Review

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Microbial dinner-party conversations: the role of LuxS in interspecies communication

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Bacteria have a tendency to be gregarious by nature. Whether on abiotic surfaces in the environment or on the mucosal surfaces of humans, bacteria accumulate in complex multi-species communities. In these dynamic accretions, bacteria can be densely packed and often depend on each other for the provision of metabolic substrates. Under these circumstances, it will be advantageous for bacteria to be able to detect the presence of their neighbours, to communicate with them and to co-ordinate various physiological activities. Such cell–cell sensing and communication systems can be established through the release and detection of chemical signalling molecules. While originally considered a feature characteristic of eukaryotes, the exchange of chemical signals has now been demonstrated in many bacterial species and ecosystems. Indeed, it has even been suggested that assemblages of bacterial species can be considered as proto-multicellular organisms, whereby biological processes are controlled for the benefit of the entire community. Regardless of the extent to which bacterial communication represents a step on the road to multicellularity, it is becoming increasingly apparent that the signalling systems devised by bacteria are essential for successful relationships with other bacteria and with eukaryotic hosts.

The chattering classes

Population density is used by bacteria as a cue for the regulation of diverse cellular functions. Quorum (density-dependent) sensing involves the synthesis of small signalling molecules that accumulate in the local environment. As the bacterial population density increases, the concentration of signalling molecules also increases until a threshold, or quorum, is reached, triggering the expression of target genes. In Gram-negative bacteria, the best-described and most widespread signalling molecule is acyl homoserine lactone (AHL) (Fuqua *et al.*, 2001). In the archetypal quorum-sensing system of the marine symbiotic bacterium *Vibrio fischeri*, AHL is synthesized by LuxI protein. At a threshold concentration, the membrane-diffusible AHL interacts with and activates the intracellular LuxR transcriptional activator resulting, in the case of *V. fischeri*, in expression of genes required for bioluminescence (Fig. 1a). LuxI/R homologues have now been described in a large group of phylogenetically diverse bacteria including numerous plant and animal pathogens and, in many instances, two or more LuxI/R systems may function in a single species. AHL-based quorum sensing is known to regulate a range of cellular functions including bioluminescence, motility, production of secondary metabolites, expression of virulence factors and plasmid conjugal transfer (Fuqua *et al.*, 2001; Miller & Bassler, 2001;

Schauder & Bassler, 2001). Signalling through AHLs has also been shown to be important for biofilm formation in some organisms (Davies *et al.*, 1998), although other factors such as hydrodynamics appear to be at least as important (Kjelleberg & Molin, 2002; Purevdorj *et al.*, 2002). The AHLs produced by LuxI enzymic activity are system specific, and AHLs have been described with *N*-linked acyl side-chain lengths varying between 4 and 14 carbons, with various substitutions at the C3 position of the side chain. The LuxR proteins are exquisitely sensitive to their cognate AHL; however, they may respond to non-cognate AHLs, albeit at higher concentrations of the AHL (Fuqua *et al.*, 2001; Parsek & Greenberg, 2000).

LuxI/R systems have not yet been described in Gram-positive bacteria. Instead, many genera possess quorum-sensing systems based on modified or unmodified peptides. Not only is the language of communication different, but the means by which Gram-positive bacteria detect and act upon a threshold level of quorum-sensing signal may also differ from the LuxI/R system (Dunny & Leonard, 1997). One of the best-described systems is regulation of competence for genetic transformation in *Streptococcus pneumoniae* (Claverys *et al.*, 2000; Morrison & Lee, 2000). The *comCDE* locus, encoding competence-stimulating peptide (CSP), histidine kinase receptor and cognate response regulator, is transcribed at low levels during growth. The 41 amino acid product of *comC* is secreted from cells, as an active mature

Abbreviation: AHL, acyl homoserine lactone.

