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Quorum Sensing and Interspecies Interactions in *Stenotrophomonas maltophilia*

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Author's contribution

The only author performed the whole research work. Author RA wrote the first draft of the paper. Author RA read and approved the final manuscript.

Review Article

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ABSTRACT

Stenotrophomonas maltophilia is a gram-negative bacterium that is widespread in the environment and that has become important in the last years as an emerging opportunistic pathogen. Quorum sensing (QS) is a bacterial cell–cell communication process that involves the production, detection, and response to extracellular signalling molecules called autoinducers.

S. maltophilia has a diffusible signal factor (DSF) that controls cell–cell communication and many functions such as motility, extracellular protease production and microcolonies formation in artificial sputum medium. This DSF signalling mediates also interspecies interactions between S. maltophilia and Pseudomonas aeruginosa such as susceptibility to polymixin and its influence on biofilm formation.

The traditional approach for the treatment of infectious diseases is to kill or inhibit the growth of bacteria using antibiotics. In response to the rise in antibiotic resistance, the development and use of QS inhibition based drugs to attenuate bacterial pathogenicity is now highly required in the microbiological and clinical fields.

Keywords: Stenotrophpmonas maltophilia; quorum sensing; interspecies interactions; inhibition.

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1. INTRODUCTION

S. maltophilia is a Gram-negative bacterium that is widespread in the environment and that has become important in the last years as an emerging opportunistic pathogen associated with nosocomial colonization and infection. *S. maltophilia* is frequently isolated from clinical specimens and is implicated in catheter-related bacteremia and septicemia, urinary and respiratory tract infections, and endocarditis [1,2,3]. Infections occur in cystic fibrosis and burn patients and are common in individuals with impaired defenses who are susceptible to opportunistic infections [2].

S. maltophilia is associated with crude mortality rates ranging from 14 to 69% in patients with bacteremia [4]. Infections associated with *S. maltophilia* include (most commonly) respiratory tract infections (pneumonia and acute exacerbations of chronic obstructive pulmonary disease [COPD] [5]; bacteremia [6,7]; biliary sepsis [8]; infections of the bones and joints, urinary tract, and soft tissues [9,10]; endophthalmitis [11]; eye infections (keratitis, scleritis, and dacryocystitis [12]; endocarditis [13,14]; and meningitis [15]. *S. maltophilia* is a significant pathogen in cancer patients, particularly those with obstructive lung cancer [16].

S. maltophilia is an environmental multi-drug resistant organism. It's incidence in hospital-acquired infections is increasing, particularly in the immunocompromised patient population, and cases of community-acquired *S. maltophilia* have also been reported. *S. maltophilia* infections can occur in both children and adults.

The transmission of *S. maltophilia* to susceptible individuals may occur through direct contact with the source. The hands of health care personnel have been reported to transmit nosocomial *S. maltophilia* infection in an intensive care unit (ICU) [17]. *S. maltophilia* has been cocultured with *P. aeruginosa* in respiratory samples obtained from CF patients. Cough generated aerosols from CF patients have the potential to provide airborne transmission of *S. maltophilia* [18].

The treatment of *S. maltophilia* infections is problematic, as isolates are resistant to many clinically useful antibiotics. A number of laboratories have begun to address the molecular bases for the broad antibiotic resistance and for virulence in *S. maltophilia* [19,20].

2. QUORUM SENSING

Quorum sensing (QS) is a bacterial cell–cell communication process that involves the production, detection, and response to extracellular signaling molecules called autoinducers (AIs). AIs accumulate in the environment as the bacterial population density increases, and bacteria monitor this information to track changes in their cell numbers and collectively alter gene expression. When the autoinducer reaches a critical level the, the population responds through the coordinated expression of specific target genes [21] (Fig. 1). QS controls genes that direct activities that are beneficial when performed by groups of bacteria acting in synchrony. Processes controlled by QS include bioluminescence, sporulation, competence, antibiotic production, biofilm formation, and virulence factor secretion [22,23].

