language: DSF-based quorum sensing in Gram-negative bacteria. *Chem. Rev.* 111, 160–173
7. Zhou, L. *et al.* (2017) The DSF family of quorum sensing signals: diversity, biosynthesis, and turnover in *Xanthomonas*. *Trends Microbiol.* 25, 293–303
8. Tran, T.M. *et al.* (2020) The bacterial quorum sensing signal DSF hijacks *Arabidopsis thaliana* sterol biosynthesis to suppress plant innate immunity. *Life Sci. Alliance* 3, e202000720
9. Ma, Z. *et al.* (2021) Formin nanoclustering-mediated actin assembly during plant flagellin and DSF signaling. *Cell Rep.* 34, 108884
10. Zhu, H. *et al.* (2022) Bacterial quorum sensing signal DSF inhibits LPS-induced inflammations by suppressing Toll-like receptor signaling and preventing lysosome mediated apoptosis in zebrafish. *Int. J. Mol. Sci.* 23, 7110
11. He, Y.W. *et al.* (2008) Quorum sensing and virulence regulation in *Xanthomonas campestris*. *FEMS Microbiol. Rev.* 32, 842–857
12. Dow, J.M. (2016) Diffusible signal factor-dependent quorum sensing in pathogenic bacteria and its exploitation for disease control. *J. Appl. Microbiol.* 122, 2–11
13. Wang, M. *et al.* (2022) The *cis*-2-dodecenoic acid (BDSF) quorum sensing system in *Burkholderia cenocepacia*. *Appl. Environ. Microbiol.* 88, e0234221
14. An, S.Q. *et al.* (2018) Diffusible signal factor signaling regulates multiple functions in the opportunistic pathogen *Stenotrophomonas maltophilia*. *BMC Res. Notes* 11, 569
15. Li, L. *et al.* (2019) Diffusible signal factor (DSF)-mediated quorum sensing modulates expression of diverse traits in *Xanthomonas citri* and responses of citrus plants to promote disease. *BMC Genomics* 20, 55
16. Feng, T. *et al.* (2019) Interspecies and intraspecies signals synergistically regulate *Lysobacter enzymogenes* twitching motility. *Appl. Environ. Microbiol.* 85, e01742
17. Ling, J. *et al.* (2019) LbDSF, the *Lysobacter brunescens* quorum-sensing system diffusible signaling factor, regulates anti- *Xanthomonas* XSAC biosynthesis, colony morphology, and surface motility. *Front. Microbiol.* 10, 1230
18. Alcaraz, E. *et al.* (2019) The *rpf*/DSF signalling system of *Stenotrophomonas maltophilia* positively regulates biofilm formation, production of virulence-associated factors and β-lactamase induction. *FEMS Microbiol. Lett.* 366, fnz069
19. Yero, D. *et al.* (2020) Genetic variants of the DSF quorum sensing system in *Stenotrophomonas maltophilia* influence virulence and resistance phenotypes among genotypically diverse clinical isolates. *Front. Microbiol.* 11, 1160
20. Bellenberg, S. *et al.* (2021) Diffusible signal factor signaling controls bioleaching activity and niche protection in the acidophilic, mineral-oxidizing *Leptospirilli*. *Sci. Rep.* 11, 16275
21. Singh, P. *et al.* (2022) The diffusible signal factor synthase, RpfF, in *Xanthomonas oryzae* pv. *oryzae* is required for the maintenance of membrane integrity and virulence. *Mol. Plant Pathol.* 23, 118–132

22. Zhou, L. *et al.* (2015) The multiple DSF-family QS signals are synthesized from carbohydrate and branched-chain amino acids via the FAS elongation cycle. *Sci. Rep.* 5, 13294
23. He, Y.W. *et al.* (2006) Dual signaling functions of the hybrid sensor kinase RpfC of *Xanthomonas campestris* involve either phosphorelay or receiver domain–protein interaction. *J. Biol. Chem.* 281, 33414–33421
24. Zhou, L. *et al.* (2015) Identification and characterization of naturally occurring DSF-family quorum sensing signal turnover system in the phytopathogen *Xanthomonas*. *Environ. Microbiol.* 17, 4646–4658
25. He, Y.W. *et al.* (2007) *Xanthomonas campestris* cell-cell communication involves a putative nucleotide receptor protein Clp and a hierarchical signalling network. *Mol. Microbiol.* 64, 281–292
26. Tao, F. *et al.* (2010) The cyclic nucleotide monophosphate domain of *Xanthomonas campestris* global regulator Clp defines a new class of cyclic di-GMP effectors. *J. Bacteriol.* 192, 1020–1029
27. Ryan, R.P. *et al.* (2010) Cell-cell signal-dependent dynamic interactons between HD-GYP and GGDEF domain proteins mediate virulence in *Xanthomonas campestris*. *Proc. Natl. Acad. Sci. U. S. A.* 107, 5989–5994
28. Tao, F. *et al.* (2010) Quorum sensing modulation of a putative glycosyltransferase gene cluster essential for *Xanthomonas campestris* biofilm formation. *Environ. Microbiol.* 12, 3159–3170
