The evolution of bacterial LuxI and LuxR quorum sensing regulators

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Quorum sensing is a widespread form of bacterial communication in which individual cells produce and respond to specific *N*-acyl homoserine lactone signal metabolites. The different autoinducer synthases that generate these signals and the receptor/activator proteins that mediate the cell's response to them constitute evolutionarily conserved families of regulatory proteins known as the LuxI and LuxR families, respectively. We have performed a phylogenetic analysis of 76 individual LuxI and LuxR homologues present in diverse members of the Gram-negative *Proteobacteria*. The results were consistent with an early origin for these regulators during the evolution of the *Proteobacteria*, with functional pairs of *luxI* and *luxR* genes possibly coevolving as regulatory cassettes. In many cases, specific LuxI and LuxR family members appeared to have been inherited horizontally. In particular, those species containing multiple LuxI and/or LuxR homologues usually appeared to have obtained each individual homologue or functional pair of homologues from an independent source. Because multiple homologues interact to form regulatory cascades, this finding suggests that hierarchical signalling pathways can potentially evolve by the sequential integration of pre-existing regulatory circuits acquired from diverse sources.

Keywords: intercellular signalling, cell–cell communication, horizontal gene transfer

INTRODUCTION

Intercellular communication plays a central role in the physiology and development of living organisms. Even unicellular organisms such as bacteria will frequently coordinate their activities so that populations of cells can function as multicellular entities (Shapiro, 1998). Such coordination necessarily requires cell–cell communication, often by means of the production and release of specific signal compounds by individual cells. The extracellular accumulation of these self-produced signals denotes the presence of a relatively dense population of cells and thus the appropriateness of coordinated group behaviour. Bacterial cell–cell communication of this sort is known as quorum sensing.

The best-studied mechanisms of quorum sensing are found among the Gram-negative *Proteobacteria*, which use *N*-acyl homoserine lactone 'autoinducers' as signal molecules (Greenberg, 2000; Parsek & Greenberg, 2000; Swift *et al*., 1999). With few exceptions, the proteins that synthesize these autoinducers constitute an evolutionarily conserved family of homologues known as the LuxI family of autoinducer synthases. The proteins that act as the specific response elements for autoinducers form another evolutionarily conserved group known as the LuxR family of transcriptional regulators.

LuxI and LuxR homologues have been identified in diverse species throughout the Gram-negative *Proteobacteria*, including phototrophic purple non-sulfur bacteria, marine vibrios, rhizosphere bacteria, enteric commensals and opportunistic pathogens of plants and animals. The LuxI and LuxR homologues present in these species regulate such disparate functions as exoenzyme synthesis, conjugation, antibiotic production, luminescence and biofilm formation (Greenberg, 2000; Parsek & Greenberg, 2000; Swift *et al*., 1999).

The genetic organization of LuxI and LuxR family members is as diverse as their function. Homologues of *luxI* and *luxR* genes have been identified both on bacterial chromosomes and on extrachromosomal elements such as the symbiosis (Sym) and tumour-inducing

