



Quorum signaling and sensing by nontypeable *Haemophilus influenzae*

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Quorum signals are diffusible factors produced by bacteria that coordinate communal responses. For nontypeable *Haemophilus influenzae* (NTHi), a series of recent papers indicate that production and sensing of quorum signals are determinants of biofilm formation/maturation and persistence *in vivo*. In this mini-review I will summarize the current knowledge about quorum signaling/sensing by this organism, and identify specific topics for additional study.

Keywords: biofilm, *Haemophilus influenzae*, otitis media, quorum signals, chronic

INTRODUCTION

Bacterial quorum sensing involves community-dependent regulation of bacterial gene expression by means of soluble signal molecules that are released in a density-dependent manner (Miller and Bassler, 2001; Henke and Bassler, 2004; Waters and Bassler, 2005). These signaling molecules are chemically diverse, and include the so-called competence factor peptides of pneumococci and other gram-positive bacteria, homoserine lactones, and derivatives of the bacterial metabolic by-product dihydroxy-pentanedione (DPD), which is also referred to as autoinducer-2 (AI-2). Because production of DPD is widely conserved among bacterial species, it has been referred to as an interspecies quorum signal (Surette et al., 1999).

Quorum sensing has been shown to coordinate group activities among bacterial populations, including formation and maturation of biofilm communities (Parsek and Singh, 2003; Henke and Bassler, 2004; Irie and Parsek, 2008; Shrout et al., 2011). In this mini-review, I discuss the current knowledge about quorum signaling and sensing in nontypeable *Haemophilus influenzae* (NTHi), and highlight potential areas for future study.

RELATIONSHIP BETWEEN DPD (AI-2) QUORUM SIGNALS AND VIRULENCE

The current published work concerning NTHi quorum signaling and sensing is summarized in **Table 1**. Quorum signaling for this species was first suggested by presence of the *luxS* genetic determinant of DPD production in the *H. influenzae* Rd genomic sequence (Surette et al., 1999). Later, Daines and colleagues showed that numerous NTHi strains produced quorum signal as detected by the *Vibrio harveyi* bioluminescence assay (Daines et al., 2005). Also in this study, *luxS* transcript levels were shown to be increased during infection of epithelial cells, and mutants lacking *luxS* were also shown to retain the capacity

to form biofilms, have increased invasion of epithelial cells, and cause more severe otitis media disease in the chinchilla infection model.

Later work from our laboratory expanded on this work to show that while isogenic NTHi mutants lacking *luxS* do retain the capacity to form biofilms, quantitative assessment of the biofilm structure by confocal microscopic analysis showed significantly reduced thickness and density, which was restored by complementation or by co-culture with the parental strains (Armbruster et al., 2009). Chinchilla infection studies also revealed that *luxS* mutants caused a more acute, inflammatory infection and that long-term persistence of *luxS* mutants was significantly reduced as compared with the parental strain (Armbruster et al., 2009). These changes in biofilm were correlated with shifts in the lipooligosaccharide glycolipids on the bacterial surface, which had previously been shown by our group to promote biofilm maturation and persistence *in vivo* (Hong et al., 2007a,b).

More recently, the RbsB protein was shown to mediate uptake of DPD quorum signals for NTHi 86-028NP (Armbruster et al., 2011). RbsB is a periplasmic binding protein which functions as part of an ABC transporter for ribose sugars (Park et al., 1999), and has been shown to function in AI-2 uptake in other bacterial species (Shao et al., 2007). Similar to *luxS* mutants, isogenic *rbsB* mutants were demonstrated to produce biofilms with significantly reduced thickness and density as compared to the parental NTHi strain. These changes in biofilm were correlated with changes in the lipooligosaccharide content and a persistence defect in the chinchilla infection model (Armbruster et al., 2011). However, given the genomic diversity among NTHi strains, it is important to note that there is a strong possibility for other determinants of quorum signal uptake (or absence of RbsB) in other strains (Pereira et al., 2009). In support of this

Table 1 | Current knowledge about quorum signaling/sensing in *H. influenzae*.

