The Evolutionary History of Quorum-Sensing Systems in Bacteria

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Communication among bacterial cells through quorum-sensing (QS) systems is used to regulate ecologically and medically important traits, including virulence to hosts. QS is widespread in bacteria; it has been demonstrated experimentally in diverse phylogenetic groups, and homologs to the implicated genes have been discovered in a large proportion of sequenced bacterial genomes. The widespread distribution of the underlying gene families (LuxI/R and LuxS) raises the questions of how often QS genes have been transferred among bacterial lineages and the extent to which genes in the same QS system exchange partners or coevolve. Phylogenetic analyses of the relevant gene families show that the genes annotated as LuxI/R inducer and receptor elements comprise two families with virtually no homology between them and with one family restricted to the γ-Proteobacteria and the other more widely distributed. Within bacterial phyla, trees for the LuxS and the two LuxI/R families show broad agreement with the ribosomal RNA tree, suggesting that these systems have been continually present during the evolution of groups such as the Proteobacteria and the Firmicutes. However, lateral transfer can be inferred for some genes (e.g., from Firmicutes to some distantly related lineages for LuxS). In general, the inducer/receptor elements in the LuxI/R systems have evolved together with little exchange of partners, although loss or replacement of partners has occurred in several lineages of γ-Proteobacteria, the group for which sampling is most intensive in current databases. For instance, in *Pseudomonas aeruginosa*, a transferred QS system has been incorporated into the pathway of a native one. Gene phylogenies for the main LuxI/R family in *Pseudomonas* species imply a complex history of lateral transfer, ancestral duplication, and gene loss within the genus.

Introduction

In bacteria, the perception of the environment can direct cellular differentiation by influencing the expression of genes underlying a variety of biological pathways. For example, such systems are used for deciding to migrate according to a nutrient gradient or to adopt new modes of growth, such as the formation of biofilms, according to cell density. A well-known mechanism of cell-cell communication is called quorum sensing (QS). In that process, bacteria communicate via secreted signaling molecules called autoinducers, which contribute to the regulation of the expression of particular genes. The first such system was described in *Vibrio fischeri* (Neaslon and Hastings 1979), a symbiotic species that provides its marine eukaryotic hosts with light. Light emission depends on transcription of the luciferase operon, which occurs when the cell-population density is sufficient to produce a threshold accumulation of a secreted autoinducer, a specific acylated homoserine lactone (AHL). The biosynthesis of AHL is controlled by the gene *luxI*. Above a certain concentration, the autoinducer binds the LuxR protein, and the resulting complex activates target gene transcription by binding the luciferase promoter at a specific site called the lux box.

Although initially considered to be a specialized system of *V. fischeri* and related species, experimental work later revealed homologous systems with diverse biological roles in other proteobacterial species. These included *Pseudomonas aeruginosa*, a human pathogen in which two circuits act in parallel to control the expression of a number of virulence factors (Jones et al. 1993; Passador et al. 1993; Brint and Ohman 1995), *Erwinia carotovora*, a plant pathogen with separate QS systems for expression of enzymes that attack the host (Jones et al. 1993; Pirhonen et al. 1993) and production of antibiotics (Bainton et al. 1992), and *Agrobacterium tumefaciens*, a plant pathogen whose *luxI*/*R* homologs (*traI*/*R*) are located on a plasmid and control conjugal transfer of the plasmid between bacteria (Fuqua and Winans 1994). The nature of the autoinducer varies among species and among systems in the same species. Each pathway responds to its own autoinducer signal.

The characterization of further QS systems has revealed different levels of evolutionary conservatism for different autoinducer systems. For example, in the free-living marine bacterium *Vibrio harveyi*, the LuxI/R system is absent, and light production is controlled by two nonhomologous parallel pathways (Bassler et al. 1993; Bassler, Wright, and Silverman 1994). In one of those, the autoinducer is not an AHL, but a furanosyl borate diester (Chen et al. 2002), for which synthesis is controlled by the gene *luxS*, and the receptor is a membrane-associated sensor kinase, LuxQ. This inducer has been found in a large range of gram-negative and gram-positive bacteria, including some pathogens (Surette, Miller, and Bassler 1999; Miller and Bassler 2001). Because this autoinducer and its biosynthetic pathway are the same among all bacterial species that possess *luxS*, it has been proposed that this system could be used in interspecies communication (Mok, Wingreen, and Bassler 2003). In some species, *luxS* has been linked to regulatory functions, such as control of toxin production in *Clostridium perfringens* (Ohtani, Hayashi, and Shimizu 2002) and of the virulence cascade in *Vibrio cholerae*, *Escherichia coli*, and *S. typhimurium* (Surette, Miller, and Bassler 1999; Miller et al. 2002), but the role of the autoinducer remains largely unknown.

The genes underlying QS are distributed in a discontinuous manner among the bacteria (Surette, Miller, and Bassler 1999; Miller and Bassler 2001), suggesting that they have been subject to loss or horizontal transfer. In this regard, gene phylogenies for the components of QS systems can provide evidence as to whether they are

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ancestral and lost in some species or have been acquired from distantly related lineages. Knowledge of the evolutionary mechanisms of such genes is of particular importance because they are increasingly being considered as potential targets in new antimicrobial strategies.

Here, we present phylogenetic analyses of genes underlying the two QS systems, LuxI/R and LuxS. The genes that are regularly referred to as the LuxI/R families in fact constitute two families in each case. Although these could be very ancient paralogs, the sequence similarity is not sufficient to allow their inclusion in the same phylogenetic analysis. We use these phylogenies to address the basis for the observed distributions of QS genes in bacteria. Specifically, we examine (1) the extent to which LuxI and LuxR have coevolved versus the extent to which they have switched partners and (2) the extent to which the distributions of LuxI/R and LuxS reflect vertical transmission along the organismal phylogeny versus horizontal transmission among lineages.

Material and Methods

Data

The sequences of 55 LuxR, 55 LuxI, and 44 LuxS proteins of 90 species have been retrieved from GenBank (Benson et al. 2002) (see table 1 and Supplementary Material online for the sequence names and accession numbers). Some of the genes were identified experimentally, and others have been identified on the basis of sequence homology (i.e., those from genome sequencing projects).

Determining the Families of LuxI/R

We assigned membership to the two LuxI/R families using the following method: In a BlastP search, the degree of similarity is given by the bit score (Altschul et al. 1997). The bit score is dependent upon the scoring system employed and takes into account both the degree of similarity and the length of the alignment between the query and the match sequences. A BlastP search was performed for each protein against all proteins in our set, including the query protein itself. The self-match for each sequence yields the maximal bit score. We inferred that a protein that matched with the query protein can be considered as its homolog if its bit score value is not less than 20% to 25% of the maximal bit score; values less than this threshold reflect random matches between sequences (Lerat, Daubin, and Moran 2003). We, thus, obtained two families for the LuxI sequences, one family for the LuxS sequences, and two families for the LuxR sequences.