Table 1. Quorum sensing homologues included in this study

Organism	LuxI homologue*	LuxR homologue*	Orientation of adjacent genes	GenBank accession no. or reference
α-Proteobacteria				
Agrobacterium tumefaciens (pTiA6; pTiR10) (oct)†	TraI	TraR	Non-adjacent	L17024; L08596
Agrobacterium tumefaciens (pTiR10) (oct)†	–	TrlR‡	Non-adjacent	U60011
Agrobacterium tumefaciens (pTiC58) (nop)†	TraI	TraR	Non-adjacent	L22207; Z15003
Agrobacterium tumefaciens (pTi-SAKURA) (nop)†	TraI	–	Non-adjacent	AB006858
Agrobacterium vitis (pTi2608) (o/c)†	–	TraR	Non-adjacent	Z22732
Rhizobium etli§	RaiI	RaiR	→ →	U92712; U92713
Rhizobium leguminosarum§	CinI	CinR	→ →	AF210630
Rhizobium leguminosarum (pRL1JI)§	RhiI	RhiR	Non-adjacent	Rodelas *et al.* (1999); M98835
Rhizobium leguminosarum (pRL1JI)§	–	BisR	Non-adjacent	J. A. Downie (unpublished results)
Rhizobium leguminosarum (pRL1JI)§	–	TriR	Non-adjacent	J. A. Downie (unpublished results)
Rhizobium sp. NGR 234 (pSym)§	TraI	TraR	Non-adjacent	AE000068; AE000069
Rhizobium sp. NGR 234 (pSym)§	–	Y4qH	Non-adjacent	AE000093
Rhodobacter sphaeroides	CerI	CerR	→ →	AF016298
β-Proteobacteria				
Burkholderia cepacia	CepI	CepR	← →	AF019654
Chromobacterium violaceum	CviI	CviR	→ ←	M. K. Winson (unpublished results)
Ralstonia solanacearum	SolI	SolR	← →	AF02184
γ-Proteobacteria, aeromonads				
Aeromonas hydrophila	AhyI	AhyR	← →	X89469
Aeromonas salmonicida	AsaI	AsaR	← →	U65741
γ-Proteobacteria, enterobacteria				
Pantoea (*Enterobacter*) *agglomerans*	EagI	*EagR*	→ ←	X74300
Erwinia carotovora subsp. *carotovora* SCC3193	ExpI	ExpR	→ ←	X72891; X80475
Erwinia carotovora subsp. *carotovora* 71	HslI (ExpI)	*HslR* (ExpR)	→ ←	L40174
Erwinia carotovora subsp. *carotovora* GS101	CarI (ExpI)	*RexR* (ExpR)	→ ←	X74299
Erwinia carotovora subsp. *carotovora* GS101	–	CarR	Non-adjacent	U17224
Erwinia carotovora subsp. *carotovora* SCRI 193	–	CarR	Non-adjacent	Holden *et al.* (1998)
Erwinia carotovora subsp. *betavasculorum*	EcbI	EcbR	→ ←	AF001050
Erwinia chrysanthemi NCPPB 1066	EchI (ExpI)	EchR (ExpR)	→ ←	U45854
Escherichia coli	–	SdiA	Non-adjacent	AE000284
Pantoea (*Erwinia*) *stewartii*	EsaI	EsaR	→ ←	L32184
Erwinia chrysanthemi 3937	ExpI	ExpR	→ ←	X96440
Salmonella typhimurium	–	SdiA	Non-adjacent	U88651
Serratia liquefaciens	SwrI	SwrR	→ ←	U22823; M. Givskov & M. K. Winson (unpublished results)
Serratia marcescens	SmaI	SmaR	→ ←	AJ275980
Serratia marcescens	–	CarR	Non-adjacent	AF012907
Yersinia enterocolitica	YenI	YenR	→ ←	X76082
γ-Proteobacteria, pseudomonads				
Pseudomonas aeruginosa PAO1	LasI	LasR	→ →	D90832; L04681
Pseudomonas aeruginosa PAO1	RhlI	RhlR	→ →	U40458
Pseudomonas aeruginosa PAO1	VsmI (RhlI)	VsmR (RhlR)	→ →	U15644
Pseudomonas aeruginosa PG201	RhlI	RhlR	→ →	L08962
Pseudomonas aureofaciens	PhzI	PhzR	→ ←	L33724; L32729
Pseudomonas fluorescens	PhzI	PhzR	→ ←	L48616
Pseudomonas syringae pv. tabaci	PsyI	*PsyR*	→ ←	U39802
Pseudomonas syringae pv. syringae	AhlI	*AhlR*	→ ←	AF072537

Table 1 (cont).

