



Cell to cell communication by autoinducing peptides in gram-positive bacteria

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Abstract

While intercellular communication systems in Gram-negative bacteria are often based on homoserine lactones as signalling molecules, it has been shown that autoinducing peptides are involved in intercellular communication in Gram-positive bacteria. Many of these peptides are exported by dedicated systems, posttranslationally modified in various ways, and finally sensed by other cells via membrane-located receptors that are part of two-component regulatory systems. In this way the expression of a variety of functions including virulence, genetic competence and the production of antimicrobial compounds can be modulated in a co-ordinated and cell density- and growth phase-dependent manner. Occasionally the autoinducing peptide has a dual function, such as in the case of nisin that is both a signalling pheromone involved in quorum sensing and an antimicrobial peptide. Moreover, biochemical, genetic and genomic studies have shown that bacteria may contain multiple quorum sensing systems, underlining the importance of intercellular communication. Finally, in some cases different peptides may be recognised by the same receptor, while also hybrid receptors have been constructed that respond to new peptides or show novel responses. This paper provides an overview of the characteristics of autoinducing peptide-based quorum sensing systems, their application in various gram-positive bacteria, and the discovery of new systems in natural and engineered ecosystems.

Introduction

It has been shown for several bacteria that a variety of physiological changes in the bacterial population are dependent on specific cell densities and growth phases. This phenomenon of cell density-dependent gene expression has been termed quorum sensing, and was first found in the control of bioluminescence in *Vibrio fischeri* (Fuqua et al. 1994). Usually quorum sensing occurs at high cell densities, but in some cases also at relatively low densities, as was shown for genetic competence in *Streptococcus pneumoniae* (Håvarstein et al. 1995b). Many other quorum sensing systems have been discovered since, in both Gram-negative and Gram-positive bacteria. Examples in Gram-negative bacteria include biofilm-formation

and virulence in *Pseudomonas aeruginosa* (Winson et al. 1995; Singh et al. 2000), root-nodule formation by *Rhizobium leguminosarum* (Gray et al. 1996), and swarming motility of *Serratia liquefaciens* (Eberl et al. 1996). A comprehensive review on quorum sensing in Gram-negative bacteria is given in Whitehead et al. (2001). In Gram-positive bacteria quorum sensing regulation has been shown for: genetic competence in *Bacillus subtilis* (Tortosa & Dubnau 1999) and *Streptococcus pneumoniae* (Cheng et al. 1997), virulence in *Staphylococcus aureus* (Novick 1999), and the production of antimicrobial peptides, including bacteriocins and lantibiotics, in lactic acid bacteria (Kleerebezem et al. 1997a; Risøen et al. 2000). In these quorum sensing systems bacteria produce extracellular signalling molecules that are responsible for the com-