Despite differences in regulatory components and molecular mechanisms, all known QS systems depend on three basic principles. First, the members of the community produce AIs, which are the signaling molecules. At low cell density (LCD), AIs diffuse away, and, therefore, are present at concentrations below the threshold required for detection. At high

cell density (HCD), the cumulative production of AIs leads to a local high concentration, enabling detection and response [24]. Second, AIs are detected by receptors that exist in the cytoplasm or in the membrane. Third, in addition to activating expression of genes necessary for cooperative behaviors, detection of AIs results in activation of AI production. This promotes synchrony in the population [25].

Thus QS, is a regulatory mechanism, enables bacteria to make collective decisions with respect to the expression of a specific set of genes that involve the production, release and subsequent detection of chemical signaling molecules.

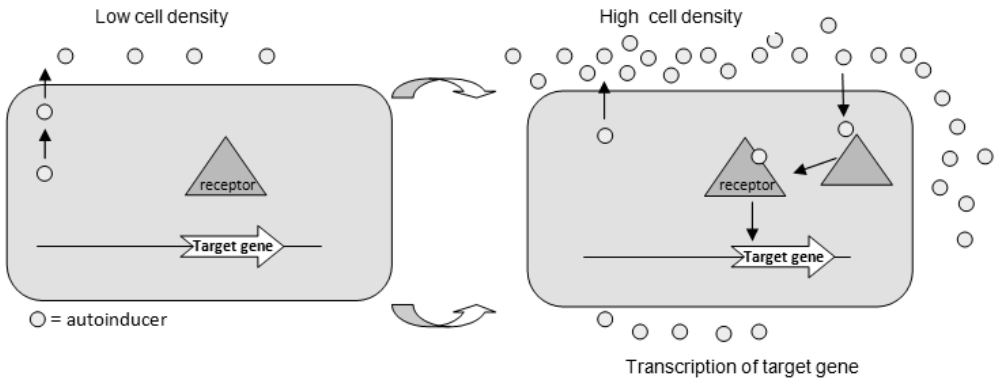
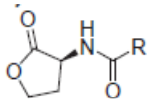
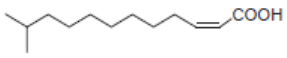


Fig. 1. Diagram showing bacterial quorum sensing regulation

2.1 Quorum Sensing in Gram Negative Bacteria.

Gram-negative bacteria communicate using small molecules as AIs. These are either acylhomoserine lactones (AHLs) (Table 1) or other molecules whose production depends on S-adenosylmethionine (SAM) as a substrate [26]. AIs are produced in the cell and freely diffuse across the inner and outer membranes. When the concentration of AIs is sufficiently high, which occurs at HCD, they bind cytoplasmic receptors that are transcription factors. The AI-bound receptors regulate expression of the genes in the QS regulon [27].

Table 1. QS signaling molecules

Compound & structure	producer organisms	QS system	Ref.
N-acyl homoserine lactones 	*Pseudomonas aeruginosa*	LasIR, RhlIR	[28]
DSF Diffusible signal factor 	*Stenotrophomonas maltophilia*	Rpf system	[29]

2.2 Quorum Sensing in *S. maltophilia*.

S. maltophilia has a diffusible signal factor (DSF) (Table 1) system that was first identified in *Xanthomonas campestris pv. Campestris* [30]. The DSF activity of *S. maltophilia* strain WR-C is due to cis-2-11-methyl-dodecenoic acid and seven structural derivatives [31]. rpfF, part of the rpf (regulation of pathogenicity factors) gene cluster of *S. maltophilia* K279a, complemented the rpfF mutant of X. campestris, resulting in DSF production [32]. The rpfF mutant of *S. maltophilia* K279a demonstrated reduced motility, reduced extracellular protease production, altered LPS, and reduced tolerance to select antibiotics and heavy metals. In contrast to wild-type *S. maltophilia*, the rpfF *S. maltophilia* mutant is unable to form microcolonies in artificial sputum medium. The exogenous addition of DSF (1_Mor from *S. maltophilia* extracts) restored the ability of the rpfF *S. maltophilia* mutant to form microcolonies and restored motility and extracellular protease production. In a nematode model, the rpfF *S. maltophilia* mutant demonstrated reduced killing activity, in contrast to wild-type *S. maltophilia* [32]. The rpfF gene regulates the expression of FecA, an outer membrane receptor used for ferric citrate uptake [30].