Organism	LuxI homologue*	LuxR homologue*	Orientation of adjacent genes	GenBank accession no. or reference
γ-Proteobacteria, vibrios				
Vibrio anguillarum	VanI	VanR	→ ←	U69677
Vibrio (Photobacterium) fischeri ATCC 7744	LuxI	LuxR	← →	M19039
Vibrio (Photobacterium) fischeri ES114	LuxI	LuxR	← →	M96844
Vibrio (Photobacterium) fischeri MJ1	LuxI	LuxR	← →	Y00509

* When different names are reported for what may be the same genetic locus, the alias is listed followed by the first-reported name in parentheses. Homologues written in italics were unavailable for this study.

† The tumour-inducing (Ti) plasmids of *Agrobacterium* spp. are grouped according to type as either octopine-producing (oct), nopaline-producing (nop) or octopine/cucumopine-producing (o/c). The homologues listed are present on the Ti plasmids specifically.

‡ The *trlR* gene contains a frameshift mutation in the 3′ end of the normal reading frame (Oger *et al.*, 1998; Zhu & Winans, 1998). This point mutation was corrected to generate a gene product that more accurately reflects its relationship to other members of the LuxR family.

§ Homologues listed for *Rhizobium* spp. are present on the chromosome unless a symbiosis (Sym) plasmid is designated (e.g. pRL1JI), in which case the homologues are present on the Sym plasmid itself.

(Ti) plasmids of *Rhizobium* and *Agrobacterium* spp., respectively. Many bacterial species even contain multiple LuxI and/or LuxR homologues for the production or detection of multiple, distinct signals. Multiple LuxR homologues can also permit the independent activation of different gene functions in response to a single autoinducer signal (McGowan *et al.*, 1995; Pirhonen *et al.*, 1993).

The question of where these two families of quorum sensing regulators first arose and how they came to be disseminated across such wide evolutionary distances has yet to be addressed. We have therefore performed a phylogenetic analysis of 76 LuxI and LuxR family members. Our results suggest that these quorum sensing regulators arose early in the evolution of the *Proteobacteria* and subsequently diverged within each group of organisms, although instances of horizontal gene transfer have also occurred. Surprisingly, most bacteria that contain multiple LuxI and/or LuxR family members appear to have acquired each of their different homologues from separate sources. This result provides new insights into the possible evolution of hierarchical signalling cascades in bacteria.

METHODS

DNA sequences. The LuxI and LuxR homologues considered in this analysis are listed in Table 1. Published sequences were obtained from GenBank, while unpublished sequences were generously provided by individual colleagues. MalT of *Escherichia coli* (accession no. M13585), EpsR of *Ralstonia solanacearum* (M61197) and FixJ of *Sinorhizobium meliloti* (Z21854) were tested as outgroups for the analysis of LuxR family members (Henikoff *et al.*, 1990). Prealigned 16S rRNA gene sequences were obtained from the rRNA WWW Server (Van de Peer *et al.*, 1998).

Sequence alignment and analysis. Amino acid sequences

were aligned using the default parameters of CLUSTALX (Higgins & Sharp, 1989) and adjusted by eye using GeneDoc (Nicholas & Nicholas, 1997). For purposes of comparison, a second set of alignments was made based on the published alignments of 17 LuxI homologues (Fuqua & Eberhard, 1999) and 15 LuxR homologues (Stevens & Greenberg, 1999). Sites containing gaps were excluded from the analysis to reduce systematic errors. Alignments were analysed using PAUP* version 4.0b4 (Swofford, 2000) to produce maximum-parsimony trees. Confidence in maximum-parsimony trees was determined by analysing 100 bootstrap replicates. Neighbour-joining trees were produced with the MEGA program (Kumar *et al.*, 1994) using the γ model of amino acid substitution with a shape parameter a of 2·0. Confidence in neighbour-joining trees was determined by analysing 500 bootstrap replicates. All trees were drawn with nodes collapsed where bootstrap support was less than 50 %.