Finding	Reference
<i>NTHi luxS</i> mutants have increased invasion	Daines et al., 2005
<i>NTHi luxS</i> mutants cause acute otitis media with greater inflammation	Daines et al., 2005
Quorum signaling promotes <i>NTHi</i> biofilm maturation	Armbruster et al., 2009
Quorum signaling affects lipooligosaccharide composition	Armbruster et al., 2009
<i>NTHi</i> quorum signals affect <i>M. catarrhalis</i>	Armbruster et al., 2010
RbsB is a determinant of quorum signal uptake for strain <i>NTHi</i> 86-028NP	Armbruster et al., 2011

idea, examination of the 18 *NTHi* publicly accessible genomic sequences reveals that while the majority of strains (12/18) would be predicted to only have the Rbs system for uptake, orthologs of the Lsr system associated with quorum signal uptake and sensing in other bacterial species are found in 3/18 strains; in one of these the Rbs transporter is not found. Notably, in 3/18 strains there were no predicted sequences for either transporter. Thus, the potential exists for significant mechanistic diversity in quorum signal uptake (and presumably, sensing) among different *NTHi* strains. We are currently addressing this important topic.

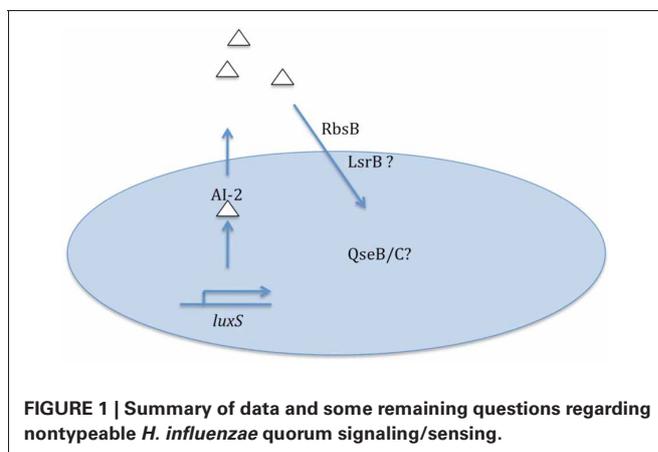
INTERSPECIES QUORUM SIGNALING

In addition to the impact of quorum signaling on *NTHi* biofilms, recent work has demonstrated that *NTHi* quorum signals may impact *Moraxella catarrhalis*, an opportunistic pathogen that inhabits many of the same host environments within the airway. Growth of *M. catarrhalis* within a polymicrobial biofilm with nontypeable *H. influenzae* was shown to promote antibiotic resistance and persistence within the chinchilla infection model (Armbruster et al., 2010). In support of quorum signaling as a mechanism for these effects on *M. catarrhalis*, there was no significant benefit in terms of antibiotic resistance or persistence *in vivo* in parallel experiments using an isogenic *NTHi luxS* mutant strain. Notably, no AI-2 quorum signal production was detected for any *M. catarrhalis* strain, and recent data analyzing a number of sequenced *M. catarrhalis* genomes show that none have a homologue for the *luxS* genetic determinant of this quorum signal (Davie et al., 2011). Moreover, *M. catarrhalis* bacteria had the capacity to take up purified DPD from culture supernatants, and addition of purified DPD to *M. catarrhalis* bacteria also promoted biofilm density and antibiotic resistance *in vivo* (Armbruster et al., 2010). Taken together, these results show that *M. catarrhalis* “eavesdrops” on *NTHi* quorum signals to coordinate its biofilm development. These results are consistent with epidemiologic data that indicate a significant correlation of *M. catarrhalis* with *NTHi* co-infection in clinical samples from patients with otitis media or other opportunistic airway infections (Pettigrew et al., 2008; Verhaegh et al., 2011).