Fig. 1. Schematic diagrams of representative quorum-sensing systems. (a) LuxI/R quorum sensing. LuxI autoinducer synthase produces AHL molecules, which freely diffuse across the cytoplasmic membrane. At a critical concentration, AHL binds LuxR, generating an active response regulator that activates transcription of target genes. (b) Peptide-mediated quorum sensing in development of competence. The *comC* locus encodes a peptide pheromone precursor that is secreted as a mature signalling peptide (CSP) by a dedicated ABC transporter (ComAB). At a critical concentration, CSP binds to, and induces autophosphorylation of, the cognate histidine kinase (ComD) at the cytoplasmic membrane. The kinase subsequently phosphorylates and activates the response regulator (ComE). (c) Quorum sensing in *V. harveyi*. Two quorum-sensing circuits integrate cell-density information at the shared LuxU phosphorelay component. AI-1 and AI-2 are detected at the cell surface by LuxN and LuxQ, respectively (the periplasmic LuxP protein that is involved in recognition of AI-2 is not shown for simplicity).

heptadecapeptide, by a dedicated ATP-binding-cassette (ABC) transporter that is encoded by the unlinked *comAB* locus. The threshold level of CSP is detected at the cell surface by the ComD histidine kinase, which subsequently phosphorylates the response regulator ComE. Thus activated, ComE switches on the genes required for DNA uptake and processing (Fig. 1b). Competence in other streptococci, and the expression of antimicrobial peptides by a range of Gram-positive bacterial genera, is regulated in a similar fashion. In *Bacillus subtilis*, sporulation and competence are regulated by a complex network of phosphorylation and dephosphorylation events influenced by numerous peptide signals (Grossman, 1995). Some peptide signals are detected at the cell surface by a histidine kinase, while others are taken up by oligopeptide permeases and function intracellularly. The phosphorelay system allows the integration of multiple signals from metabolic and environmental conditions that impact on the decision of whether or not to sporulate.

The Babel fish

The cell-cell signalling systems described so far can be

considered by and large to function in self-perception, coordinating a cellular response with a specific density of like-minded cells. More recently, a novel communication system was described in the marine bacterium *Vibrio harveyi* that employs a signalling molecule that might function as a universal bacterial language. Bioluminescence in *V. harveyi* is regulated by using two distinct signalling molecules that are detected by independent signal-transduction systems that subsequently converge in a common pathway to regulate bioluminescence gene expression (Fig. 1c) (Miller & Bassler, 2001). Autoinducer (AI)-1 is a well-characterized derivative of an AHL that is highly species-specific for *V. harveyi*. LuxL and LuxM proteins are required for the synthesis of AI-1, and it is detected by LuxN sensor protein associated with the cytoplasmic membrane (Bassler *et al.*, 1993). It became clear from molecular studies of *V. harveyi* that there existed a second signalling system, based on an unrelated autoinducer (AI-2), that was species non-specific (Bassler *et al.*, 1994). The *luxS* gene encoding AI-2 synthase revealed no similarity to other autoinducer synthase genes (Surette *et al.*, 1999). Moreover, by using a mutant strain of *V. harveyi* able to detect AI-2 but not AI-1, it was soon demonstrated that culture supernatants of strains of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) were able to induce bioluminescence, indicating that AI-2 production was not restricted to *V. harveyi* and that interspecies communication was possible (Surette *et al.*, 1999). In *V. harveyi*, the proteins required for signal sensing were identified as LuxQ, showing homology to LuxN, and LuxP. LuxP is similar to periplasmic ribose-binding proteins of *E. coli* and *S. Typhimurium* (Bassler *et al.*, 1994). The story was completed with the identification of LuxU and LuxO, components of a phosphorelay cascade, which serve to integrate information from the receptors for AI-1 (LuxN) and AI-2 (LuxQ) and to regulate the expression of bioluminescence genes in response to cell density (Fig. 1c) (Freeman & Bassler, 1999a, b).

Talking the talk and walking the walk

Following its discovery in *V. harveyi*, *in silico* analyses revealed homologues of *luxS* in a wide range of organisms, including a number of important human pathogens. Most of these species were also able to produce functional AI-2, as measured by induction of bioluminescence in the *V. harveyi* reporter strain. As these organisms are not bioluminescent, nor likely to colonize the light organs of marine animals, the role of AI-2 must extend beyond light induction. Intriguingly, it appears that different organisms co-opt LuxS-based signalling for different purposes. Utilizing microarrays, Sperandio *et al.* (2001) found that a *luxS* null mutant of enterohaemorrhagic *E. coli* (EHEC) demonstrated differential expression of over 400 genes; remarkably, around 10 % of the total number of genes on the array. LuxS-downregulated genes included those involved in cell division, along with ribosomal and tRNA genes. Upregulated genes included several virulence factors such as genes involved in flagella biogenesis, motility and chemotaxis. The same group has also