Alignments and Phylogenies

The alignments for each group of sequences were created using the ClustalW program version 1.8 (Thompson, Higgins, and Gibson 1994) and then were corrected by hand using the SEAVIEW sequence editor (Galtier, Gouy, and Gautier 1996). The Tree-Puzzle program version 5.1 (Schmidt et al. 2002) was used to determine the alpha parameter from the data sets for the gamma-based method for correcting the heterogeneity in substitution rates among sites (γ correction).

We used several methods to reconstruct phylogenies for each protein alignment: a maximum likelihood reconstruction with the JTT model of substitution (Jones, Taylor, and Thorton 1992) and the γ correction in the PROML module of PHYLIP version 3.6 (Felsenstein 2002); the neighbor-joining (NJ) method with 500 bootstrap replicates, the Poisson correction distance and the γ correction using the MEGA program (Kumar, Tamura, and Nei 1993); the minimum-evolution method with the γ correction also using the MEGA program; and the NJ method with the Poisson correction distance and with 500 bootstrap replicates implemented in the Phylo_win program (Galtier, Gouy, and Gautier 1996). We used several methods to determine the robustness of the results to artifacts. Because we obtained identical topologies from all methods, we present only those from NJ as implemented in MEGA.

The SSU rRNA tree of all species was reconstructed using the NJ method with 500 bootstrap replicates and with the γ correction using MEGA (Kumar, Tamura, and Nei 1993).

Our goal was to determine whether the phylogenies for each QS gene family showed overall agreement with the organismal phylogeny as reflected in the SSU rRNA tree. We wished to identify any cases in which lack of congruence clearly indicated the occurrence of lateral gene transfers (LGT) in particular lineages. We did not perform statistical tests of congruence between the SSU rRNA tree and the different trees obtained from the protein alignments, because detection of significant conflict could be explained through several processes, including LGT in any of the lineages.

Results and Discussion

Two Families in the LuxI/R Proteins

We obtained a single family of homologous genes for the LuxS proteins. However, for LuxI and LuxR, the proteins are clearly subdivided into two different families of homologous genes. Although their functional similarity suggests that these two families could be ancient paralogs, the resulting alignment cannot be considered in a single phylogenetic analysis because of the extent of sequence divergence. This finding contradicts a previous analysis of the evolution of QS genes (Gray and Garey 2001), in which all LuxI and LuxR were forced into an alignment and considered in the same phylogenetic analysis, despite lack of evident homology. In that study, the two families were resolved as two clades diverging at the base of the tree.

The first family (family A) grouped Proteobacteria from different divisions (α, β, and γ), whereas family B contained only γ-Proteobacteria. One interpretation is that family B has arisen relatively recently within the γ-Proteobacteria. The most likely timing for this origin is after the divergence of *Xanthomonas* species and *Xylella*, which lack homologs (Bhattacharyya et al. 2002), but before the divergence from *Pseudomonas* species, which do contain homologs of these genes.

Table 1
Names of Species and Genes Used in the Analyses

Group	Organism	Symbol	Inducer	Receptor
Gamma proteo				
	Vibrio fischeri	Vfis	**LuxI**	**LuxR**
			LuxS[a]	?
	Vibrio anguillarum	Vang	**VanI**	**VanR**
	Vibrio harveyi	Vhar	LuxS	LuxP, LuxQ
	Vibrio cholerae	Vcho	LuxS	?
	Vibrio parahaemolyticus	Vpara	LuxS	?
	Vibrio vulnificus	Vvul	LuxS	?
	Photorhabdus luminescens	Plum	LuxS	?
	Proteus mirabilis	Pmir	LuxS	?
	Pseudomonas aeruginosa	Paer	**RhlI**	**RhlR**
			LasI	**LasR**
			?	**PhzR**
	Pseudomonas fluorescens	Pflu	AfmI	AfmR
			RhlI	**RhlR**
			PhzI	**PhzR**
			MupI	**MupR**
	Pseudomonas chlororaphis = P. aureofaciens	Pchl	**PhzI**	**PhzR**
			CsaI	**CsaR**
	Pseudomonas syringae	Psyr	AhlI	?
			PsmI	PsmR
			PsyI	PsyR
	Salmonella typhimurium	Sty	/	**SdiA**
			LuxS	?
	Salmonella enterica	Sent	/	**SdiA**
			LuxS	?
	Shigella flexneri	Sfle	LuxS	?
			/	**SdiA**
	Shigella sonnei	Sson	/	**SdiA**[a]
	Shigella dysenteriae	Sdys	/	**SdiA**[a]
	Shewanella oneidensis	Sone	LuxS	?
	Escherichia coli K12	EcoliK12	/	**SdiA**
			LuxS	?
	Escherichia coli O157:H7	EcoliO157	/	**SdiA**
			LuxS	?
	Aeromonas hydrophila	Ahyd	**AhyI**	**AhyR**
	Aeromonas salmonicida	Asalm	**AsaI**	**AsaR/AhyR**
	Yersinia pestis	Ypes	LuxS	?
			YpeI	YpeR
			YspI	YspR
	Yersinia pseudotuberculosis	Ypseu	YpsI	YpsR
			YtbI	YtbR
	Yersinia ruckeri	Yruc	YruI/YukI	YruR/YukR
	Yersinia enterocolitica	Yent	YenI	YenR
			LuxS[a]	?
	Erwinia chrysanthemi = Pectobacterium chrysanthemi	Echr	ExpI	ExpR
			EchI	EchR
	Erwinia carotovora = Pectobacterium carotovorum	Ecar	ExpI/CarI	ExpR
				CarR
			EcbI	EcbR
	Erwinia stewartii = Pantoea stewartii	Estew	EsaI	EsaR
	Serratia liquenfaciens	Sliq	SwrI	SwrR
	Serratia sp	serratia	SmaI	SmaR
	Serratia marcescens	Smar	/	CarR
			SpnI	SpnR
	Serratia proteamaculans	Spro	SprI	SprR
	Hafnia alvei	Halv	HalI	HalR
	Pantoea agglomerans	Paggl	EagI	No sequence
	Pasteurella multocida	Pmult	LuxS	?
	Haemophilus influenzae	Hinfl	LuxS	?
Beta proteo	*Burkholderia cepacia*	Bcep	**CepI**	**CepR**
			BviI	**BviR**
	Burkholderia stabilis	Bsta	**CepI**	**CepR**
	Burkholderia vietnamiensis	Bvie	**CepI**	**CepR**
	Burkholderia ambifaria	Bamb	**BafI**	**BafR**
	Burkholderia multivorans	Bmult	**CepI**	**CepR**
	Burkholderia pseudomallei	Bpseu	**BspI**	