When tree topologies disagreed with the groupings suggested by rRNA analysis, alternative trees were tested statistically both by a pairwise parsimony method (Templeton, 1983) and by comparison of log-likelihood differences (Kishino & Hasegawa, 1989), each as implemented by PAUP*. Analysis of the LuxI family tree was performed using a strict consensus of the eight most parsimonious trees derived from the modified CLUSTAL analysis. Analysis of the LuxR family tree was performed using a strict consensus of the two most parsimonious trees, while analysis of a combined LuxI/LuxR tree was performed on a strict consensus of the six most parsimonious trees. In all cases, alternative topologies were considered significantly different if P was less than 0·05.
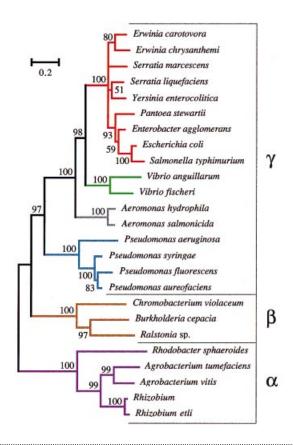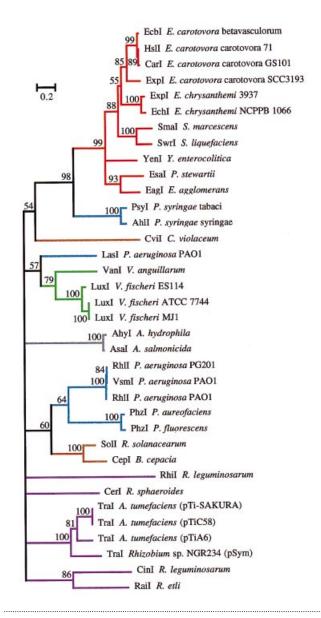
RESULTS AND DISCUSSION

Host bacterial phylogeny as determined by rRNA analysis

The bacteria included in this study can be grouped according to 16S rRNA gene sequences into the α-*Proteobacteria* (comprising the genera *Agrobacterium*,

Fig. 1. Neighbour-joining tree of 16S rRNA sequences from bacteria that contain LuxI and/or LuxR family members. Species belonging to the α-, β- and γ-*Proteobacteria* are grouped within brackets. Different colours within the γ-*Proteobacteria* identify pseudomonads (blue), aeromonads (grey), vibrios (green) and enterobacteria (red). Scale bar indicates the mean number of substitutions per site. Bootstrap values as the percentage of 500 replicates are shown for each branch.

Rhizobium and *Rhodobacter*), the β-*Proteobacteria* (*Burkholderia*, *Chromobacterium* and *Ralstonia*) and the γ-*Proteobacteria* (Fig. 1; Woese, 1987). The γ-*Proteobacteria* can be subdivided into families representing the pseudomonads (*Pseudomonas*), aeromonads (*Aeromonas*), vibrios (*Vibrio*) and enterobacteria (*Enterobacter*, *Erwinia*, *Escherichia*, *Pantoea*, *Salmonella*, *Serratia* and *Yersinia*) (Table 1, Fig. 1). These 16S rRNA groupings were used as the basis for comparison with phylogenetic trees derived from our analysis of LuxI and LuxR family members.

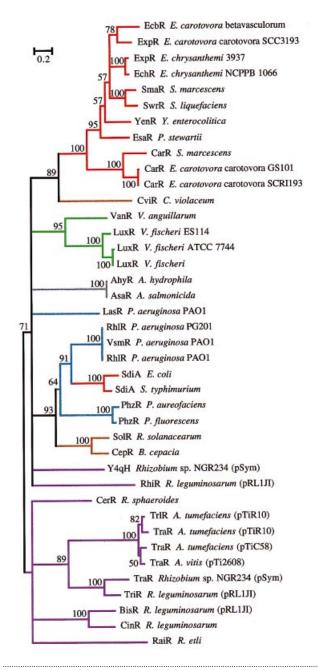### Phylogenetic analysis of the LuxI and LuxR families

Neighbour-joining trees generated from the aligned LuxI and LuxR sequences indicated that the majority of proteins in each family clustered within the same groups defined by 16S rRNA sequences (Figs 2 & 3). Although individual exceptions to this rule were observed, the overall congruity between the quorum sensing and the rRNA trees is consistent with an ancient origin for quorum sensing proteins within the *Proteobacteria*.