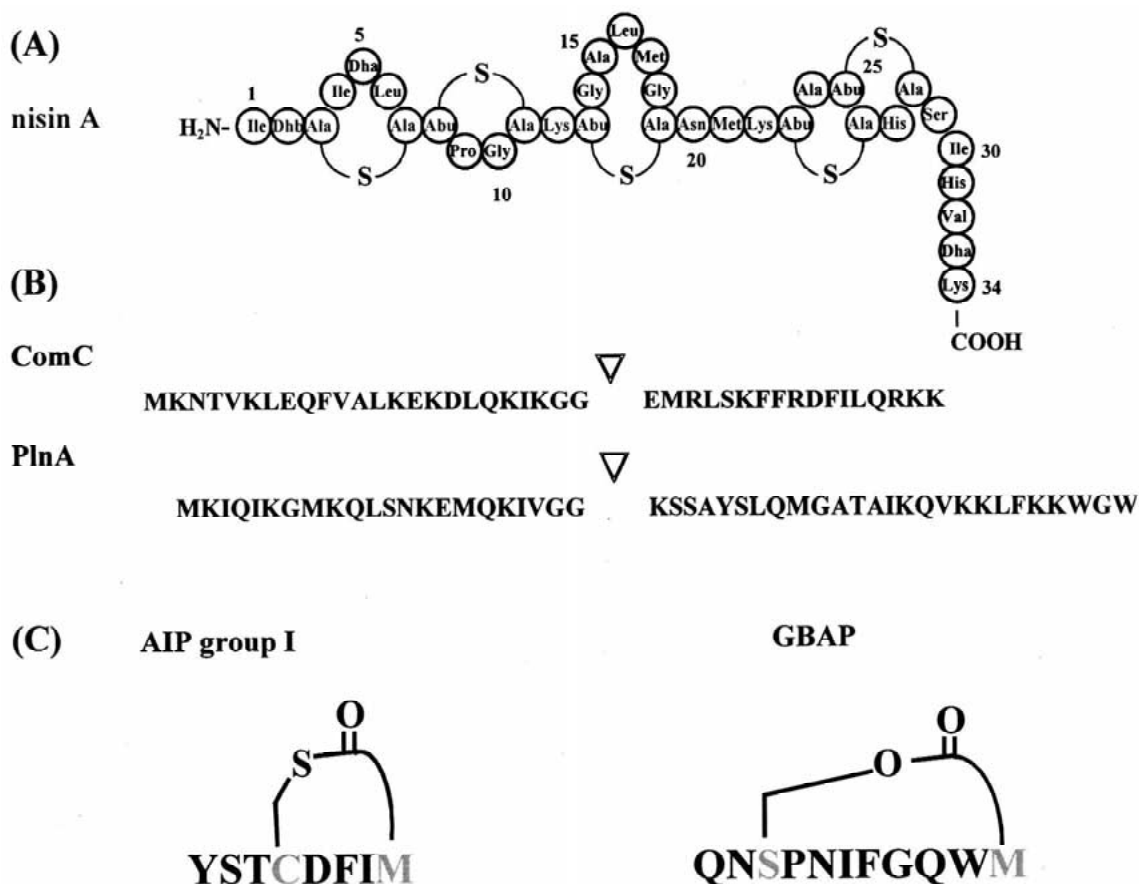


Figure 1. Structural diversity of autoinducing peptides found in Gram-positive bacteria. (A) Lantibiotic nisin A of *Lactococcus lactis* (Ala-S-Ala: lanthionine, Abu-S-Ala: β -methyl-lanthionine, Dha: dehydroalanine, Dhb: dehydrobutyrine). (B) Double-glycine-type prepeptides involved in the regulation of class II AMP production (PlnA of *Lactobacillus plantarum* C11) and competence development (ComC of *Streptococcus pneumoniae*); (∇) indicates the cleavage-position between the leader-peptide and the mature autoinducing peptide. (C) Cyclic thiolactone- and lactone-peptides. AIP group I: group I cyclic thiolactone-peptide of *Staphylococcus aureus*; GBAP: cyclic lactone-peptide GBAP (gelatinase biosynthesis-activating pheromone) of *Enterococcus faecalis*.

munication between bacterial cells. Several distinct families of communication molecules have been identified so far, including (*N*-acyl) homoserine lactones, 4-quinolones, diketopiperazines and peptides (Holden et al. 2000). While many Gram-negative bacteria communicate via *N*-acyl-homoserine lactones, peptides are the most common and well-studied signalling molecules in Gram-positive bacteria, here referred to as autoinducing peptides (AIP). These peptides show a variety of structures but share a small size, are ribosomally synthesised, and are in some cases subject to post-translational modifications that add to their stability and functionality (Figure 1). Recently, it has been suggested that genes for autoinducing peptides may also be present in Gram-negative bacteria, implying that peptide-based signalling is a general system in bacteria (Michiels et al. 2001).

This review provides an overview of our current knowledge of the presence and functions of peptide-based quorum sensing systems in Gram-positive bacteria, their application in genetic tools for manipulating industrial species, and the detection of novel quorum sensing genes in natural and engineered ecosystems.

Two-component regulatory systems involved in quorum sensing

Various ways are known by which peptide-based quorum sensing operates in Gram-positive bacteria (Figure 2). All involve one or both elements of a two-component regulatory signal transduction system, consisting of a membrane-located receptor histidine

