Bacterial communication: when does a metabolite become a signal?

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Bacterial communication has risen to prominence in microbiology as a dynamic research topic, both because of its role in microbial ecology and evolution [1] and for the opportunity it offers to control pathogenic microbial activity [2]. Bacterial communication has evolved from the metabolic processes of prokaryotic cellular life, in which the biosynthesis and breakdown of chemical compounds in central metabolism generates secondary metabolites with ambiguous utility in natural selection.

Here we review evidence for the transformation of secondary metabolites into bacterial signalling molecules by the forces of evolution. A signalling molecule involved in an act of communication is distinguished here from chemical cues or manipulations by the following definition – bacterial signals are compounds produced by bacterial cells to which other organisms have evolved responses beneficial to both the producer and receiver.

Two recognised bacterial intercellular signalling mechanisms serve to illustrate how small organic molecules can evolve signalling functions and other distinct roles in population and community ecology. The first involves 4,5-dihydroxy-2,3-pentanedione (DPD, the AI-2 precursor) produced by lineages widespread throughout the bacterial domain and the second involves acylated homoserine lactones (AHLs) produced by taxa in 7% of genera in the alpha, beta and gamma classes of the proteobacterial phylum [3]. For detailed descriptions of the molecular biology of these systems, the reader is referred to the many reviews available [4, 5].

MICRO-FACT

The smallest genome for free living bacteria is 1.6 Mb for Pseudomonas [Brevundimonas] diminuta. *The genome of* E. coli *is 4.6 Mb.*

Central bacterial metabolism harvests energy and building blocks from the environment to construct the biochemical materials and power necessary for cell function.

S-adenosyl methionine (SAM) is one such central metabolite responsible for delivering methyl groups to proteins, nucleic acids and lipids. Methyl transferase reactions involving SAM generate S-adenosylhomocysteine (SAH), which inhibits methyl transferase reactions and must therefore be removed from the cell. In most taxa, the breakdown of SAH involves the enzymatic cleavage of the adenine and sulphur containing moieties, catalysed by LuxS, resulting in the production of DPD, which is an unstable molecule that spontaneously forms a small number of benign furanones [6] (collectively referred to here as AI-2). Cells that do not produce AI-2 dispose of SAH by a single step hydrolysis generating homocysteine and adenosine [7].

While AI-2 has remained solely the product of this metabolic waste disposal system for most of the taxa that produce it, members of the *Vibrio* genus within the Gammaproteobacteria have evolved an additional function for AI-2. Because AI-2 is freely diffusible across bacterial cell membranes, it has the capacity to accumulate in the extracellular milieu in response to excessive production by populations of cells and barriers to free diffusion away from producing cells. Vibrios rely on the accumulation of AI-2 to orchestrate the expression of phenotypes such as bioluminescence and virulence factor production in clonal populations. The communal expression of bioluminescence or virulence factors is beneficial both to AI-2 producer and recipient cells and thus constitutes genuine communication.

While it has been proposed that AI-2 is also involved in communication between distantly related bacterial lineages [8], the definitive criterion of mutual benefit has yet to be tested for many of these organisms. Many of the phenotypes affected in LuxS mutants may be due to interruptions to the activated methyl cycle and not quorum sensing *per se*.

However, in a recent report, it was demonstrated that mutualistic biofilm growth of two oral commensal bacteria was dependent on AI-2 and that pure synthetic AI-2 could restore this phenotype in *luxS* mutants (LuxS is the AI-2 synthase) [9]. Thus, further investigations may indeed identify other genera that have evolved to use AI-2 as a signal but, for now, it appears that the use of AI-2 for communication (production, response and mutual benefit) is mainly restricted to the Vibrios.

Beyond its role in transferring methyl groups, SAM also reacts with acylated acyl carrier proteins involved in fatty acid biosynthesis to form AHLs [10]. AHLs are a group of molecules with a homoserine lactone ring joined through an amide group to an acyl chain of varying length and modifications. Like AI-2 in Vibrios, AHLs represent genuine communication signals through accumulation in

the extracellular environment and orchestration of the expression of various phenotypes, including bioluminescence, virulence and siderophore, exoenzyme and surfactant production [5].