Table 1
Continued

Group	Organism	Symbol	Inducer	Receptor
	Burkholderia mallei	Bmal	**LuxI**	
	Ralstonia solanacearum	Rsol	**SolI**	**SolR**
	Neisseria meningitidis B	NmenB	LuxS	?
	Neisseria meningitidis A	NmenA	LuxS	?
	Neisseria gonorrhoeae	Ngor	LuxS[a]	?
Alpha proteo	*Rhodobacter sphaeroides*	Rsph	**CerI**	**CerR**
	Rhizobium meliloti	Rmel	**TraI**	
	Rhizobium loti	Rloti	**TraI**	
	Rhizobium sp	Rhizobium	**TraI**	
	Agrobacterium tumefaciens	Atum	**TraI**	**TraR**
	Agrobacterium rhizogenes	Arhi	**TraI**	**TraR**
	Rhizobium leguminosarum	Rleg	**RaiI**	**RaiR**
	Rhizobium etli	Retli	**RaiI**	**RaiR**
	Ruegeria sp	Ruegeria	**TraI**	
Epsilon proteo	*Campylobacter jejuni*	Cjej	LuxS	?
	Helicobacter pylori	Hpyl	LuxS	?
	Helicobacter pylori J99	HpylJ99	LuxS	?
Firmicutes	*Clostridium perfringens*	Cperf	LuxS	?
	Bacillus halodurans	Bhal	LuxS	?
	Bacillus anthracis	Bant	LuxS	?
	Bacillus cereus	Bcer	LuxS	?
	Bacillus subtilis	Bsub	LuxS	?
	Staphylococcus aureus	Saur	LuxS	?
	Listeria innocua	Linno	LuxS	?
	Listeria monocytogenes	Lmono	LuxS	?
	Lactococcus lactis	Llac	LuxS	?
	Lactobacillus plantarum	Lplan	LuxS	?
	Oceanobacillus iheyensis	Oihe	LuxS	?
	Staphylococcus epidermidis	Sepi	LuxS	?
	Streptococcus pyogenes	Spyo	LuxS	?
	Streptococcus pneumoniae	Spneu	LuxS	?
	Streptococcus gordonii	Sgord	LuxS	?
	Streptococcus mutans	Smut	LuxS	?
	Streptococcus agalactiae	Saga	LuxS	?
	Clostridium acetobutylicum	Cace	LuxS	?
Actinobacteria	*Bifidobacterium longum*	Blon	LuxS	?
Deinococcus	*Deinococcus radiodurans*	Drad	LuxS	?
Spirochaete	*Borrelia burgdorferi*	Bbur	LuxS	?

[a] No accession number (unfinished genomes).

The existence of two families in the LuxI/R alignments raises questions about their differences in function or structure, despite involvement in similar processes. Investigations of the molecular aspects of the activation and the binding of the receptor proteins (Qin et al. 2000) have shown that the TraR protein of *A. tumefaciens* exists in a monomer form without the presence of the inducer. The binding of the AHL induces the formation of a stable homodimer that binds the promoter of the target genes to activate their transcription (Qin et al. 2000). Such a mechanism is also found for LuxR in *V. fischeri* and LasR in *P. aeruginosa*; in the latter case, there is even multimerization after fixation of the signal molecule (Kiratisin, Tucker, and Passador 2002). All of these proteins are members of family A. In contrast, members of family B, CarR and ExpR in *E. carotovora* and EsaR in *Erwinia stewartii*, have been shown to dimerize and to bind to the promoter sequences in absence of the inducer (Welch et al. 2000). In the case of EsaR, it has been demonstrated that the protein acts as a repressor rather than as a transcription activator in the presence of the inducer molecule (Minogue et al. 2002). This difference in mechanism of regulation suggests that the two families might have diverged in the very ancient past. Further investigations will be needed to determine if differences in mechanism apply to all proteins of each family. Already one counterexample exists. In *P. aeruginosa*, the RhlI/R system belongs to family A but exists in the cell as a homodimer in absence of signal (Ventre et al. 2003).

We failed to find a correlation between gene family and a particular type of AHL, the phenotype expressed by the QS system, or the environment of the bacterium. This lack of pattern may be a result of the lack of information for many species. The discovery of homologs from the two families in other species may give more insight into the evolutionary lability of particular QS systems.

Evolution of LuxI/R Family A

Tree Reconstruction

Figures 1*A* and *B* show the tree reconstructions of the LuxI and LuxR proteins for family A. As the different methods led to identical topologies, only the NJ trees obtained in MEGA are represented.

The two trees are globally congruent. Particularly in the γ-Proteobacteria, in which several systems of this