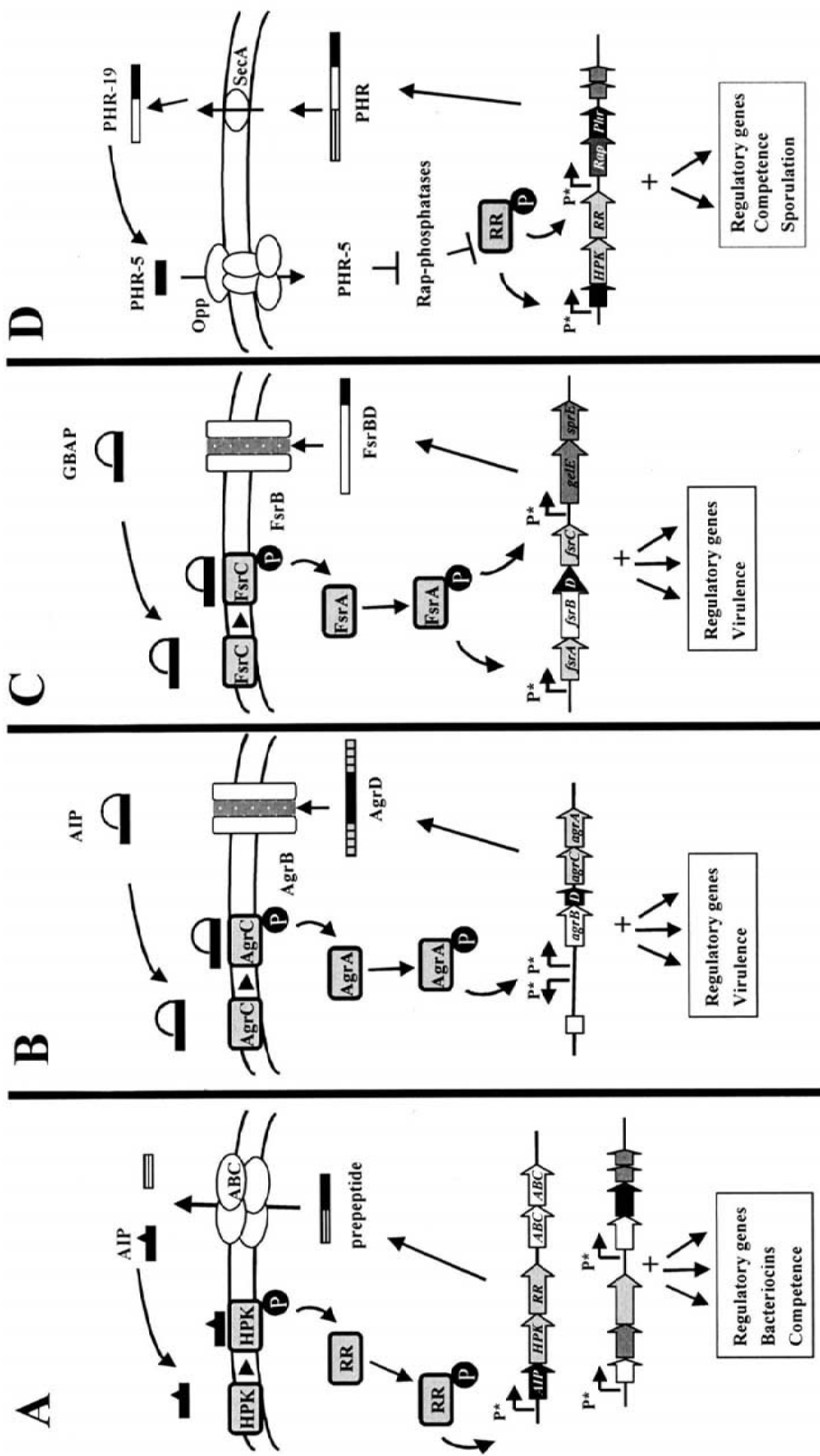


Figure 2. Models of peptide-mediated regulatory systems found in Gram-positive bacteria. P⁺ indicates regulated promoters in Gram-positive bacteria. (A) General system for Gram-positive bacteria. HPK: histidine protein kinase; RR: response regulator; ABC: ABC transporter-system; P: phosphate. (B) Staphylococcal *agr*-system. AgrA: response regulator; AgrB: putative AIP-processing/transporter protein; AgrC: histidine protein kinase; AgrD: AIP prepeptide (C) *Enterococcus faecalis* *fsr*-system. FsrA: response regulator; FsrB: putative GBAP-processing/transporter protein; FsrBD: FsrB-GBAP fusion protein; FsrC: histidine protein kinase; gelE: gelatinase biosynthesis-activating pheromone; sprE: serine protease gene. (D) Phr-peptide regulatory system of *Bacillus subtilis*. Opp: oligopeptide-permease; SecA: SecA-dependent transport system; PHR: Phr-protein; PHR-19: Phr-prepeptide; PHR-5: Phr-pentapeptide; Rap: Rap-phosphatase.