Fig. 2. Neighbour-joining tree of LuxI family members. Scale bar indicates the mean number of substitutions per site. Bootstrap values as the percentage of 500 replicates are shown for each branch with values of 50% or greater. Branches with less than 50% bootstrap support are shown collapsed. Colours indicate species belonging to the α-*Proteobacteria* (purple), β-*Proteobacteria* (brown), pseudomonads (blue), aeromonads (grey), vibrios (green) and enterobacteria (red).

Similar results were obtained with maximum-parsimony trees. Trees generated by either method using alignments based on previously published comparisons of a more limited number of sequences (Fuqua & Eberhard, 1999; Stevens & Greenberg, 1999) were indistinguishable from those produced using our own alignments.
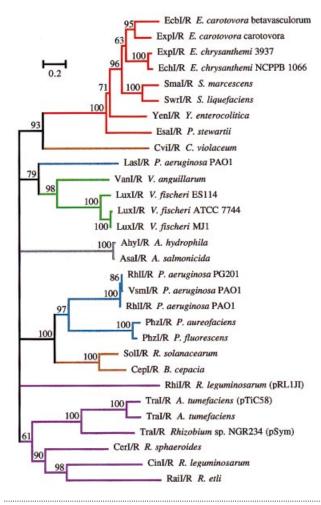
Unfortunately, the amino acid sequences of individual LuxI homologues did not resolve deeper relationships among taxa in this family, as the previously identified groups simply joined with a few unaffiliated homologues

Fig. 3. Neighbour-joining tree of LuxR family members. Scale bar, bootstrap values and colours are the same as for Fig. 2.

Fig. 4. Neighbour-joining tree of combined LuxI/LuxR sequences from functionally linked ('cognate') gene products. Scale bar, bootstrap values and colours are the same as for Fig. 2.

into a single polytomy (Fig. 2). The LuxR family tree showed a similar pattern: in this case, a basal branch separated most of the α-proteobacterial sequences from the remaining groups, but these were once again united as a large polytomy (Fig. 3). This basal separation of the α-*Proteobacteria* sequences had relatively weak (71 %) bootstrap support in the neighbour-joining trees and was absent from the maximum-parsimony trees, which more closely resembled the single polytomy of the LuxI tree.

In an attempt to resolve these uncertain deeper branches further, we decided to consider the combined sequences

of 'cognate' pairs of LuxI and LuxR homologues. Cognate pairs are defined as those that are functionally linked, with the LuxI homologue producing the specific acyl homoserine lactone required to activate the corresponding LuxR protein. In most cases, cognate *luxI* and *luxR* gene homologues are located adjacently to each other, with ORFs oriented tandemly, divergently or convergently (Table 1). In addition, the TraI and TraR proteins of *Agrobacterium tumefaciens* and the RhiI and RhiR proteins of *Rhizobium leguminosarum* are known to function as cognate pairs, despite the fact that their genes are not adjacently positioned. Because functionally related gene pairs have the potential to coevolve, we could repeat our analysis using the combined sequences of these cognate LuxI and LuxR homologues as single taxa.

The resulting phylogenetic tree from the combined LuxI and LuxR sequences was more robust in terms of bootstrap support than those generated from LuxI or LuxR sequences alone, but was otherwise the same as the previous trees (Fig. 4). The fact that this 'combined'

tree was consistent with the individual LuxI and LuxR trees supported the hypothesis that cognate *luxI/luxR* gene pairs may have become physically and/or functionally linked fairly early in their history and subsequently coevolved as unified regulatory cassettes. The relative orientation of the ORFs, however, was not a reliable indicator of phylogenetic relationship. As an example, the divergently oriented homologues of *Vibrio fischeri* grouped most closely with the convergently oriented homologues of *Vibrio anguillarum*, while both sets of *Vibrio* sequences clustered with the tandemly oriented *Pseudomonas aeruginosa* LasI/LasR sequences (Fig. 4, Table 1).