In contrast to AI-2, AHLs have no known role in the central metabolism of the Proteobacteria that produce them. The primary evidence for this is that mutants lacking the AHL synthase gene are viable under common culturing conditions. This raises the question of whether AHLs once played an essential role in metabolism, as for AI-2, or whether it has always been selected for its communication function at the level of the population. Like AI-2, it has been suggested that AHL production may historically have been a means of disposing of SAH [3]. However, the fact that SAH does not serve as a substrate for contemporary AHL synthases is at odds with this possibility [11].

In addition to its role in methyl transferase activities, SAM is also a key substrate for polyamine biosynthesis. While polyamines are essential for bacterial cells, at high pH and at high concentrations, they can also be harmful to cells. Thus, it is also possible that AHL production was a low cost mechanism to by-pass polyamine synthesis during unfavourable conditions, perhaps stationary phase, where cell densities were high.

It has also been suggested that AHLs may once have been used as metal chelating agents that have been superseded by contemporary molecules such as siderophores [3]. Unfortunately, such speculation does not preclude the possibility that AHLs were directly co-opted from the by-products of central metabolism into a communication role.

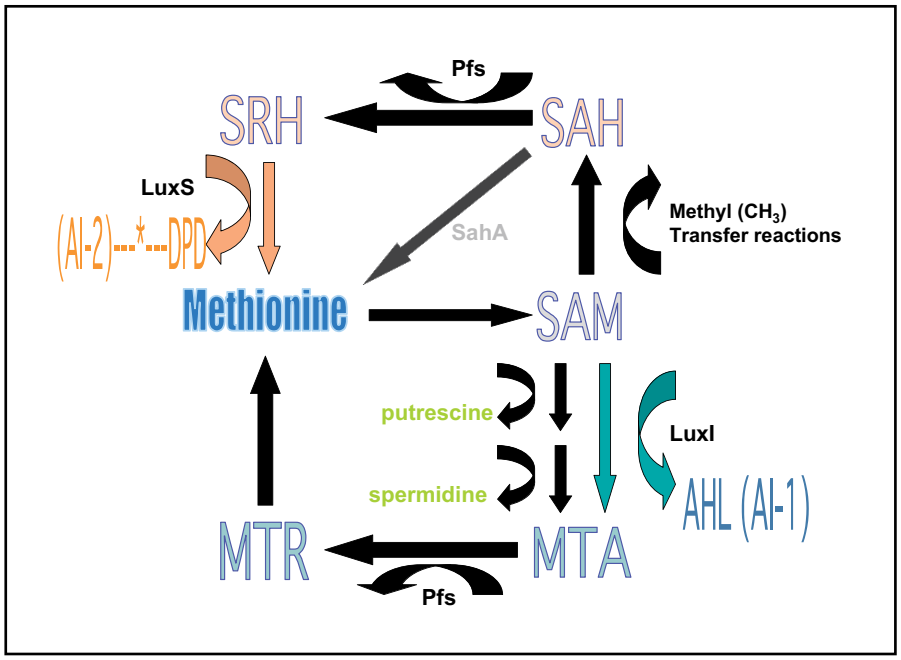

Figure 1. Metabolic pathways of quorum signal production. Enzymes appear as black text while colours arrows indicate signal synthesis steps. Methionine is converted into S-adenosyl-methionine (SAH), which can be converted into either AI-1 (Acylated homoserine lactone, lower pathway) or AI-2 (upper pathway) signals.
For AI-2 synthesis, SAM is converted into S-adenosyl-homocysteine (SAH) during methyl transfer reactions. SAH is converted by Pfs into S-ribosyl-homocysteine (SRH), which LuxS then converts into methionine and DPD. The DPD spontaneously converts into the AI-2 signal, noted by the asterisk.
The grey arrow, for SahA, is the one step pathway for conversion of SAH into methionine and organisms either have the Pfs-LuxS pathway or the SahA. For AHL synthesis, SAM is converted into methyl-thio-adenosine (MTA) and the AHL signal by LuxI.
Bacteria can also convert SAM into MTA via a two step process during the synthesis of polyamines (noted by the products of putrecine and spermidine). MTA is converted into methyl-thio-ribose (MTR) by Pfs, which is subsequently recycled into methionine.