protein kinase and an intracellular response regulator (Parkinson 1995; Grebe & Stock 1999). Autoinducing peptides are ribosomally synthesised as precursor peptides and exported from the cells. During this process, but also at other stages, the precursor peptides may be subject to one or more posttranslational modification events, till the active and usually stable autoinducing peptide is produced. In most cases the autoinducing peptide has no other known function than being the communication molecule, but exceptions include the lantibiotics nisin and subtilin which have not only signalling activity but also show antimicrobial activity (see below). While the autoinduction loop always proceeds via a response regulator that upon phosphorylation activates transcription of target genes as well as genes involved in production of the autoinducing peptides, the mechanism by which the precursor peptides are exported and finally sensed by the cells varies considerably (Figure 2).

The simplest quorum sensing systems that were the first to be discovered, involve a dedicated ABC exporter and all elements of a two-component regulatory system (Kleerebezem et al. 1997a) (Figure 2). The autoinducing peptide is assumed to be produced at a low level during growth and at a certain cell-density reaches a threshold concentration, upon which activation of the receptor kinase takes place. The receptor kinase is then auto-phosphorylated, and subsequently phosphorylates the response regulator that transcriptionally activates the structural gene for the autoinducer, the genes for the two-component regulatory system, and often also the ABC exporter genes, resulting in autoinduction in a dynamic range (Figure 2A). This simple quorum sensing system has first been described in *Lactococcus lactis* and *Streptococcus pneumoniae* (Kuipers et al. 1995; Håvarstein et al. 1995b). Subsequently, it was found in many other genera, including *Bacillus*, *Carnobacterium*, *Enterococcus*, and *Lactobacillus* spp. (Diep et al. 1996; Quadri et al. 1997; Nilsen et al. 1998; Kleerebezem et al. 2002). In addition to induction of the regulatory genes, many other genes are transcriptionally activated by the quorum sensing system, such as those coding for bacteriocin-production, competence-development, or those involved in modification of the autoinducing peptides (for reviews see Kleerebezem et al. 1997a; Nes & Eijsink 1999; Kleerebezem et al. 2001b).

A modification of the general peptide-based quorum sensing system is that found for the *agr*- and *fsr*-systems in *Staphylococcus aureus* and *Enterococcus faecalis*, respectively (Ji & Novick 1997;

Nakayama et al. 2001). The *agr*-system in staphylococci encodes a receptor kinase AgrC, a response regulator AgrA, an autoinducing peptide derived from prepeptide AgrD and additionally AgrB, a protein that may be involved in processing and posttranslational modification of AgrD (Figure 2B). The corresponding regulatory proteins and a fused FsrBD protein can also be found in the *fsr*-system (see below) (Figure 2C). Another difference is the nature of the peptide export-system, which is not an ABC transporter but a dedicated protein, which has been suggested to be AgrB or FsrB in staphylococci and *E. faecalis*, respectively (Saenz et al. 2000; Nakayama et al. 2001). In all cases, the genes involved in the production, modification and export of the autoinducing peptide as well as those coding for the two-component regulatory system are induced. Moreover, various other genes are induced and these include known virulence determinants in *S. aureus* or those implied in virulence such as the *E. faecalis* gelatinase and serine protease (Figure 2B and C).

Another type of peptide-mediated regulatory system is found for the *phr* self-signalling system in *Bacillus subtilis* (Perego 1999; McQuade et al. 2001) (Figure 2D). Phr-prepeptides are exported by SecA-dependent systems, and the resulting 19-residue peptide is extracellularly processed to the active pentapeptide, which is subsequently imported by an oligopeptide transport-system (Opp) (Jiang et al. 2000). Phr-pentapeptides inactivate Rap-phosphatases that dephosphorylate response regulators of two-component systems. The production of the *phr* peptides may result in an activation of their own production and this represents the autoregulation loop that is characteristic of quorum sensing systems. This specific peptide-based system has so far only been reported to be present in *B. subtilis* and hence will not be further discussed here.