29. Rai, R. *et al.* (2015) Cell-cell signaling promotes ferric iron uptake in *Xanthomonas oryzae* pv. *oryzicola* that contribute to its virulence and growth inside rice. *Mol. Microbiol.* 96, 708–727
30. Ionescu, M. *et al.* (2016) Promiscuous diffusible signal factor production and responsiveness of the *Xylella fastidiosa rpf* System. *mBio* 7, e01054
31. He, Y.W. *et al.* (2006) Genome scale analysis of diffusible signal factor regulon in *Xanthomonas campestris* pv. *campestris*: identification of novel cell-cell communication-dependent genes and functions. *Mol. Microbiol.* 59, 610–622
32. Barel, V. *et al.* (2015) Virulence and in planta movement of *Xanthomonas hortorum* pv. *pelargonii* are affected by the diffusible signal factor (DSF)-dependent quorum sensing system. *Mol. Plant Pathol.* 16, 710–723
33. Deng, Y. *et al.* (2010) Structural and functional characterization of diffusible signal factor family quorum-sensing signals produced by members of the *Burkholderia cepacia* complex. *Appl. Environ. Microbiol.* 76, 4675–4683
34. Suppiger, A. *et al.* (2016) Evidence for the widespread production of DSF family signal molecules by members of the genus *Burkholderia* by the aid of novel biosensors. *Environ. Microbiol. Rep.* 8, 38–44
35. Cui, C. *et al.* (2018) A novel two-component system modulates quorum sensing and pathogenicity in *Burkholderia cenocepacia*. *Mol. Microbiol.* 108, 32–44
36. Waldron, E.J. *et al.* (2019) Structural basis of DSF recognition by its receptor RpfR and its regulatory interaction with the DSF synthase RpfF. *PLoS Biol.* 17, e3000123
37. Yang, C. *et al.* (2017) *Burkholderia cenocepacia* integrates cis-2-dodecenoic acid and cyclic dimeric guanosine monophosphate signals to control virulence. *Proc. Natl. Acad. Sci. U. S. A.* 114, 13006–13011
38. Amari, D.T. *et al.* (2013) The putative enoyl-coenzyme A hydratase DspI is required for production of the *Pseudomonas aeruginosa* biofilm dispersion autoinducer *cis*-2-decenoic acid. *J. Bacteriol.* 195, 4600–4610
39. Rahmani-Badi, A. *et al.* (2015) Dissection of the cis-2-decenoic acid signaling network in *Pseudomonas aeruginosa* using microarray technique. *Front. Microbiol.* 6, 383
40. Liu, L. *et al.* (2018) Structural and functional studies on *Pseudomonas aeruginosa* DspI: implications for its role in DSF biosynthesis. *Sci. Rep.* 8, 3928
41. Coquant, G. *et al.* (1827) (2020) Impact of N-acyl-homoserine lactones, quorum sensing molecules, on gut immunity. *Front. Immunol.* 2020, 11
42. Hughes, D.A. *et al.* (2022) Clinical characteristics of *Pseudomonas* and *Aspergillus* co-infected cystic fibrosis patients: a UK registry study. *J. Cyst. Fibros.* 21, 129–135
43. Ryan, R.P. *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
44. Twomey, K.B. *et al.* (2012) Bacterial *cis*-2-unsaturated fatty acids found in the cystic fibrosis airway modulate virulence and persistence of *Pseudomonas aeruginosa*. *ISME J.* 6, 939–950
45. Deng, Y. *et al.* (2013) *Cis*-2-dodecenoic acid signal modulates virulence of *Pseudomonas aeruginosa* through interference with quorum sensing systems and T3SS. *BMC Microbiol.* 13, 231
46. Xing, Y. *et al.* (2020) *Bacillus* spore awakening: recent discoveries and technological developments. *Curr. Opin. Biotechnol.* 64, 110–115
47. Rastogi, G. *et al.* (2012) Leaf microbiota in an agroecosystem: Spatiotemporal variation in bacterial community composition on field-grown lettuce. *ISME J.* 6, 1812–1822
48. Deng, Y. *et al.* (2014) Diffusible signal factor (DSF) quorum sensing signal and structurally related molecules enhance the antimicrobial efficacy of antibiotics against some bacterial pathogens. *BMC Microbiol.* 14, 51
49. Deng, Y. *et al.* (2016) Diffusible signal factor family signals provide a fitness advantage to *Xanthomonas campestris* pv. *campestris* in interspecies competition. *Environ. Microbiol.* 18, 1534–1545
50. Fira, D. *et al.* (2018) Biological control of plant pathogens by *Bacillus species*. *J. Biotechnol.* 285, 44–55
51. Kingry, L.C. *et al.* (2014) Comparative review of *Francisella tularensis* and *Francisella novicida*. *Front. Cell. Infect. Microbiol.* 4, 35
52. Dean, S.N. *et al.* (2015) *Burkholderia* diffusible signal factor signals to *Francisella novicida* to disperse biofilm and increase siderophore production. *Appl. Environ. Microbiol.* 81, 7057–7066