In a final attempt to resolve deeper relationships among the different groups of quorum sensing regulators, we performed an outgroup analysis on the LuxR family tree. LuxR family members are part of a larger superfamily of regulatory proteins that share similarity only in their C-terminal DNA binding domains (Henikoff *et al.*, 1990; Slock *et al.*, 1990). Three members of this superfamily, FixJ from *Sinorhizobium meliloti*, EpsR from *Ralstonia solanacearum* and MalT from *Escherichia coli*, were therefore used as representative outgroups from the α-, β- and γ-Proteobacteria, respectively. Unfortunately, these outgroup sequences were so different from the quorum sensing family sequences that the resulting alignments were too weak to provide any useful information in terms of basal associations with the LuxR homologues. As a result, we were unable to establish which of the quorum sensing homologues appeared to be associated most closely with the outgroup sequences.

The observed groupings of the *Chromobacterium violaceum* CviI/CviR sequences with the enterobacterial cluster and of the *Pseudomonas aeruginosa* LasI/LasR sequences with the *Vibrio* cluster (Fig. 4) represented notable exceptions to the overall agreement between the 16S rRNA gene tree and the combined LuxI/LuxR tree. The validity of these anomalous groupings was tested by placing each pair of sequences into the appropriate host group as identified by 16S rRNA analysis and subsequently determining the statistical significance of the resulting differences in the two trees (Templeton, 1983; Kishino & Hasegawa, 1989).

When the combined CviI/CviR sequences were moved to join the base of the β-proteobacterial cluster (Fig. 4), the resulting tree was significantly longer than the original ($P = 0.0007$ and 0.0012 for the Kishino–Hasegawa and Templeton tests, respectively). The same result was true of CviR in the LuxR tree alone ($P = 0.0002$ and 0.0005), but not of CviI in the LuxI tree ($P = 0.0513$ and 0.0744). These results suggest that the *Chromobacterium* CviR is more closely related to the enterobacterial LuxR homologues than it is to those of the other β-Proteobacteria. As the association of CviI with the enterobacterial LuxI sequences was not supported statistically, its proper affiliation remains open to question. By contrast, moving the combined LasI/LasR sequences of *Pseudomonas aeruginosa* into the pseudo-

monad cluster also resulted in a significantly longer tree ($P = 0.0137$ and 0.0139), but this difference primarily reflected the contribution of LasI rather than LasR. Moving LasR alone did not produce a significantly longer tree ($P = 0.2971$ and 0.2985), while moving LasI alone did result in a significant difference ($P = 0.0024$ and 0.0027). These are intriguing results, given our previous finding concerning the possible coevolution of functionally linked LuxI/LuxR pairs. Apparently CviI/CviR and LasI/LasR are unusual pairs of cognate quorum sensing homologues in that one member of each pair appears to be affiliated with a specific group of sequences, while the other does not.

Horizontal transfer of individual LuxI and LuxR homologues

Many other differences between the rRNA and quorum sensing trees were observed among the unlinked homologues found within the separate LuxI and LuxR trees. For example, the LuxI homologues of two pathovars of *Pseudomonas syringae* grouped strongly (98 % bootstrap support) at the base of the enterobacterial cluster, well apart from other *Pseudomonas* LuxI homologues (Fig. 2). The appearance of the *Pseudomonas syringae* sequences within this cluster suggests that this species may have acquired its quorum sensing regulators by means of horizontal gene transfer. Moving these sequences to the base of the pseudomonad cluster resulted in a significantly longer tree ($P = 0.005$ and 0.006), indicating that they properly belong with the enterobacterial group.