Processing and modification of autoinducing peptides

Autoinducing peptides are processed during export from the cell and are in several cases subject to posttranslational modification before or after export. Lantibiotics, like nisin in *Lactococcus lactis* or subtilin in *Bacillus subtilis* are class I antimicrobial peptides that contain modifications such as dehydrated amino acids and typical (β -methyl)-lanthionines (see Figure 1A). The modification reactions occur intracellularly on the precursor peptide and involve several modification enzymes, which are all encoded by genes that

are also subject to autoregulation (de Vos et al. 1995; Kleerebezem et al. 1997a; Kleerebezem et al. 2002). The modified precursor peptides are exported by a dedicated ABC exporter and activated by proteolytic removal of the pre-sequence, also known as the leader sequence (Klein et al. 1992; van der Meer et al. 1993). In several cases this is catalysed by a specific leader peptidase, encoded by a gene which is also autoregulated (Kleerebezem et al. 1997a; McAuliffe et al. 2001).

Autoinducing peptides involved in the production of class II antimicrobial proteins (also known as bacteriocins) and genetic competence are linear peptides (Figure 1B). These are not known to be modified but contain a typical double-glycine-type leader peptide. This leader peptide is cleaved from the precursor peptide during export by the dedicated ABC-transporter system, encoded by a gene which is also autoregulated. For this function the ABC exporter has an N-terminal extension which contains a characteristic peptidase domain (Håvarstein et al. 1995a; Nes and Eijsink 1999). This typical processing of autoinducing peptides is widely spread and has been reported for the competence-inducing peptide ComC in *Streptococcus pneumoniae* (Håvarstein et al. 1995b), and the bacteriocin-inducing peptides IP-673 in *Lactobacillus sake* LTH673 (Brurberg et al. 1997), PlnA in *Lactobacillus plantarum* C11 (Diep et al. 1996), CbnB2 and CbnS in *Carnobacterium piscicola* LV17B (Quadri et al. 1997; Kleerebezem et al. 2001a), and CTC492 in *Enterococcus faecium* CTC492 (Nilsen et al. 1998).

A specific type of modification is found in the autoinducing peptides that are involved in the *agr*- and *fsr*-systems in *S. aureus* and *E. faecalis*, where the peptides contain, respectively, a cyclic thiolactone- or cyclic lactone-structure (Figure 1C). Here the actual autoinducing peptide is cleaved off as an internal part from the prepeptide and then modified, probably during export. This is thought to be catalysed via an unknown mechanism by the suggested processing and exporting protein, the homologous AgrB and FsrB in *S. aureus* and *E. faecalis*, respectively (Ji & Novick 1997; Nakayama et al. 2001) (Figure 2B and C). Remarkably, it has been found that the FsrB protein is in fact a fusion protein consisting of an N-terminal exporter-domain and a C-terminal extension including the autoinducing peptide, gelatinase biosynthesis-activating pheromone (GBAP), which is cleaved off (Nakayama et al. 2001).

Finally, a new type of modification recently has been predicted for the ComX pheromone that is in-

involved in competence development in *Bacillus subtilis* (Magnuson et al. 1994). This 10-amino acid pheromone is processed from a 55-amino-acid prepeptide and modified at a tryptophan residue, probably by protein ComQ (Lazazzera et al. 1999). By mutational analysis of protein ComQ, it was found that a putative isoprenoid-binding domain in ComQ was required for its *in vivo* function, thus implying that the modification on ComX is likely to be an isoprenoid. This was also consistent with the mass of the modification that was determined (Ansaldi et al. 2002; Bacon-Schneider et al. 2002).

Diversity and specificity of quorum sensing systems

The regulatory genes involved in quorum sensing show high genetic polymorphism in between species and even strains. The highest sequence diversity is found in the N-terminal and linker part of the receptor histidine protein kinase, and in the autoinducing peptide and peptide-processing genes. Diversity and specificity has been well studied for the *agr*-system in *S. aureus* (Jarraud et al. 2000; Dufour et al. 2002), and the competence-systems in *B. subtilis* (Tortosa et al. 2001) and *Streptococcus* spp. (Håvarstein et al. 1997; Whatmore et al. 1999). For these systems the induction activity of each autoinducing peptide appeared to be specific towards its cognate receptor, indicating the existence of distinct phenotypes. However, in some cases the signalling molecule can not only be recognised by its cognate species, but also acts on different strains of the same or a related species. This intra- and interspecies cross-talk can be either inhibitory or inducing. In staphylococci to date four AIP-subgroups have been found in *S. aureus* isolates, while non-*S. aureus* AIPs have been detected in *S. epidermidis* and *S. lugdunensis* (Jarraud et al. 2000; Otto et al. 2001; Dufour et al. 2002). Autoinducing peptides produced by one *S. aureus* strain were shown to inhibit *agr*-expression in some of the other strains. In addition, cross-inhibition between *S. aureus* strains and *S. epidermidis* or *S. lugdunensis* was detected (Ji et al. 1997; Otto et al. 2001). Specific activation or inhibition of *agr*-expression was clearly shown to be dependent on both amino acid sequence and stereochemical structure of the staphylococcal AIP, with a cyclic peptide-structure in all cases being essential for biological activity. For specific self-activation it seems furthermore important that the AIP contains a cyclic thiolactone linkage, although activation by lactam analogs of these AIPs was also found in some cases, albeit