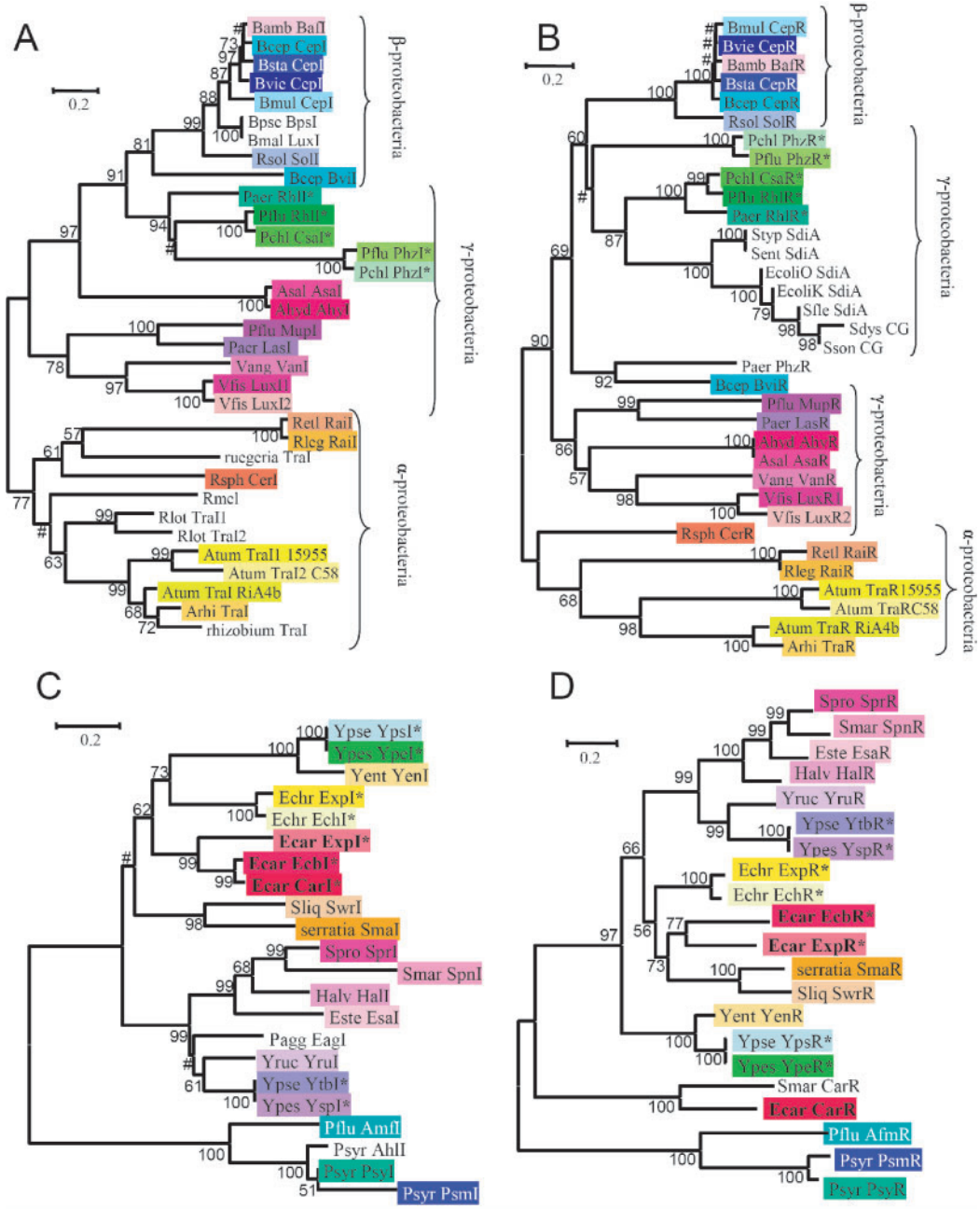

FIG. 1.—(*A*) Tree based on the protein alignment of the LuxI members of family A. (*B*) Tree based on the protein alignment of the LuxR members of family A. (*C*) Tree based on the protein alignment of the LuxI members of family B. (*D*) Tree based on the protein alignment of the LuxR members of family B. Species abbreviations as in table 1. Different phylogenetic methods yielded the same topology, and we present trees obtained using NJ and γ correction. Asterisks (*) indicate paralogous copies. The number sign (#) indicates nodes with bootstrap values less than 50%.

family coexist, the receptor and its corresponding inducer branch at corresponding nodes in the topologies. An implication is that the receptor and inducer gene typically remain linked and that changes in partnership between inducer and receptor genes are rare.

Both of the groups formed by the inducers RhlI, CsaI, and PhzI in *Pseudomonas* and their corresponding receptors (RhlR, CsaR, and PhzR) show a close relationship to genes of the β-proteobacterial species.

This group of receptors is closely related to the SdiA (for suppressor of cell division inhibitor) proteins, which are known to play a variety of roles in enterobacteria, including the regulation of cell division by the activation of the ftsQAZ operon (Wang, de Boer, and Rothfield 1991), and the expression of virulence genes (Ahmer et al. 1998; Kanamaru et al. 2000). The species possessing SdiA do not have a corresponding inducer and are therefore unable to produce an AHL. In *S. typhimurium*, it was shown that SdiA can detect AHL produced by other bacterial species (Smith and Ahmer 2003), suggesting that

this protein could be involved in recognizing the presence of foreign bacteria.

The only clear-cut incongruence between the two trees involves the BviR protein of *Burkholderia cepacia* (β-Proteobacteria), which groups with the PhzR protein of *P. aeruginosa* (γ-Proteobacteria), suggesting a replacement of the receptor in *B. cepacia*. Whereas BviI probably underlies production of the inducer for BviR in *B. cepacia*, no corresponding inducer gene for PhzR seems to be present in the complete genome of *P. aeruginosa*. Interestingly, these two human pathogens occasionally coinfect the same individual and have been demonstrated to be able to communicate and coordinately regulate virulence factors (Lewenza, Visser, and Sokol 2002).

Gene Duplications and Acquisitions

Although the LuxI and LuxR trees are globally congruent, indicating fidelity of the inducer and receptor genes to one another, it is still possible that they have been transferred as a pair between different organisms, and this possibility is strengthened by the fact that they often occur in tandem on the bacterial chromosome (Gray and Garey 2001). Using the SSU rRNA tree to represent the organismal phylogeny, the LuxI and LuxR family A trees show substantial agreement with the SSU rRNA tree (fig. 2). Besides the case of *B. cepacia* discussed above, both β-proteobacterial and α-proteobacterial genes are monophyletic. In contrast, genes from the γ-Proteobacteria are paraphyletic, and they are also the only group in which homologs are found within a single genome. In the case of *Pseudomonas chlororaphis* and *P. fluorescens*, pairs of paralogs (RhlI and PhzI in *P. fluorescens* and CsaI and PhzI in *P. chlororaphis*) appear to have arisen from a duplication in the ancestral genome of these two species, followed by speciation. The same pattern is seen for the receptor genes, providing a clear case of parallel evolution for the receptor and the inducer genes. Interestingly, PhzI/R in *P. chlororaphis* and *P. fluorescens* regulate the same gene expression: they are implicated in the production of phenazine antibiotics (Mavrodi et al. 1998). However, the two other orthologs, CsaI/R and RhlI/R, do not have homologous functions. In *P. fluorescens*, RhlI/R regulate the expression of antifungal products (Flavier et al., personal communication), and, in *P. chlororaphis*, CsaI/R are implicated in the biosynthesis of cell surface components (Zhang and Pierson 2001).

Pseudomonas species also possess more distant paralogs that fall into the clade containing genes of other γ-Proteobacteria: MupI/R and LasI/R in *P. fluorescens* and *P. aeruginosa*, respectively. The observed pattern is most consistent with retention of the ancestral gene pair leading to MupI/R and LasI/R, combined with an ancient horizontal transfer from β-Proteobacteria of the gene pair leading to the RhlI/R, PhzI/R, and CsaI/R clades, followed by duplication and speciation in the transferred gene lineage. This scenario is depicted in figure 3*A*. Other explanations for the observed gene phylogenies require the same or more steps of transfer, duplication and/or gene loss. Our finding is congruent with observations by Boucher et al. (2003). Therefore, the incongruence with the accepted species phylogeny is best explained by a combination of horizontal transfer with ancestral gene duplications. Interestingly, the LasI/R and the RhlI/R systems in *P. aeruginosa* interact with each other, with the RhlI/R system being dependent upon the LasI/R system (Whitehead et al. 2001). The two systems have been demonstrated as being important for the pathogenesis of *P. aeruginosa*. Thus, the acquisition of the RhlI/R system might have been a step in the establishment of new virulence properties during the evolution of this species. Moreover, the PhzI/R system in *P. chlororaphis* and *P. fluorescens* is implicated in the production of antibiotics, suggesting that it may have been horizontally acquired in the ancestral species and maintained because of the competitive advantage it conferred.