Similarly, the unlinked LuxR homologues, both named SdiA, of *Escherichia coli* and *Salmonella typhimurium* did not group with other enterobacterial homologues. Instead, they appeared most closely related to the *Pseudomonas aeruginosa* RhlR sequences (Fig. 3), suggesting the horizontal transfer of a pseudomonad homologue to one or both of these enterobacterial species. Moving the SdiA proteins to the base of the enterobacterial cluster created a significantly longer topology ($P = 0.0018$ and 0.0019). The SdiA proteins are physiologically unusual in that no specific autoinducer is known to be involved in their activation, although it has been proposed that they might respond to autoinducers produced by other bacteria (Ahmer *et al.*, 1998). Both *Escherichia coli* and *Salmonella typhimurium* have been shown to produce extracellular signals (Surette & Bassler, 1998), but these compounds do not appear to be acylated homoserine lactones (Surette *et al.*, 1999), nor is there any evidence of a functional link between them and the SdiA proteins (Surette & Bassler, 1999).

An alternative interpretation of these results is that differences between the quorum sensing and the rRNA trees might reflect gene duplication events followed by the subsequent loss of certain duplicated genes over time. The enterobacterial-like LuxI homologues of *Pseudomonas syringae* might therefore represent genes that were lost or remain unidentified in the majority of

pseudomonads. While this argument cannot be ruled out entirely, it does not satisfactorily explain many of the results from this analysis. For example, the complete genome of *Escherichia coli* (Blattner *et al*., 1997) contains no LuxI homologues and only the single LuxR homologue SdiA, which resembles a pseudomonad rather than an enterobacterial sequence (Fig. 3). To account for this result by gene duplication, *Escherichia coli* would have to have lost the LuxI and LuxR homologues retained by almost all other enterobacteria, while retaining instead the pseudomonad-like SdiA, which has yet to be found in the other enterobacterial taxa. In addition, there are several examples (described below) in which multiple quorum sensing regulators are found within a single bacterial species, yet most of these homologues cluster within the same rRNA group that defines the host. As a result, even in situations where we know that multiple homologues exist, those examples do not support the argument that early gene duplication adequately explains the appearance of a pseudomonad-like homologue within an enterobacterial host. We therefore propose that horizontal gene transfer is the best explanation for these instances of observed non-agreement between the quorum sensing regulator and the rRNA trees.

Sequence variability within species

Surprisingly, the variation observed among quorum sensing homologues from different laboratory strains of the same bacterial species was sometimes greater than the variation observed between closely related species. For example, the LuxI and LuxR proteins of *Vibrio fischeri* ES114 are distinct from those of strains MJ1 and ATCC 7744 (Fig. 4), while the LuxI homologue ExpI of *Erwinia carotovora* SCC3193 differs from the synonymous HslI and CarI sequences of strains 71 and GS101 (Fig. 2). By comparison, the quorum sensing proteins found in *Aeromonas hydrophila* and *Aeromonas salmonicida* are more closely related to each other, as are those of *Pseudomonas fluorescens* and *Pseudomonas aureofaciens* (Fig. 4). This disparity of greater intraspecific than interspecific variability may reflect either recent horizontal transfer events or simply the recent speciation of these closely related taxa. In either case, it is curious that such extreme sequence diversity should exist within regulatory proteins shared by different strains of a single species.

Evolution of multiple homologues within a species

Six species of bacteria included in this study, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Pseudomonas aeruginosa*, *Rhizobium* sp. NGR234, *Rhizobium leguminosarum* and *Serratia marcescens*, contain more than one LuxI and/or LuxR family member (Table 1). If these multiple homologues arose endogenously by means of recent gene duplication, one would expect them to group most closely with each other in this analysis. Among 13 such possible pairings, however, only two of them – TraR and TrlR of *Agrobacterium*

tumefaciens, and CinR and BisR of *Rhizobium leguminosarum* – appeared as closest relatives (Fig. 3). This suggests that in most cases the multiple quorum sensing genes present within a single species were each acquired independently of the other.