at very high concentrations. Cross-group inhibiting peptides should contain a cyclic structure with either a thiolactone, lactone or lactam linkage (Mayville et al. 1999; Lyon et al. 2000). Further experiments have shown that activating and inhibiting AIPs interact with the AIP receptor at overlapping binding sites, although the exact mechanisms still have to be determined (Lyon et al. 2002). Cross-induction but not cross-inhibition was also observed for the ComX-peptides from *B. subtilis* and *B. mojavensis* strains (Tortosa et al. 2001), the ComC-peptides in *Streptococcus pneumoniae* isolates (Whatmore et al. 1999) and the SalA lantibiotic peptides in *Streptococcus salivarius* and *Streptococcus pyogenes* (Upton et al. 2001).

Some autoinducing peptides can also have a dual function: the autoinducing peptide may have both signalling and antimicrobial activity. The most extensively studied example of this is the lantibiotic nisin from *L. lactis*, which acts as a signal molecule that induces its own biosynthesis (Kuipers et al. 1995; Kleerebezem et al. 1999). This also holds for the related subtilin that is produced by *Bacillus subtilis* (Kleerebezem et al. 2001b). In addition, the linear peptide CbnB2 in *Carnobacterium piscicola* LV17B has been found to be both a bacteriocin (carnobacteriocin) and an autoinducing peptide (Quadri et al. 1997). For the nisin molecule it has been shown that the induction capacity and antimicrobial activity are mediated via different mechanisms. The induction capacity is determined by interactions of specific amino acid residues with the N-terminal domain of the sensor protein, while the antimicrobial activity depends on general structural properties, that cause pore-formation in the cytoplasmic membrane (Gao et al. 1991; Kuipers et al. 1995; Van Kraaij et al. 1998; Kleerebezem et al. 2001b). The former has been substantiated by the construction of a hybrid sensor kinase of the nisin system, in which the extracellular and trans-membrane domains of the nisin sensor NisK were replaced by those derived from the related subtilin sensor SpaK. Instead of reacting on nisin as autoinducing peptide, this hybrid sensor now reacts on subtilin (Kleerebezem et al. 2001b).

Quorum sensing in ecosystems

Regulation of physiological responses by quorum sensing could be important to most bacteria, as it may ensure co-ordinated gene expression in the population. High cell-density ecosystems with many microbial interactions, where there is also a diffusion barrier, could

be selective for the development of these quorum sensing systems in bacteria. Co-ordinated responses should lead to competitive advantages for the population, more effective adaptation and responses to changing environmental conditions, or co-ordination of interactions between bacteria and their abiotic and sometimes biotic environment.

Cross-talk by autoinducing peptides between strains and species is a phenomenon that could play an important role in the way microbial ecosystems develop and function. Cross-inhibition of other bacteria is likely to be advantageous to a species during: colonisation of plant material that acts as substrate; other raw materials such as milk that provide an excellent growth-substrate for many bacteria; or surfaces such as the skin or mucosal epithelium in the gastrointestinal tract (Swift et al. 2000). Cross-signalling could also lead to co-ordinated co-metabolism among bacterial species or interference with host responses. In general however, specificity of the autoinducing peptides towards their cognate receptors should avoid too much cross-interference.

Peptide-induced production of antimicrobial peptides is a phenomenon involved in competition and has been extensively studied in lactic acid bacteria that are competing with other bacteria (McAuliffe et al. 2001; Ennahar et al. 2000). By producing these natural inhibitors, growth of competing microorganisms is controlled, and in this way the species producing antimicrobial peptides may have an advantage in colonisation of ecological niches or accessing nutrients.