Independent of whether their function was initially central in the cellular sense or revolutionary in that it encoded multicellular behaviour, it is likely that AHLs have not played an essential role in the metabolism of bacteria since before the divergence of the alpha, beta and gamma *Proteobacteria*. This assertion is derived from the fact that the last common ancestor of the alpha, beta and gamma *Proteobacteria* produced AHLs [12] and that 93% of the genera in these classes now have no known AHL producing representatives. This implies that the selection pressure maintaining AHL production is relatively weak, which is consistent with the hypothesis that it is selected for at the level of the population rather than the individual.

Further, to suggest that AHLs were not involved in communication when the *Alphaproteobacteria* diverged from the Beta and Gamma proteobacterial clade, is to intimate that AHLs independently evolved signalling function in multiple instances. Given that AI-2 has roles outside of communication and that the signalling function is restricted to the *Vibrio* genus, it suggests that the signalling function of AI-2 evolved more recently relative to that of the AHLs.

While AHLs have no apparent role in central metabolism, they do appear to have roles outside of their function in clonal communication as chemical cues or manipulations mediating interactions across species boundaries. There is, for example, compelling evidence that *Pseudomonas aeruginosa* can stimulate AHL mediated gene expression in *Burkholderia cepacia*, while co-habiting *in vitro* and *in situ* (mouse lung) biofilms [13].

In addition, *Escherichia coli* has the ability to alter gene expression and phenotype in response to AHLs produced by other bacterial species by means of SdiA, a LuxR homologue for which there is no cognate AI synthase [14]. It should be noted that LuxR and its homologs are the AHL (or AI-1) receptor proteins. The converse phenomenon is apparent in the discovery that *Bacillus* species, which do not produce AHLs, produce lactonases that can modulate AHL mediated gene expression in AHL producing species through degradation of the signalling molecule [15]. Such manipulations are likely rife throughout the prokaryotic world, but are most certainly not restricted to it.

As an example of inter-domain interactions mediated by AHLs, this promiscuous bacterial metabolite has been shown to manipulate gene expression in eukaryotes, with the finding that interleukin-8 production was stimulated in respiratory epithelial cells by an AHL produced by *P. aeruginosa* [16].

In addition to manipulations, it has also been demonstrated that AHLs act as a chemical cue for eukaryotic immune responses [17]. In the marine environment, AHLs have been shown to act as a settlement queue for motile zoospores of the green alga *Enteromorpha* [18], hinting that AHL producing bacteria may have a direct impact on algal biogeography. In the rhizopshere environment, Mathesius *et al.* provided proteomic evidence for extensive and specific responses to AHLs in the model legume *Medicago trunculata* [19]. These discoveries suggest that the chemical nature of AHLs appears to confer promiscuous bioactivity on this molecule at high levels of biological organisation such as populations and communities.

One of the most striking questions in biological communication, which is inherently a group activity, is how it can perpetuate under the threat of non-signalling or 'cheating' mutant genotypes. The question arises from the fact that signal production incurs a cost to the producing cells, so mutations that negate signal production in cells that still reap the benefit of group behaviour are strongly selected for at the cellular level.

In the case of AI-2, the fact that its signalling function is additional to a role in cellular metabolism offers some insurance against its loss through mutation. In the case of AHLs, however, there is no insurance at the cellular level against the loss of AHL production through random mutation. The signal producing genotype is therefore likely maintained through successive rounds of colony formation and dispersal, where colonies emerging from signalling cells are more successful than colonies emerging from non-cooperative mutants. Having made this point, the fact that selection at the cellular level is a more immediate pressure than at the population level might explain why, in the vast majority of proteobacterial lineages, AHL production and response have been lost.

In conclusion, the forces of evolution appear to co-opt small organic molecules to mediate information transfer between bacterial cells. Bacterial communication represents a mechanism by which bacteria have evolved higher levels of organisation and complexity. This refined understanding of how bacteria function at the cellular, population and community level, offers opportunities to enhance or interfere with bacterial activity according to context.

References

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MICRO-FACT

The smallest genomes of the reproducing organisms are Mycoplasma at 0.6 Mb. They have no cell-wall.