This result has important implications for the evolution of hierarchical signalling cascades in general. As an example, *Rhizobium leguminosarum* contains a pair of linked homologues (*cinI* and *cinR*) on its chromosome as well as the *luxI* homologue *rhiI* and three additional unlinked *luxR* homologues (*bisR*, *rhiR* and *triR*) on the Sym plasmid pRL1JI (Table 1). According to our analysis, each of the LuxI and LuxR family members present on this Sym plasmid appears to have been acquired from a separate source (Figs 3 & 4), with only BisR suggesting a possible endogenous origin from the chromosomal CinR (or vice versa). Given this mosaic pattern of inheritance, it will be especially intriguing to understand how the functions of these different homologues are integrated into a unified global regulatory system. The sheer abundance of these diverse regulators within this species may provide continuing opportunities for innovation in the specific role each plays within this global hierarchy.

Similarly, the two LuxR family members CarR and ExpR of *Erwinia carotovora* subsp. *carotovora* (Table 1) allow the independent activation of different sets of genes in response to a single autoinducer signal (McGowan *et al*., 1995; Pirhonen *et al*., 1993). Of these two homologues, CarR groups at the base of the enterobacterial cluster with a homologue of the same name found in *Serratia marcescens*, while ExpR clusters with the other *Erwinia* sequences (Fig. 4). The same pattern is seen with the two *Serratia marcescens* homologues CarR and SmaR, which group separately with the *Erwinia carotovora* CarR and the *Serratia liquefaciens* SwrR, respectively (Fig. 4). These results again suggest that each of the two LuxR homologues found in these species were acquired independently of each other. In the case of the two CarR proteins, these data further imply that horizontal gene transfer may have occurred between *Erwinia* and *Serratia*. Both of the CarR proteins activate carbapenem antibiotic synthesis in their respective hosts (Cox *et al*., 1998; Holden *et al*., 1998; Thomson *et al*., 2000). This conservation of function may result from the fact that each homologue is genetically linked to a carbapenem biosynthesis gene cluster (Cox *et al*., 1998; Holden *et al*., 1998; Thomson *et al*., 2000), thus allowing the inheritance of biosynthetic and regulatory functions as a single cassette.

Finally, the independent acquisition of multiple homologues is also evident in *Pseudomonas aeruginosa*. As previously noted, the LasI and LasR proteins of *Pseudomonas aeruginosa* showed no affiliation with any of the other pseudomonad homologues, including RhlI and RhlR from the same species (Fig. 4). This suggests that the *lasI* and *lasR* genes were acquired independently of *rhlI* and *rhlR*. This is an especially significant result given that LasI and LasR serve as dominant regulators of

rhlI and *rhlR* gene expression in *Pseudomonas aeruginosa* (Latifi *et al.*, 1996; Pesci *et al.*, 1997).

Collectively, these results provide additional support for the potential importance of horizontal gene transfer in the evolution of bacteria (Lawrence, 1997; Lawrence & Ochman, 1998). The horizontal transfer of LuxI and LuxR family members is especially noteworthy, however, because these represent regulatory rather than structural genes, and it has been proposed that regulatory functions are not routinely acquired by means of horizontal transfer (Jain *et al.*, 1999). Our results therefore suggest that bacteria not only can gain new functional capabilities through horizontal gene transfer, but that they can gain new regulatory circuits and/or signalling abilities as well. As a specific example, *Escherichia coli* and *Salmonella typhimurium* may have the ability to respond to acyl homoserine lactone autoinducers generated by other organisms (Ahmer *et al.*, 1998; Sitnikov *et al.*, 1996), primarily because the ability to process that information through a LuxR homologue was itself acquired from other organisms. The functional organization of the *Pseudomonas aeruginosa* LasI/LasR and RhlI/RhlR quorum sensing systems provides a further example of the potential for acquired regulatory functions to be incorporated within existing regulatory pathways. These data therefore suggest that integrated, hierarchical signalling cascades can potentially arise through the sequential addition of independently acquired regulatory genes.

ACKNOWLEDGEMENTS

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