The quorum sensing-regulated competence systems found in *Bacillus* and *Streptococcus* spp. enables these bacteria to take up free DNA from the environment, and to integrate parts of this foreign DNA into their genomes. This can lead to new features in these bacteria, and thereby to increased adaptation to the environment or competitive advantages, or it could have a function in DNA repair mechanisms. Induction of these natural transformation systems at high cell densities increases the probability of naked DNA being available, as free DNA is continuously being produced by lysis of bacterial cells and excretion. Indications for such transformations in natural ecosystems originated from several soil microcosm studies on *B. subtilis* strains (Lorenz & Wackernagel 1994). The specificity of the quorum sensing-regulated competence system involved might enhance the probability of taking up DNA from closely related strains, and thus reducing

the risk of gene disruption and incorporation of toxic genes (Tortosa & Dubnau 1999).

An interesting example of potential functional co-ordination between commensal bacteria and their host is found for the regulatory gene cluster involved in the production of the lantibiotic ruminococcin A (RumA) in *Ruminococcus gnavus* FRE1. Production of this lantibiotic was dependent on the presence of trypsin while it was shown to be resistant to digestive proteases. Trypsin could be involved in processing of a putative extracellular autoinducing peptide, which then should act as the inducing signal for the regulatory two-component system involved in RumA production. Such activation could be a specific adaptation to the intestinal environment, in which this strain naturally resides (Dabard et al. 2001; Gomez et al. 2002; Swift et al. 2000).

Impact of genomics on quorum sensing studies

With the increasing availability of completed genome sequences, genomic data-mining can be used for analysing bacterial genomes and detecting potential quorum sensing systems. These can be analysed in detail for their functionality by mutation and transcriptional profiling studies that may reveal genome-wide effects of autoinducing peptides.

Detection of genes involved in quorum sensing

Systematic genome searches are simple approaches that have been applied to two-component systems of *S. pneumoniae*, the genome of which has now been reported (Tettelin et al. 2001). A similar approach for the recently sequenced *Lactobacillus plantarum* WCFS1 genome is in progress. In this way 13 putative two-component systems were identified in *S. pneumoniae* and their function was analysed by mutational analysis (Lange et al. 1999; Throup et al. 2000). This revealed several growth-phase regulated two-component systems, at least one of which was involved in a peptide-dependent cell-to-cell signalling. In the sequenced *L. plantarum* strain 15 putative two-component systems were identified, several of which showed significant homologies to the Agr-like systems. Analysis on the functions and regulation of these putative quorum sensing systems is in progress (Sturme, unpubl.).

Although many examples of quorum sensing in Gram-positive bacteria are now known, little research has been done on the presence and activity

of these systems in natural microbial ecosystems. Indications of a widespread distribution of quorum sensing in ecosystems comes from the discovery of (putative) peptide-controlled bacteriocin production in gastrointestinal Gram-positive bacteria such as: *Lactobacillus plantarum* (Diep et al. 1995), *Enterococcus faecium* (Nilsen et al. 1998) and *Ruminococcus gnavus* (Gomez et al. 2002); or ruminal bacteria like *Butyrivibrio fibrisolvens* (Kalmokoff & Teather 1997; Kalmokoff et al. 1999).

Several recent studies have addressed the presence, expression and function of peptide-based communication systems in microbial communities. For this purpose, molecular approaches have been developed to detect novel or known quorum sensing genes. Oligonucleotide probes based on the amino acid sequence of purified autoinducing peptides or bacteriocins have been used to detect genomic DNA-fragments carrying the genes involved in their biosynthesis. In this way the regulatory gene cluster involved in the production of ruminococcin A (RumA) in *Ruminococcus gnavus* FRE1 was discovered, by using a probe against the RumA gene (Dabard et al. 2001; Gomez et al. 2002). Degenerate Oligonucleotide Primer PCR (DOP-PCR) is another powerful method to detect quorum sensing systems and has been applied with degenerate primers that are designed based on the amino acid similarity of quorum sensing two-component systems, notably the conserved regions in the histidine protein kinases. Various potential quorum sensing regulatory genes were discovered by DOP-PCR in several Gram-positive bacteria (Morel-Deville et al. 1997), *L. lactis* (O'Connell-Motherway et al. 2000) and other lactic acid bacteria (Nakayama et al. 2002). Moreover, this approach was also used to detect these systems in the complex ecosystem of the human gastro-intestinal tract (J. Nakayama & M. Sturme, unpublished observations).