Burkholderia cepacia also possesses two systems: CepI/R and BviI/R. The first is implicated in the regulation of extracellular virulence factor production (Lewenza et al. 1999), and orthologs are found in other *Burkholderia* species (Lutter et al. 2001). The case of BviI/R, first described in *B. vietnamiensis* (Dennis and Zylstra 1998), is more puzzling. The receptor is more closely related to the PhzR of *P. aeruginosa* than with the other receptor of the β-Proteobacteria. A gene transfer between these two lineages seems the most plausible explanation, with the inducer gene acquiring a new receptor as partner in the signaling system.

Evolution of LuxI/R Family B

Tree Reconstruction

The phylogenies reconstructed for the members of family B (figs. 1*C* and *D*) display globally similar topologies, with the position of the inducer mirroring that of the corresponding receptor. (Because the different methods led to identical topologies, only the NJ trees obtained in MEGA are presented.) Three groups appear in the two trees.

The first group contains sequences of *Yersinia*, *Pantoea*, *Serratia*, and *Hafnia*. The second group contains sequences from *Yersinia*, *Erwinia*, and *Serratia*. The third group encompasses *Pseudomonas* sequences. One explanation for these groupings is that the first two groups correspond to paralogs originating from duplications that occurred after the divergence from *Pseudomonas* but before the speciation of all the other organisms; this explanation would imply a large number of independent losses.

The principal strongly supported difference between the two trees concerns the position in a basal part of the tree of the CarR proteins of *Serratia marcescens* and *E. carotovora*, which form a sister group quite distinct from their paralogs. This could indicate a horizontal acquisition of the receptor in these species, resulting in a change in the receptor partner of CarI within *E. carotovora*. In *E. carotovora*, the CarI/R system is implied in the production of carbapenem, an antibiotic. In the majority of LuxI/R systems in family B, the two genes corresponding to the inducer and the receptor are adjacent. However, *carI* (also named *expI*) and *carR* are not linked (von Bodman, Bauer, and Coplin 2003), supporting the hypothesis of the

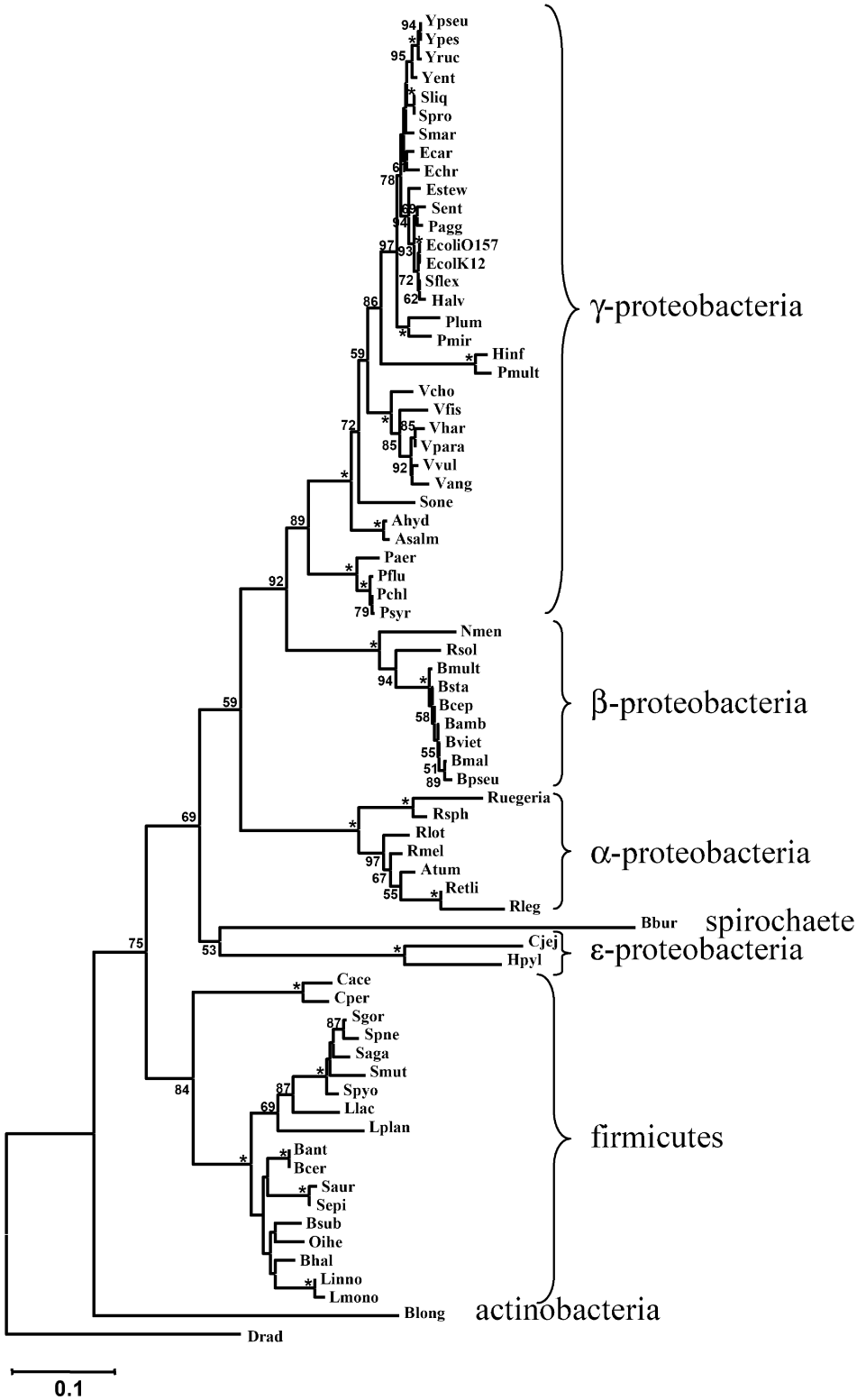

FIG. 2.—Tree based on the alignment of the SSU rRNA of each species. Species abbreviations as in table 1. Asterisks (*) indicate nodes with bootstrap values less than 50%.

independent acquisition of *carR*. Moreover, a different gene, *expR*, is adjacent to *carI*, but no genes regulated by ExpR have been identified (Andersson et al. 2000). This could indicate that, after the acquisition of *carR*, some *expR* functions, particularly the activation of *expI* expression have been assumed by the new gene. If the carbapenem operon was acquired at the same time as *carR*, the selective advantage of being able to produce this