shown that the type-III secretion system, along with intimin and its translocated receptor, are regulated by LuxS in both EHEC and enteropathogenic *E. coli* (Sperandio *et al.*, 1999). Similarly, DeLisa *et al.* (2001) found 242 genes (approx. 5 % of the genome) of *E. coli* that responded to the presence of AI-2, delivered in conditioned medium. Many of these genes are related to virulence or to processes such as cell division, morphogenesis and cell-surface architecture. A role for LuxS signalling as both a global regulator and as important for expression of virulence traits is thus emerging. In *Porphyromonas gingivalis*, AI-2 can control expression of genes involved in haemin uptake and in haemagglutination (Burgess *et al.*, 2002; Chung *et al.*, 2001). This periodontal pathogen has a requirement for haemin as a source of iron. Furthermore, haemin levels can also regulate expression of virulence properties by the organism. AI-2 signal is also required for formation of a mixed species biofilm with the oral commensal *Streptococcus gordonii* (McNab *et al.*, 2003). Consistent with its role as a species-non-specific signal, AI-2 from *S. gordonii* can complement a *luxS* mutation in *P. gingivalis*. Furthermore, *P. gingivalis* can also respond to AI-2 signal from another oral pathogen, *Actinobacillus actinomycescomitans* (Fong *et al.*, 2001). In *A. actinomycescomitans* itself, production of leukotoxin, an important virulence factor, and iron-uptake proteins is regulated in response to LuxS (Fong *et al.*, 2001). The *luxS* gene is also present in a range of pathogenic Gram-positive bacteria including *Streptococcus mutans*, *Streptococcus pyogenes* and *Clostridium perfringens* (Lyon *et al.*, 2001; Ohtani *et al.*, 2002; Wen & Burne, 2002). In the latter two instances, LuxS may be associated with virulence through the regulation of expression or activity of virulence factors, including haemolytic activity and SpeB extracellular cysteine protease, and secreted toxins, respectively.

Nonetheless, despite the frequently observed connection between LuxS and pathogenicity, results of studies testing *luxS* mutants in models of disease have been disappointing. *P. gingivalis luxS* mutants were found not to be attenuated in a murine lesion model of infection nor to be deficient in intracellular invasion of host epithelial cells (Burgess *et al.*, 2002; Chung *et al.*, 2001). Similarly, while *Shigella flexneri* LuxS modulates expression of *virB*, a transcription factor necessary for the expression of invasion loci, mutants deficient in AI-2 were fully virulent in the *in vivo* Sereny test (Day & Maurelli, 2001). An exception to this was the demonstration that *luxS* mutants of *Neisseria meningitidis* are defective for bacteraemia in a rat model (Winzer *et al.*, 2002c). Complementation of the mutation by *luxS* *in trans* restored virulence to wild-type levels. The influence of LuxS on pathogenicity may, therefore, be subtle and interconnected with other regulatory pathways such that a loss of LuxS is only manifest during certain stages of disease.

Talking with their mouths full

The LuxS system was originally discovered as a second quorum-sensing circuit controlling bioluminescence in *V. harveyi*. Moreover, in *V. harveyi*, the LuxQ sensor kinase