Finally, new developments in the construction of environmental gene libraries are promising. Especially the so-called bacterial artificial chromosome (BAC) libraries are gaining more interest, as large genomic fragments (usually 50–150 kb) can be cloned from DNA of entire microbial ecosystems (termed *metagenome*: Rondon et al. 2000). BAC libraries could provide a useful tool for studying genetic and functional diversity of quorum sensing systems in bacterial communities, of which a large part can not be cultured yet. Some of the promising applications of BAC libraries have already been described in studies on

soil and marine microbial communities (Rondon et al. 2000; Béjà et al. 2000; MacNeil et al. 2001).

Transcriptome analysis of quorum sensing-regulated genes

In *S. pneumoniae* a bacteriocin-like peptide (*blp*) operon belonging to the Agr-family was identified by genome analysis. It encodes for a signalling peptide, response regulator, histidine protein kinase, and peptide-transporters. Microarray-based analysis of expression profiles in *S. pneumoniae* revealed several genes that are regulated by this bacteriocin-like autoinducing peptide (De Saizieu et al. 2000). Most of these genes were located near the *blp* operon, suggesting a functional link with it, and appeared to be cell density-dependent regulated. Microarray analysis of the *B. subtilis* competence quorum sensing-system showed differences between cells expressing ComK (competence transcription factor) and vegetatively growing cells. In addition to the known competence genes, also ComK-regulation of many unsuspected genes was detected (Berka et al. 2002). Similarly, transcription profiling of genes regulated by the *agr* and *sarA* systems in *S. aureus* revealed the regulation of non-characterised putative virulence factors next to the expected virulence genes, and additionally regulation of more general non-virulence genes (Dunman et al. 2001). Microarray studies therefore appear to be valuable for identifying quorum sensing-regulated genes and elucidating the regulatory mechanisms involved.

Concluding remarks and perspectives

Until now research on quorum sensing has mainly focused on the autoinducing signals and the regulatory systems involved. These studies have revealed many new quorum sensing systems in individual species and have led to a wealth of sequence information of their genes and regulation. Our current knowledge indicates that cell-to-cell communication by autoinducing peptides plays an important role in regulating cell-density-dependent phenotypes in Gram-positive bacteria, and involves two-component regulatory systems. The autoinducing peptides showed high specificity and diversity, which is reflected in the differences in primary structure as well as in the extent of posttranslational modification.

The advanced knowledge of the regulation and functions of peptide-based quorum sensing systems

may lead to the development of new tools in molecular biology and applications in medicine. Quorum sensing-regulated systems could be useful for designing controlled gene-expression systems in Gram-positive bacteria, as can be illustrated by the food-grade nisin-controlled expression system (NICE), which is based on the autoregulatory nisin gene cluster from *L. lactis* (De Ruyter et al. 1996; Kuipers et al. 1997; De Vos et al. 1997). It is a transferable system consisting of a regulatory plasmid carrying the *nisRK* regulatory genes and an expression vector with the cloned gene of interest under control of the *nisA* promoter. Successful implementation of the NICE-system was demonstrated in various lactic acid and other Gram-positive bacteria including: *Lactococcus lactis*, *Leuconostoc lactis*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Enterococcus faecalis*, *Streptococcus thermophilus*, *Bacillus subtilis*, *Streptococcus pyogenes* and *Staphylococcus aureus* (Kleerebezem et al. 1997b; Eichenbaum et al. 1998; Pavan et al. 2000; Bryan et al. 2000). Insight into the effects of peptide-structure on the activity of autoinducing peptides might lead to the rational design of synthetic peptides that are able to inhibit quorum sensing-regulated phenotypes. The potential of such a strategy has already been shown in studies concerning inhibition of virulence and infections caused by *S. aureus*. In these studies quorum sensing-blocking synthetic peptides were designed based on knowledge of *S. aureus*-AIP structure-function relationships (Mayville et al. 1999; Lyon et al. 2000; McDowell et al. 2001). Similar approaches that target quorum sensing signals may also be applied to the quorum sensing systems involved in the production of virulence factors in *Enterococcus faecalis*, a main cause of hospital infections for which few therapeutics exist (Nakayama et al. 2001; Haas et al. 2002).

The rapid increase in sequence information and recent insights into regulation of quorum sensing genes can also be applied to investigate the genes that are regulated by these systems. Genome-wide approaches such as those based on transcriptome or proteome analysis can impact substantially on this area by revealing complex global regulatory mechanisms, and this knowledge may ultimately be used to study the *in situ* activity and the role of quorum sensing systems in natural ecosystems.

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