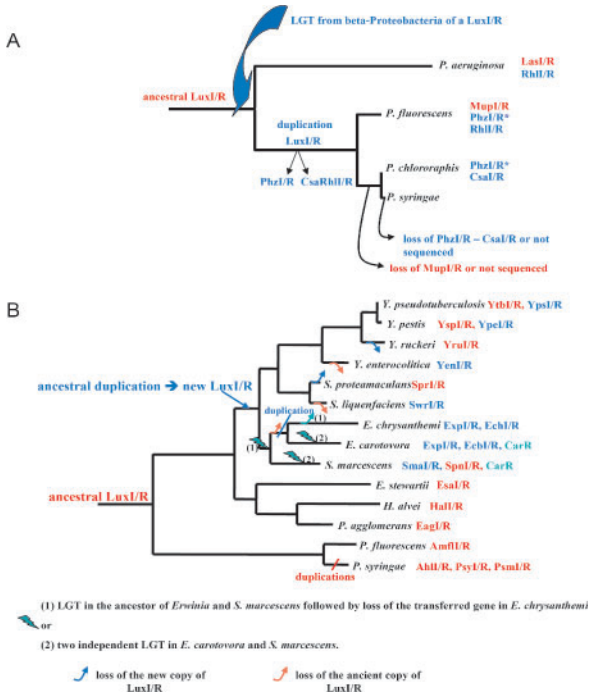

FIG. 3.—(*A*) Hypothetical scenario for the observed pattern of genes from family A in *Pseudomonas* species. Asterisks (*) indicate LuxI/R systems with the same target genes. (*B*) Hypothetical scenario underlying the distribution and relationships of genes of family B in the γ-Proteobacteria.

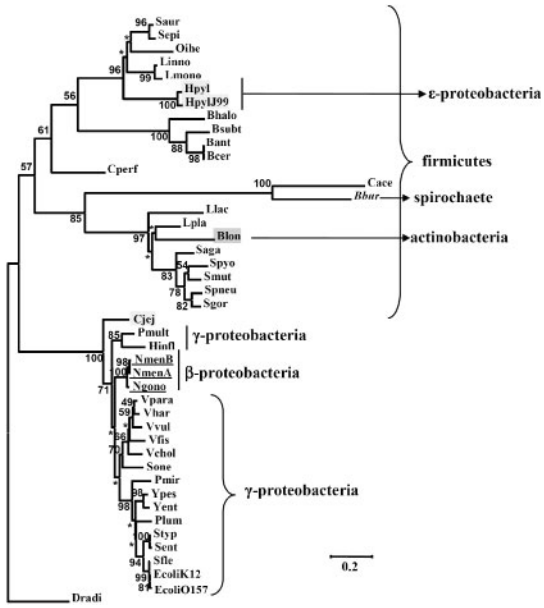

FIG. 4.—Tree based on the protein alignment of the LuxS members with the NJ method with 500 bootstrap replicates and with the γ correction. The other phylogenetic methods gave a similar topology. Species abbreviations as in table 1. Asterisks (*) indicate nodes with bootstrap values less than 50%.

antibiotic would explain the switch between *carR* and *expR*. It has been shown that overexpression of *expR* decreases the virulence of *E. carotovora* when the ExpR protein is attached to the inducer (Andersson et al. 2000). This suggests that *expR* is still functional but acts as a repressor of virulence by sequestering inducer molecules. In *S. marcescens*, CarR is also implicated in the production of carbapenem, and the gene is also located near the first gene *carA* of the operon (Thomson et al. 2000). There is a difference between the two mechanisms. In *Serratia*, the production of carbapenem is independent of the production of a diffusible signal (Cox et al. 1998). *S. marcescens* and *E. carotovara* are the only gram-negative bacteria known to be able to produce this antibiotic. The other bacteria known to possess this property are *Streptomyces* species, gram-positive bacteria.

Gene Duplications

The two trees are globally in agreement with the SSU rRNA phylogeny for relationships within major clades (fig. 2).

Yersinia pestis and *Y. pseudotuberculosis* are the only two species possessing genes from the two groups of paralogs (both are present in *Serratia* but not in the same genome). We performed a BlastP search against the uncompleted sequence for the genomes of *E. chrysanthemi*, *E. carotovora*, and *Y. enterolitica* to find homologous genes of YtbI/R and YspI/R. However, no genes other than those used in this study were detected. The sporadic distribution of the paralogous genes suggests that horizontal transfer occurred in an ancestor of *Yersinia*. However, ancient duplication followed by extensive loss of one or the other paralog could also explain the observed pattern. One scenario involving ancestral duplication is presented in figure 3*B*. Several other explanations are also consistent with the observations but require numerous events of duplication, transfer, and/or loss.

Evolution of LuxS

We have reconstructed the phylogenetic tree of the LuxS protein (fig. 4). The tree presented was obtained using the NJ method, with 500 bootstrap replicates and with the γ correction for the distribution of changes among sites, and the other methods yielded the identical topology. As in the SSU RNA tree (fig. 2), the Firmicutes forms a clade separate from the Proteobacteria. Inside the Proteobacteria, each division (γ, b, and ε) is distinct. Although the γ-Proteobacteria are paraphyletic in this tree, this branching pattern is not well supported.

Within the monophyletic group Firmicutes, there are four unexpected sequences. The most surprising is the position of the LuxS of *Helicobacter pylori* as sister group to *Staphylococcus*, *Listeria*, and *Bacillus* species and distant from *Campylobacter jejuni*, also in the ε-Proteobacteria and a close relative in the SSU rRNA tree (fig. 2). This suggests that *H. pylori* acquired the *luxS* gene from the firmicutes. The spirochete *Borrelia burgdorferi*, the agent responsible for Lyme disease, is also grouped among the Firmicutes and is placed as sister group to *Clostridium acetobutylicum*.

We also find an actinobacterium, *Bifidobacterium longum*, as sister group to the Firmicutes *Lactobacillus plantarum* and *Lactococcus lactis*, and several *Strepto-*

coccus. *B*. *longum* colonizes the human gastrointestinal tract and is considered as an important commensal (Schell et al. 2002). Interestingly, *L*. *plantarum* and *L*. *lactis* are known to be natural inhabitants of the human gastrointestinal tract (Bolotin et al. 2001; Kleerebezem et al. 2003). Thus, the proximity of the habitat of these species might explain the possibility of gene transfers among them.

The cohesion of the group of the γ-Proteobacteria indicates that the presence of *luxS* is ancestral in this group, possibly predating the interaction of these bacteria with metazoan hosts.

Conclusions

These phylogenetic results, particularly the general agreement between the SSU rRNA tree and the trees for individual components of QS systems, indicate that these systems are ancient in many species, suggesting that the mechanisms underlying QS were established very early in the evolution of bacteria. Furthermore, only rarely has an inducer or a receptor gene acquired a new partner. In most lineages, the genes are contiguous on the chromosome and retain their pairwise functional relationship, indicating that most gene partners have shared histories.