protein that detects AI-2 is upstream of LuxU, an integrator protein that is common to the AI-1 circuit (Freeman & Bassler, 1999a, b). These elegant studies from Bassler's group firmly established *V. harveyi* LuxS/AI-2 as a quorum-sensing mechanism in the tradition of the LuxR/LuxI system. The discovery of *luxS* homologues and AI-2 activity in other non-bioluminescent bacteria led to the understandable assumption that this also constituted LuxS-dependent quorum sensing. However, in their enthusiasm to explore this emerging field of bacterial gene regulation, investigators (such as ourselves!) rarely demonstrated formally that LuxS-based signalling was strictly cell-density dependent. While immaterial to the issue of what LuxS controls, the notion that the primary role of LuxS-based signalling may not be in cell-to-cell communication has been proposed by Winzer *et al.* (2002a). Based on the premise that a true signalling molecule generates responses beyond a physiological mechanism required to metabolize or detoxify the signal, LuxS can be viewed in terms of metabolic regulation. LuxS is an enzyme that converts S-ribosylhomocysteine (RH) to 4,5-dihydroxy-2,3-pentanedione (DPD) and homocysteine (Fig. 2) (Schauder *et al.*, 2001). In the presence of boron, DPD will form a furanosyl borate diester that has AI-2 activity (Chen *et al.*, 2002). Upstream of the LuxS-catalysed step, RH is produced by the action of the Pfs enzyme (encoding a 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase) on S-adenosylhomocysteine (SAH). SAH is formed from S-adenosylmethionine (SAM), a major methyl donor, following methylation of a number of substrates including DNA, RNA and proteins. LuxS is thus a component of an important SAH-degradation pathway that reduces feedback inhibition of SAM-dependent methylation and will also allow the scavenging of SAH constituents (Fig. 2) (Winzer *et al.*, 2002b). The subsequent accumulation of AI-2 may be damaging to the cellular DNA and, hence, AI-2 is extruded from the cell. As more methylation reactions are required

Fig. 2. Simplified diagram of the active methyl cycle. The conversion of S-ribosylhomocysteine (RH) to homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD) is catalysed by LuxS. The conversion of DPD to AI-2 or to 4-hydroxy-5-methyl-3(2H)-furanone (not shown) is thought to occur spontaneously. See text for more detail.

when cells are growing rapidly, the increase in extracellular AI-2 signal during exponential phase may simply reflect the activity of the methyl cycle rather than density dependence. Furthermore, AI-2 may be taken up and degraded by the cells later in order to recapture constituent molecules. A number of experimental observations can be interpreted as supporting this hypothesis. For example, in *P. gingivalis*, *pfs* and *luxS* form an operon (Chung *et al.*, 2001). Moreover, in the absence of glucose, AI-2 activity in *S. Typhimurium* is reduced (Surette & Bassler, 1999). This may be the result of AI-2 degradation in order to provide the cells with an additional metabolic substrate, the explanation favoured by Winzer *et al.* (2002a). As noted by Surette & Bassler (1999), however, the loss of AI-2 activity could equally well result from termination of production. Furthermore, these authors also reported that *S. Typhimurium* growing on several other carbon sources (acetate, glycerol, citrate and serine), and thus presumably utilizing the activated methyl cycle, does not produce AI-2 signal activity. More recently, Beeston & Surette (2002) reported that, in *S. Typhimurium*, expression of *luxS* is constitutive and that neither *luxS* nor *pfs* expression is regulated by AI-2. They concluded that AI-2 production is regulated at the level of LuxS substrate availability and that AI-2 signalling is a reflection of the metabolic state of the cell. Certainly, therefore, a persuasive case can be made for LuxS as having been originally designed as a component of a metabolic/detoxification pathway. Nevertheless, the large number and range of genes that appear to be controlled by LuxS would suggest relevance beyond the activated methyl cycle.

Noise annoys

Another question that remains to be answered relates to interference, or background 'noise', that would be inherent with a universally detected signal. On the basis that culture supernatant from many diverse bacteria can induce bioluminescence in *V. harveyi*, AI-2 can certainly be considered species non-specific. What is less clear, however, is whether all bacteria sense and respond equally to heterologous AI-2. Given that large numbers of genes can be controlled by AI-2 signalling, it would seem that individual bacteria in densely packed communities could be overwhelmed by AI-2 noise from other species. Is it possible, therefore, that AI-2 signalling could have a degree of specificity overlaid on its interspecies activity? One way to accomplish this would be for bacteria to produce unique modifications to the basic AI-2 structure that, while recognizable as AI-2, would also impart a degree of specificity. Some support for this concept is provided by the finding that culture supernatant from *P. gingivalis* and *S. gordonii* is about one log less active in inducing *V. harveyi* bioluminescence than is the homologous signal (Chung *et al.*, 2001; McNab *et al.*, 2003). Specificity could also occur at the level of signal transduction. In *V. harveyi*, two nutritional regulators, CRP (cAMP receptor protein) and MetR (a LysR homologue), bind to bioluminescence gene promoters (Chatterjee *et al.*, 2002). However, CRP is an activator of gene expression, whereas MetR

represses gene activity. Differential expression of regulatory proteins could, therefore, be one means of integrating multiple environmental and cell-density signals and ensuring a controlled response to AI-2. Resolution of this issue awaits the elucidation of the structure of AI-2, and of the molecular circuitry of AI-2 detection, in a wider range of organisms.