The cyclic AMP receptor protein (CRP) positively regulates rpfF transcripts; complementation studies and the presence of two potential CRP binding sites upstream of the rpfF promoter suggest that CRP is a transcriptional activator of rpfF. Transposon mutants in crp of *S. maltophilia* were defective in proteolysis and hemolysis, in contrast to wild-type *S. maltophilia* [30]. Together, these observations suggest that rpfF and crp are important for the virulence of *S. maltophilia*. Providing rpfF in trans in wild-type *S. maltophilia* and in *S. maltophilia* rpfB and rpfBF mutants resulted in swimming and radial translocation of these strains [31]. The ability of the wild type and a flagellum-defective *S. maltophilia* xanB mutant to demonstrate radial translocation in the presence of an rpfB/prpfF (plasmid prpfF contains the 975-bp rpfF native promoter and coding sequences in pBBR1MCS5) *S. maltophilia* strain suggested that the rpfB/prpfF strain secreted molecules that enabled flagellum independent translocation. High-performance liquid chromatography, electrospray ionization mass spectrometry, and gas chromatography-mass spectrometry analyses of these extracellular compounds have shown them to be derivatives of cis-2-11-methyl-dodecenoic acid. Synthetic cis-2-11-methyl-dodecenoic acid or 11-methyl-dodecenoic acid enabled the surface translocation of wild-type *S. maltophilia* carrying pBBR1MCS5 [31].

2.3 Interspecies Interactions in *S. maltophilia*.

Robert P, et al. [33] examined interspecies interactions between *S. maltophilia* and *P. aeruginosa* in mixed biofilms. Both of these organisms occur ubiquitously in the environment and are important nosocomial pathogens. They can be found together in diverse niches including the rhizosphere of plants and the cystic fibrosis (CF) lung [34].

Interspecies signalling between *S. maltophilia* and *P. aeruginosa*, mediated by DSF, influences both *P. aeruginosa* biofilm architecture and the synthesis of proteins that contribute to the resistance of this organism to cationic antimicrobial peptides (CAMPs). Furthermore they show that these effects depend upon the *P. aeruginosa* sensor kinase PA1396, which has an input domain related to the sensory input domain of RpfC [35].

Homologues of PA1396 occur in other pseudomonads, some of which are plant-pathogenic or plant-associated, as well as in unrelated bacteria. These observations suggest that modulation of bacterial behaviour through DSF-mediated interspecies signalling is a phenomenon that occurs widely [35].

It has also been noticed that DSF signalling between bacterial species is not restricted to the xanthomonads. In mixed species biofilms, *S. maltophilia* influences *P. aeruginosa* to develop structures with different architecture from those seen in *P. aeruginosa* mono-species biofilms. These findings indicated the possibility that *S. maltophilia* co-infection may have animpact on the efficacy of polymyxins, which are being are-introduced into clinical practice as agents for treatment of *P. aeruginosa* infections [35].

The DSF activity of *S. maltophilia* also alters the susceptibility of P*. aeruginosa* to polymyxin, [33]. An rpfF mutant of *S. maltophilia* does not synthesize DSF, and biofilms of the mutant are not as filamentous as those produced by wild-type *S. maltophilia* [32,33]. The complementation of the *S. maltophilia* rpfF mutant with the cloned rpfF gene or the supplementation of the mutant with DSF (10 or 50 M) restores the filamentous structure of the biofilm [33]. *P. aeruginosa* formed flat biofilms when grown in monoculture or in coculture with the *S. maltophilia* rpfF mutant. In cocultures with DSF-producing *S. maltophilia* and *P. aeruginosa*, the biofilm of *P. aeruginosa* changed from a flat to a filamentous biofilm [33].