Nonetheless, these results do indicate an important role of horizontal transfer in generating the current distribution of the QS genes across bacterial species. We document fewer instances of LGT than do Gray and Garey (2001), who considered the LuxI/R system as a single family rather than two. Combining the two families into a single analysis required the hypothesis of additional instances of LGT by these authors. More recently, Boucher et al. (2003) reanalyzed the data set of Gray and Garey (2001), adding some new sequences. They found the same LGT events, including ones that are based on the failure to separate the two families, plus some new cases of LGT. We confirm one of these additional cases, which concerns transfer from β-Proteobacteria to *P*. *aeruginosa*. Boucher et al. (2003) also propose independent events of LGT for *spnI, sprI*, and *smaI* in *Serratia* species and show that remnants of insertion sequences flank those genes. In our phylogeny, those genes do not present aberrant positions, and LGT without phylogenetic disruption or intrachromosomal rearrangements could explain these cases.

Horizontal transfer is particularly clear for the *luxS* genes transferred to several lineages of bacteria from Firmicutes, the *carR* genes in *E*. *carotovora* and *S*. *marcescens*, and certain systems of family A, such as the RhlI/R system in *P*. *aeruginosa*. The case of *carI*/*R* in *E*. *carotovora* is an interesting example of the replacement of one receptor by another, while depending on the native inducer gene and retaining the functionality of the native receptor but reassigning its role to that of negative regulator. As in the cases of the LasI/R and RhlI/R systems in *P*. *aeruginosa*, this illustrates the possibility of integrating an acquired gene into a preexisting regulatory pathway. The gain of such systems is of particular interest in pathogenic species. It provides a mechanism for regulating virulence genes with respect to the density of members of their own species and, in some cases, of other species. These capabilities can affect competitive ability, as in the case of antibiotic production by *E*. *carotovora* and *S*. *marcescens*, and thus will typically be under strong selection. Strong positive selection for the newly acquired trait has been hypothesized to be important for horizontal gene transfer (e.g., Ochman, Lawrence, and Groisman 2000).

For the LuxI/R families, receptor genes appear occasionally to lose their inducer gene partners, or vice versa, with no replacement recognizable on the basis of sequence homology. The most striking case of this is the *sdiA* receptor genes in the enteric bacteria, which appear to function in receiving signals from other bacterial species (Smith and Ahmer 2003). The variety of commensal and competitive interactions among bacteria in natural communities is expected to create many situations in which reception or emission of a signal may be independently favored.

The presence of homologous signaling systems within some genomes appears to reflect both duplication and horizontal transfer, in different cases. This indicates that the regulatory genes in bacteria can adapt to new target genes either when they are horizontally transmitted or when they are duplicated. The QS systems are regarded as new targets for antimicrobial strategies. In this perspective, it is important to consider the potential implication of horizontal transfers in the reacquisition of virulence.

Literature Cited

Ahmer, B. M., J. van Reeuwijk, C. D. Timmers, P. J. Valentine, and F. Heffron. 1998. *Salmonella typhimurium* encodes an SdiA homolog, a putative quorum sensor of the LuxR family that regulates genes on the virulence plasmid. J. Bacteriol. **180**:1185–1193.

Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**:3389–3402.

Andersson, R. A., A. R. Eriksson, R. Heikinheimo, A. Mae, M. Pirhonen, V. Koiv, H. Hyytiainen, A. Tuikkala, and E. T. Palva. 2000. Quorum sensing in the plant pathogen *Erwinia carotovora* subsp. *carotovora*: the role of *expR*(Ecc). Mol. Plant Microbe Interact. **13**:384–393.

Bainton, N. J., P. Stead, S. R. Chhabra, B. W. Bycroft, G. P. Salmond, G. S. Stewart, and P. Williams. 1992. N-(3-oxohexanoyl)-L-homoserine lactone regulates carbapenem antibiotic production in *Erwinia carotovora*. Biochem. J. **288**:997–1004.

Bassler, B. L., M. Wright, R. E. Showalter, and M. R. Silverman. 1993. Intercellular signaling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. Mol. Microbiol. **9**:773–786.

Bassler, B. L., M. Wright, and M. R. Silverman. 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.

Bhattacharyya, A., S. Stilwagen, N. Ivanova et al. (22 co-authors). 2002. Whole-genome comparative analysis of three phytopathogenic *Xylella fastidiosa* strains. Proc. Natl. Acad. Sci. USA **99**:12403–12408.

Benson, D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, B. A. Rapp, and D. L. Wheeler. 2002. GenBank. Nucleic Acids Res. **25**:3389–3402.

Bolotin, A., P. Wincker, S. Mauger, O. Jaillon, K. Malarme, J. Weissenbach, S. D. Ehrlich, and S. Sorokin. 2001. The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. Genome Res. **11**: 731–753.

Boucher, Y., C. J. Douday, R. T. Papke, D. A. Walsh, M. E. R. Boudreau, C. L. Nesbø, R. J. Case, and W. F. Doolittle. 2003. Lateral gene transfer and the origins of prokaryotic groups. Annu. Rev. Genet. **37**:283–328.

Brint, J. M., and D. E. Ohman. 1995. Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. J. Bacteriol. **177**:7155–7163.

Chen, X., S. Schauder, N. Potier, A. Van Dorsselaer, I. Pelczer, B. L. Bassler, and F. M. Hughson. 2002. Structural identification of a bacterial quorum-sensing signal containing boron. Nature **415**:545–549.

Cox, A. R. J., N.R. Thomson, B. Bycroft, G. S. A. B. Stewart, P. Williams, and G. P. C. Salmond. 1998. A pheromone-independent CarR protein controls carbapenem antibiotic synthesis in the opportunistic human pathogen *Serratia marcescens*. Microbiology **144**:201–209.

Dennis, J. J., and G. J. Zylstra. 1998. Plasposons: modular self-cloning minitransposon derivatives for rapid genetic analysis of gram-negative bacterial genomes. Appl. Environ. Microbiol. **64**:2710–2715.

Felsenstein, J. 2002. PHYLIP (phylogeny inference package). Version 3.6. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Fuqua, W. C., and S. C. Winans. 1994. A LuxR-LuxI type regulatory system activates *Agrobacterium* Ti plasmid conjugal transfer in the presence of a plant tumor metabolite. J. Bacteriol. **176**:2796–3806.

Galtier, N., M. Gouy, and C. Gautier. 1996. SeaView and Phylo_win, two graphic tools for sequence alignments and molecular phylogeny. Comput. Appl. Biosci. **12**:543–548.

Gray, K. M., and J. R. Garey, 2001. The evolution of bacterial LuxI and LuxR quorum sensing regulators. Microbiology **147**:2379–2387.