CONTROVERSIES, REMAINING QUESTIONS AND TOPICS FOR ADDITIONAL STUDY

While present data clearly establish the importance of quorum signaling/sensing for some *NTHi* model strains, much remains to be learned on this topic and its relationship to virulence (summarized in **Figure 1**). The magnitude and kinetics of quorum signal production by different *NTHi* strains is not presently known, and there is a distinct possibility that some strains may not fit with the current knowledge that has mostly been derived from *NTHi* 86-028NP. As highlighted above, genomic analyses clearly indicate presence of other potential AI-2 transporters in some *NTHi* genomes; whether these strains have greater capacity for signal uptake (and presumably sensing) is a subject for additional study. All sequenced *NTHi* strains have homologs to the QseB/C two-component signaling system that mediates sensing of AI-2/DPD signals for some other bacterial species; the role of these factors in sensing of quorum signal by *NTHi* is not presently clear. Finally, generation of *NTHi* mutant strains in which AI-2 quorum signal may be artificially induced would be of great help not only in clarifying the direct linkage of quorum signal to biofilm formation/maturation but also in defining the consequences of quorum sensing for the bacterial population.

It should also be pointed out that the relevance of biofilms for *NTHi* infection is not universally accepted (Moxon et al., 2008). Criticisms of this concept have included the lack of a defined matrix component expressed exclusively during biofilm growth, observation of aggregated bacteria with some of the descriptive qualities associated with biofilm in plate cultures, and lack of evidence for a controlled development of a differentiated community within *NTHi* biofilms/aggregates. Certainly, there was at the time of this commentary a great need for better understanding of the process of biofilm development and the role(s) of biofilms in the persistence of *NTHi* bacteria *in vivo*. For example, prior to our demonstration of *NTHi* survival within neutrophil extracellular traps (NET; Hong et al., 2009; Juneau et al., 2011), it could have been plausible to consider the possibility that the surface-adherent bacteria, rather than persisting, were being killed. Likewise, the findings discussed above regarding role(s) for synthesis and uptake of quorum signal (Armbruster et al., 2009, 2011), and restoration of biofilm phenotype by addition

**FIGURE 1 | Summary of data and some remaining questions regarding nontypeable *H. influenzae* quorum signaling/sensing.**

of culture supernatants or synthetic DPD to *luxS* mutants (Armbruster et al., 2009, 2011), provide additional evidence for coordinated development of a biofilm. It is also important to note work from other species indicating roles for nutrient composition of growth media in biofilm formation/maturation that can equal or even surpass that of quorum signaling/sensing (Shrout

et al., 2006, 2011). This will surely be an important variable for additional study with regard to NTHi biofilms.