The last word

While some aspects of bacterial communication are now well understood, much remains to be learned about the roles of the players involved in the LuxS drama. As bacteria are constantly striving to enhance their metabolic state (with subsequent increase in cell density), signalling and metabolism are intricately interconnected. Hence, it will not be a simple task to distinguish regulation of gene expression as a function of signalling activity per se from differential expression of genes induced by the metabolic state of the cell. In either event, it is a relatively common occurrence for bacteria to exploit one protein or one pathway for multiple purposes. Perhaps the more important issue is not how to define LuxS but, rather, what can LuxS-dependent systems tell us about the pathophysiology of medically relevant bacteria?

References

- Bassler, B. L., Wright, M., Showalter, R. E. & Silverman, M. R. (1993). Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. *Mol Microbiol* **9**, 773–786.
- Bassler, B. L., Wright, M. & Silverman, M. R. (1994). Multiple signalling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. *Mol Microbiol* **13**, 273–286.
- Beeston, A. L. & Surette, M. G. (2002). *pfs*-dependent regulation of autoinducer 2 production in *Salmonella enterica* serovar Typhimurium. *J Bacteriol* **184**, 3450–3456.
- Burgess, N. A., Kirke, D. F., Williams, P., Winzer, K., Hardie, K. R., Meyers, N. L., Aduse-Opoku, J., Curtis, M. A. & Cámara, M. (2002). LuxS-dependent quorum sensing in *Porphyromonas gingivalis* modulates protease and haemagglutinin activities but is not essential for virulence. *Microbiology* **148**, 763–772.
- Chatterjee, J., Miyamoto, C. M., Zouzoulas, A., Lang, B. F., Skouris, N. & Meighen, E. A. (2002). MetR and CRP bind to the *Vibrio harveyi* lux promoters and regulate luminescence. *Mol Microbiol* **46**, 101–111.
- Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczar, I., Bassler, B. L. & Hughson, F. M. (2002). Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* **415**, 545–549.
- Chung, W. O., Park, Y., Lamont, R. J., McNab, R., Barbieri, B. & Demuth, D. R. (2001). Signaling system in *Porphyromonas gingivalis* based on a LuxS protein. *J Bacteriol* **183**, 3903–3909.
- Claverys, J. P., Prudhomme, M., Mortier-Barriere, I. & Martin, B. (2000). Adaptation to the environment: *Streptococcus pneumoniae*, a paradigm for recombination-mediated genetic plasticity? *Mol Microbiol* **35**, 251–259.
- Davies, D. G., Parsek, M. R., Pearson, J. P., Iglewski, B. H., Costerton, J. W. & Greenberg, E. P. (1998). The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* **280**, 295–298.
- Day, W. A., Jr & Maurelli, A. T. (2001). *Shigella flexneri* LuxS quorum-sensing system modulates *virB* expression but is not essential for virulence. *Infect Immun* **69**, 15–23.