Jones, D. T., W. R. Taylor, and J. M. Thorton. 1992. The rapid generation of mutation data matrices from protein sequences. Comput. Appl. Biosci. **8**:275–282.

Jones, S., B. Yu, N. J. Bainton, M. Birdsall et al. (13 co-authors). 1993. The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa*. EMBO J. **12**:2477–2482.

Kanamaru, K., K. Kanamaru, I. Tatsuno, T. Tobe, and C. Sasakawa. 2000. SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic *Escherichia coli* O157:H7. Mol. Microbiol. **38**:805–816.

Kiratisin, P., K. D. Tucker, and L. Passador. 2002. LasR, a transcriptional activator of *Pseudomonas aeruginosa* virulence genes, functions as a multimer. J. Bacteriol. **184**:4912–4919.

Kleerebezem, M., J. Boekhorst, R. van Kranenburg et al. (20 co-authors). 2003. Complete genome sequence of *Lactobacillus plantarum* WCFS1. Proc. Natl. Acad. Sci. USA **100**:1990–1995.

Kumar, S., K. Tamura, and M. Nei. 1993. MEGA: molecular eEvolutionary genetics analysis. Version 1.01. The Pennsylvania State University, University Park, Pa.

Lerat, E., V. Daubin, and N. A. Moran. 2003. From gene trees to organismal phylogeny in prokaryotes: the case of the γ-Proteobacteria. PLoS Biol. **1**:101–109.

Lewenza, S., B. Conway, E. P. Greenberg, and P. A. Sokol. 1999. Quorum sensing in *Burkholderia cepacia*: identification of the LuxRI homologs CepRI. J. Bacteriol. **181**: 748–756.

Lewenza, S., M. B. Visser, and P. A. Sokol. 2002. Interspecies communication between *Burkholderia cepacia* and *Pseudomonas aeruginosa*. Can. J. Microbiol. **48**:707–716.

Lutter, E., S. Lewenza, J. J. Dennis, M. B. Visser, and P. A. Sokol. 2001. Distribution of quorum-sensing genes in the *Burkholderia cepacia* complex. Infect. Immun. **69**:4661–4666.

Mavrodi, D. V., V. N. Ksenzenko, R. F. Bonsall, R. J. Cook, A. M. Boronin, and L. S. Thomashow. 1998. A seven-gene locus for synthesis of phenazine-1-carboxylic acid by *Pseudomonas fluorescens* 2–79. J. Bacteriol. **180**:2541–2548.

Miller, M. B., and B. L. Bassler. 2001. Quorum sensing in bacteria. Annu. Rev. Microbiol. **55**:165–199.

Miller, M. B., K. Skorupski, D. H. Lenz, R. K. Taylor, and B. L. Bassler. 2002. Parallel quorum sensing systems converge to regulate virulence in *Vibrio cholerae*. Cell **110**: 303–314.

Minogue, T. D., M. Wehland-von Trebra, F. Bernhard, and S. B. von Bodman. 2002. The autoregulatory role of EsaR, a quorum-sensing regulator in *Pantoea stewartii* ssp. *stewartii*: evidence for a repressor function. Mol. Microbiol. **44**:1625–1635.

Mok, K. C., N. S. Wingreen, and B. L. Bassler. 2003. *Vibrio harveyi* quorum sensing: a coincidence detector for two autoinducers controls gene expression. EMBO J. **22**:870–881.

Neaslon, K. H., and J. W. Hastings. 1979. Bacterial bioluminescence: its control and ecological significance. Microbiol. Rev. **43**:496–518.

Ochman, H., J. G. Lawrence, and E. A. Groisman. 2000. Lateral gene transfer and the nature of bacterial innovation. Nature **405**:299–304.

Ohtani, K., H. Hayashi, and T. Shimizu. 2002. The *LuxS* gene is involved in cell-cell signaling for toxin production in *Clostridium perfringens*. Mol. Microbiol. **44**:171–179.

Passador, L., J. M. Cook, M. J. Gambello, L. Rust, and B. H. Iglewski. 1993. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. Science **260**:1127–1130.

Pirhonen, M., D. Flego, R. Heikinheimo, and E. T. Palva. 1993. A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in *Erwinia carotovora*. EMBO J. **12**:2467–2476.

Qin, Y., Z.-Q Luo, A. J. Smyth, P. Gao, S Beck von Bodman, and S. K. Farrand. 2000. Quorum-sensing signal binding results in dimerization of TraR and its release from membranes into the cytoplasm. EMBO J. **19**:5212–5221.

Schell, M. A., M. Karmirantzou, B. Snel et al. (12 co-authors). 2002. The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. Proc. Natl. Acad. Sci. USA **99**:14422–14427.

Schmidt, H. A., K. Strimmer, M. Vingron, and A. von Haeseler. 2002. TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics **18**:502–504.

Smith, J. N., and B. M. M. Ahmer. 2003. Detection of other microbial species by *Salmonella*: expression of the SdiA regulon. J. Bacteriol. **185**:1357–1366.

Surette, M. G., M. B. Miller, and B. L. Bassler. 1999. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. Proc. Natl. Acad. Sci. USA **96**: 1639–1644.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**:4673–4680.

Thomson, N. R., M. A. Crow, S. J. McGowan, A. Cox, and G. P. Salmond. 2000. Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. Mol. Microbiol. **36**:539–556.

Ventre, I., F. Ledgham, V. Prima, A. Lazdunski, M. Foglino, and J. N. Sturgis. 2003. Dimerization of the quorum sensing regulator RhlR: development of a method using EGFP fluorescence anisotropy. Mol. Microbiol. **48**:187–198.

von Bodman, S. B., W. D. Bauer, and D. L. Coplin. 2003. Quorum sensing in plant-pathogenic bacteria. Annu. Rev. Phytopathol. **41**:12.1–12.28.

Wang, X. D., P. A. de Boer, and L. I. Rothfield. 1991. A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. EMBO J. **10**:3363–3372.

Welch, M., D. E. Todd, N. A. Whitehead, S. J. McGowan, B. W. Bycroft, and G. P Salmond. 2000. N-acyl homoserine lactone binding to the CarR receptor determines quorum-sensing specificity in Erwinia. EMBO J. **19**:631–641.

Whitehead, N. A., A. M. L. Barnard, H. Slater, N. J. L. Simpson, and G. P. C. Salmond. 2001. Quorum-sensing in gram-negative bacteria. FEMS Microbiol. Rev. **25**:365–404.

Zhang, Z. and L. S. Pierson 3rd. 2001. A second quorum-sensing system regulates cell surface properties but not phenazine antibiotic production in *Pseudomonas aureofaciens*. Appl. Environ. Microbiol. **67**:4305–4315.

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