53. Golubeva, Y.A. *et al.* (2016) Intestinal long-chain fatty acids act as a direct signal to modulate expression of the *Salmonella* pathogenicity island 1 type III secretion system. *mBio* 7, e02170-15
54. Bosire, E.M. *et al.* (2020) Diffusible signal factors act through AraC-type transcriptional regulators as chemical cues to repress virulence of enteric pathogens. *Infect. Immun.* 88, e00226-20
55. Shin, J. *et al.* (2016) Analysis of the mouse gut microbiome using full-length 16S rRNA amplicon sequencing. *Sci. Rep.* 6, 29681
56. Song, D. *et al.* (2022) Gut microbiota promote biotransformation and bioaccumulation of arsenic in tilapia. *Environ. Pollut.* 305, 119321
57. Choi, S.Y. *et al.* (2017) Violacein and bacterial predation: promising alternatives for priority multidrug resistant human pathogens. *Future Microbiol.* 12, 835–838
58. Dwidar, M. *et al.* (2021) Diffusible signaling factor, a quorum-sensing molecule, interferes with and is toxic towards *Bdellovibrio bacteriovorus* 109J. *Microb. Ecol.* 81, 347–356
59. Lopes, J.P. *et al.* (2022) Pathogenesis and virulence of *Candida albicans*. *Virulence* 13, 89–121
60. Riekhof, W.R. *et al.* (2017) Quorum sensing in *Candida albicans*: farnesol versus farnesoic acid. *FEBS Lett.* 591, 1637–1640
61. Tian, J. *et al.* (2013) BDSF inhibits *Candida albicans* adherence to urinary catheters. *Microb. Pathog.* 64, 33–38
62. Vílchez, R. *et al.* (2010) *Streptococcus mutans* inhibits *Candida albicans* hyphal formation by the fatty acid signaling molecule trans-2-decenoic acid (SDSF). *Chembiochem* 11, 1552–1562
63. de Rossi, B.P. *et al.* (2014) *Stenotrophomonas maltophilia* interferes via the DSF-mediated quorum sensing system with *Candida albicans* filamentation and its planktonic and biofilm modes of growth. *Rev. Argent. Microbiol.* 46, 288–297
64. Diab, A.A. *et al.* (2019) BDSF is the predominant in planta quorum-sensing signal used during *Xanthomonas campestris* infection and pathogenesis in Chinese Cabbage. *Mol. Plant-Microbe Interact.* 32, 240–254
65. Kakkar, A. *et al.* (2015) *Xanthomonas campestris* cell-cell signaling molecule DSF (diffusible signal factor) elicits innate immunity in plants and is suppressed by the exopolysaccharide xanthan. *J. Exp. Bot.* 66, 6697–6714
66. Chatterjee, S. *et al.* (2008) Living in two worlds: the plant and insect lifestyles of *Xylella fastidiosa*. *Annu. Rev. Phytopathol.* 46, 243–271
67. Caserta, R. *et al.* (2017) Ectopic expression of *Xylella fastidiosa rpfF* conferring production of diffusible signal factor in transgenic tobacco and citrus alters pathogen behavior

and reduces disease severity. *Mol. Plant-Microbe Interact.* 30, 866–875
68. Baccari, C. *et al.* (2014) Diffusible signal factor-repressed extracellular traits enable attachment of *Xylella fastidiosa* to insect vectors and transmission. *Phytopathol* 104, 27–33
69. Newman, K.L. *et al.* (2004) Cell-cell signaling controls *Xylella fastidiosa* interactions with both insects and plants. *Proc. Natl. Acad. Sci. U. S. A.* 101, 1737–1742
70. Chatterjee, S. *et al.* (2008) Cell-cell signaling in *Xylella fastidiosa* suppresses movement and xylem vessel colonization in grape. *Mol. Plant-Microbe Interact.* 21, 1309–1315
71. Ionescu, M. *et al.* (2014) *Xylella fastidiosa* outer membrane vesicles modulate plant colonization by blocking attachment to surfaces. *Proc. Natl. Acad. Sci. U. S. A.* 111, 3910–3918
72. Feitosa-Junior, O.R. *et al.* (2019) Proteomic and metabolomic analyses of *Xylella fastidiosa* OMV-enriched fractions reveal association with virulence factors and signaling molecules of the DSF family. *Phytopathol* 109, 1344–1353
73. Lindow, S. *et al.* (2014) Production of *Xylella fastidiosa* diffusible signal factor in transgenic grape causes pathogen confusion and reduction in severity of Pierce's disease. *Mol. Plant-Microbe Interact.* 27, 244–254
74. López Nadal, A. *et al.* (2020) Feed, microbiota, and gut immunity: using the zebrafish model to understand fish health. *Front. Immunol.* 11, 114
75. Zheng, Y. *et al.* (2021) Sheng-Mai Yin exerts anti-inflammatory effects on RAW 264.7 cells and zebrafish. *J. Ethnopharmacol.* 267, 113497
76. Kim, E. *et al.* (2019) Tuberatolide B isolated from *Sargassum macrocarpum* inhibited LPS-stimulated inflammatory response via MAPKs and NF-κB signaling pathway in RAW264.7 cells and zebrafish model. *J. Funct. Foods* 52, 109–115