- DeLisa, M. P., Wu, C. F., Wang, L., Valdes, J. J. & Bentley, W. E. (2001). DNA microarray-based identification of genes controlled by autoinducer 2-stimulated quorum sensing in *Escherichia coli*. *J Bacteriol* **183**, 5239–5247.
- Dunny, G. M. & Leonard, B. A. (1997). Cell–cell communication in gram-positive bacteria. *Annu Rev Microbiol* **51**, 527–564.
- Fong, K. P., Chung, W. O., Lamont, R. J. & Demuth, D. R. (2001). Intra- and interspecies regulation of gene expression by *Actinobacillus actinomycescomitans* LuxS. *Infect Immun* **69**, 7625–7634.
- Freeman, J. A. & Bassler, B. L. (1999a). A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in *Vibrio harveyi*. *Mol Microbiol* **31**, 665–677.
- Freeman, J. A. & Bassler, B. L. (1999b). Sequence and function of LuxU: a two-component phosphorelay protein that regulates quorum sensing in *Vibrio harveyi*. *J Bacteriol* **181**, 899–906.
- Fuqua, C., Parsek, M. R. & Greenberg, E. P. (2001). Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. *Annu Rev Genet* **35**, 439–468.
- Grossman, A. D. (1995). Genetic networks controlling the initiation of sporulation and the development of genetic competence in *Bacillus subtilis*. *Annu Rev Genet* **29**, 477–508.
- Kjelleberg, S. & Molin, S. (2002). Is there a role for quorum sensing signals in bacterial biofilms? *Curr Opin Microbiol* **5**, 254–258.
- Lyon, W. R., Madden, J. C., Levin, J. C., Stein, J. L. & Caparon, M. G. (2001). Mutation of *luxS* affects growth and virulence factor expression in *Streptococcus pyogenes*. *Mol Microbiol* **42**, 145–157.
- McNab, R., Ford, S. K., El-Sabaeny, A., Barbieri, B., Cook, G. S. & Lamont, R. J. (2003). LuxS-based signaling in *Streptococcus gordonii*: autoinducer 2 controls carbohydrate metabolism and biofilm formation with *Porphyromonas gingivalis*. *J Bacteriol* **185**, 274–284.
- Miller, M. B. & Bassler, B. L. (2001). Quorum sensing in bacteria. *Annu Rev Microbiol* **55**, 165–199.
- Morrison, D. A. & Lee, M. S. (2000). Regulation of competence for genetic transformation in *Streptococcus pneumoniae*: a link between quorum sensing and DNA processing genes. *Res Microbiol* **151**, 445–451.
- Ohtani, K., Hayashi, H. & Shimizu, T. (2002). The *luxS* gene is involved in cell–cell signalling for toxin production in *Clostridium perfringens*. *Mol Microbiol* **44**, 171–179.
- Parsek, M. R. & Greenberg, E. P. (2000). Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc Natl Acad Sci U S A* **97**, 8789–8793.
- Purevdorj, B., Costerton, J. W. & Stoodley, P. (2002). Influence of hydrodynamics and cell signaling on the structure and behavior of *Pseudomonas aeruginosa* biofilms. *Appl Environ Microbiol* **68**, 4457–4464.
- Schauder, S. & Bassler, B. L. (2001). The languages of bacteria. *Genes Dev* **15**, 1468–1480.
- Schauder, S., Shokat, K., Surette, M. G. & Bassler, B. L. (2001). The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol Microbiol* **41**, 463–476.
- Sperandio, V., Mellies, J. L., Nguyen, W., Shin, S. & Kaper, J. B. (1999). Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc Natl Acad Sci U S A* **96**, 15196–15201.
- Sperandio, V., Torres, A. G., Giron, J. A. & Kaper, J. B. (2001). Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. *J Bacteriol* **183**, 5187–5197.
- Surette, M. G. & Bassler, B. L. (1999). Regulation of autoinducer production in *Salmonella typhimurium*. *Mol Microbiol* **31**, 585–595.
- Surette, M. G., Miller, M. B. & 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.
- Wen, Z. T. & Burne, R. A. (2002). Functional genomics approach to identifying genes required for biofilm development by *Streptococcus mutans*. *Appl Environ Microbiol* **68**, 1196–1203.
- Winzer, K., Hardie, K. R. & Williams, P. (2002a). Bacterial cell-to-cell communication: sorry, can't talk now – gone to lunch! *Curr Opin Microbiol* **5**, 216–222.
- Winzer, K., Hardie, K. R., Burgess, N. & 8 other authors (2002b). LuxS: its role in central metabolism and the *in vitro* synthesis of 4-hydroxy-5-methyl-3(2H)-furanone. *Microbiology* **148**, 909–922.
- Winzer, K., Sun, Y. H., Green, A., Delory, M., Blackley, D., Hardie, K. R., Baldwin, T. J. & Tang, C. M. (2002c). Role of *Neisseria meningitidis luxS* in cell-to-cell signaling and bacteremic infection. *Infect Immun* **70**, 2245–2248.