A filamentous biofilm was also observed in monocultures of *P. aeruginosa* supplemented with 10 or 50 M DSF. The PA1396 protein of *P. aeruginosa* was identified as a two-component sensor of DSF [30]. The addition of DSF or the mutation of PA1396 resulted in increased resistance to polymyxins B and E. Mutations of PA1396 also resulted in the increased expression of a number of proteins involved in stress tolerance [33]. The recent identification of cis-2-decenoic acid as a fatty acid that induces the dispersal of *P. aeruginosa* PAO1 biofilms provides further evidence for the role of select fatty acids as cell-cell signaling molecules that influence biofilm architecture [36]. Together, these observations have clinical significance for the treatment of polymicrobial infections of *S. maltophilia* and *P. aeruginosa*. The DSF system may be a target for pharmacological therapy [37].

2.4 Quorum Sensing Inhibition.

The traditional approach for the treatment of infectious diseases is to kill or inhibit the growth of bacteria using antibiotics, which has selected for resistance to these drugs, and this has particularly been the case in *P. aeruginosa*. In response to the rise of antibiotic resistance, the continued development of new drugs and the judicious use of our current arsenal of antibiotics is required [38]. In this context, the development and use of QSinhibition- based drugs to attenuate bacterial pathogenicity is attractive [39].

Inhibition of the bacterial QS system, rather than a bactericidal or bacteriostatic strategy, might be appliedin many fields such as medicine, agriculture and food technology. This approach is very attractive because it is not directly involved in the inhibition of bacterial growth and does not impose harsh selective pressure for the development of resistance. Accordingly, there is a particular interest in finding new chemical entities that inhibit bacterial QS [40].

Indeed, possibly because of their anti-biofilm effects, some quorum-sensing inhibitors (QSIs) like patulin and garlic extracts have even been found to make *P. aeruginosa* more susceptible to antibiotics, for example, tobramycin [41]. To date, one of the known anti-QS compounds of nonbacterial origin are halogenated furanones from the red alga Delisea pulchra [42]. Anti-QS activity has also been noted in a number of traditional medicinal plants [43]. Rhubarb (named Dahuang in Chinese), a medicinal plant, displays diverse pharmacological activities such as bacteriostatic, antiviral, antifungal and antitumour activities [44].

Emodin, one of the free anthraquinone compounds extracted from rhubarb, is the major active constituent [45] that inhibits the expression of the tumour necrosis factor alpha (TNF-a) gene and tumour metastasis in vitro and in vivo. It also promotes the apoptosis of human breast cancer BCap-37 cells [46]. Some studies have also reported the effect of emodin on cell death in human prostate, lung, liver, cervical and blood cancer cells [47]. In this study, compound 5 (emodin) and ampicillin acted jointly against *P. aeruginosa* more effectively than either of them did alone, suggesting that QS [48].

2.5 QS Inhibition in *S. maltophilia*

Components of some traditional Chinese medicines (TCMs) have been identified to be effective in the treatment of various inflammatory and infectious diseases such as gastritis, stomatitis and pneumonia [45]. Thus, it was considered interesting to screen compounds from known TCMs to test whether they have QSI activity. Ding et al. [48] found that emodin (an active component found in TCMs) inhibited biofilm formation in *S. maltophilia*, also it significantly inhibited biofilm formation at 20 µM and induced proteolysis of the quorum-sensing signal receptor TraR in Escherichia coli at a concentration of 3–30 µM. Emodin also increased the activity of ampicillin against *P. aeruginosa*. Therefore, emodin might be suitable for development into an antivirulence and antibacterial agent [48].

3. CONCLUSION

QS is a vital regulatory mechanism used by many bacteria to control the bacterial virulence. *S. maltophilia* has a DSF system through which it can communicate and regulate many bacterial functions. DSF system also mediates interspecies signalling between *S. maltophilia* and P aeruginosa. Inhibition of the bacterial QS system, rather than a bactericidal or bacteriostatic strategy might be appliedin many fields such as medicine, agriculture and food technology. This approach is highly required in the microbiological and clinical fields because it is not directly involved in the inhibition of bacterial growth and does not impose harsh selective pressure for the development of resistance. Accordingly, there is a particular interest in finding new chemical entities that inhibit bacterial QS.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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