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REFERENCES

- Armbruster, C., Byrd, M., Love, C., Juneau, R., Kock, N. D., and Swords, W. E. (2009). LuxS promotes biofilm maturation and persistence of nontypeable *Haemophilus influenzae* in experimental otitis media by modulation of lipooligosaccharide composition. *Infect. Immun.* 77, 4081–4091.
- Armbruster, C. E., Hong, W., Pang, B., Weimer, K. E., Juneau, R. A., Turner, J., and Swords, W. E. (2010). Indirect pathogenicity of *Haemophilus influenzae* and *Moraxella catarrhalis* in polymicrobial otitis media occurs via interspecies quorum signaling. *MBio* 1, 102–110.
- Armbruster, C. E., Pang, B., Murrah, K., Juneau, R. A., Perez, A. C., Weimer, K. E., and Swords, W. E. (2011). RbsB (NTHI-0632) mediates quorum signal uptake in nontypeable *Haemophilus influenzae* strain 86-028NP. *Mol. Microbiol.* 82, 836–850.
- Daines, D. A., Bothwell, M., Furrer, J., Unrath, W., Nelson, K., Jarisch, J., Melrose, N., Greiner, L., Apicella, M., and Smith, A. L. (2005). *Haemophilus influenzae luxS* mutants form a biofilm and have increased virulence. *Microb. Pathog.* 39, 87–96.
- Davie, J. J., Earl, J., de Vries, S. P., Ahmed, A., Hu, F. Z., Bootsma, H. J., Stol, K., Hermans, P. W., Wadowsky, R. M., Ehrlich, G. D., Hays, J. P., and Campagnari, A. A. (2011). Comparative analysis and supragenome modeling of twelve *Moraxella catarrhalis* clinical isolates. *BMC Genomics* 12, 70.
- Henke, J. M., and Bassler, B. L. (2004). Bassler, Bacterial social engagements. *Trends Cell Biol.* 14, 648–656.
- Hong, W., Juneau, R., Pang, B., and Swords, W. E. (2009). Survival of bacterial biofilms within neutrophil extracellular traps promotes nontypeable *Haemophilus influenzae* persistence in the chinchilla model for otitis media. *J. Innate Immun.* 1, 215–224.
- Hong, W., Mason, K., Jurcisek, J. A., Novotny, L. A., Bakaletz, L. O., and Swords, W. E. (2007a). Phosphorylcholine decreases early inflammation and promotes the establishment of stable biofilm communities of nontypeable *Haemophilus influenzae* strain 86-028NP in a chinchilla model of otitis media. *Infect. Immun.* 75, 958–965.
- Hong, W., Pang, B., West-Barnette, S., and Swords, W. E. (2007b). Phosphorylcholine expression by nontypeable *Haemophilus influenzae* correlates with maturation of biofilm communities *in vitro* and *in vivo*. *J. Bacteriol.* 189, 8300–8307.
- Irie, Y., and Parsek, M. R. (2008). Quorum sensing and microbial biofilms. *Curr. Top. Microbiol. Immunol.* 322, 67–84.
- Juneau, R. A., Pang, B., Weimer, K. E., Armbruster, C. E., and Swords, W. E. (2011). Nontypeable *Haemophilus influenzae* initiates formation of neutrophil extracellular traps. *Infect. Immun.* 79, 431–438.
- Miller, M. B., and Bassler, B. L. (2001). Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55, 165–199.
- Moxon, E. R., Sweetman, W. A., Deadman, M. E., Ferguson, D. J., and Hood, D. W. (2008). *Haemophilus influenzae* biofilms: hypothesis or fact? *Trends Microbiol.* 16, 95–100.
- Park, Y., Cho, Y. J., Ahn, T., and Park, C. (1999). Molecular interactions in ribose transport: the binding protein module symmetrically associates with the homodimeric membrane transporter. *EMBO J.* 18, 4149–4156.
- Parsek, M. R., and Singh, P. K. (2003). Bacterial biofilms: an emerging link to disease pathogenesis. *Annu. Rev. Microbiol.* 57, 677–701.
- Pereira, C. S., de Regt, A. K., Brito, P. H., Miller, S. T., and Xavier, K. B. (2009). Identification of functional LsrB-like autoinducer-2 receptors. *J. Bacteriol.* 191, 6975–6987.
- Pettigrew, M. M., Gent, J. F., Revai, K., Patel, J. A., and Chonmaitree, T. (2008). Microbial interactions during upper respiratory tract infections. *Emerging Infect. Dis.* 14, 1584–1591.
- Shao, H., James, D., Lamont, R. J., and Demuth, D. R. (2007). Differential interaction of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* LsrB and RbsB proteins with autoinducer 2. *J. Bacteriol.* 189, 5559–5565.
- Shrout, J. D., Chopp, D. L., Just, C. L., Hentzer, M., Givskov, M., and Parsek, M. R. (2006). The impact of quorum sensing and swarming motility on *Pseudomonas aeruginosa* biofilm formation is nutritionally conditional. *Mol. Microbiol.* 62, 1264–1277.
- Shrout, J. D., Tolker-Nielsen, T., Givskov, M., and Parsek, M. R. (2011). The contribution of cell-cell signaling and motility to bacterial biofilm formation. *MRS Bull.* 36, 367–373.
- Surette, M. G., Miller, M. B., and Bassler, B. L. (1999). Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proc. Natl. Acad. Sci. U.S.A.* 96, 1639–1644.
- Verhaegh, S. J., Snippe, M. L., Levy, F., Verbrugh, H. A., Jaddoe, V. W., Hofman, A., Moll, H. A., van Belkum, A., and Hays, J. P. (2011). Colonization of healthy children by *Moraxella catarrhalis* is characterized by genotype heterogeneity, virulence gene diversity and co-colonization with *Haemophilus influenzae*. *Microbiology* 157, 169–178.
- Waters, C. M., and Bassler, B. L. (2005). Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol.* 21, 319–346.

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