Acyl-homoserine lactone-based quorum sensing in the Roseobacter clade: complex cell-to-cell communication controls multiple physiologies

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INTRODUCTION

When acting as coordinated communities, bacterial populations are able to influence their local environment in manners that are unachievable by individual cells. It has been widely reported that phylogenetically diverse bacteria use genetic regulatory systems, known as quorum sensing (QS) systems, to coordinate gene expression in a population density dependent manner (e.g., Fuqua et al., 2001; Pappas et al., 2004; Case et al., 2008; Ng and Bassler, 2009). Among other things, QS is hypothesized to facilitate maximal access to available nutrients through the use of exoenzymes (Vetter et al., 1998; Schimel and Weintraub, 2003), the colonization of desirable niches (Nadell et al., 2008, 2009), and competitive advantages against other organisms (Folcher et al., 2001; Chin-a-Woeng et al., 2003; Barnard et al., 2007). The chemical mediators of QS are often small molecular weight diffusible molecules (Fuqua et al., 2001; Churchill and Chen, 2011). A well-characterized type of QS uses N-acyl-homoserine lactones (AHLs) and appears exclusive to Proteobacteria (Case et al., 2008). Canonical AHL-QS systems produce and respond to AHLs using two proteins that mediate signal production and response, LuxI and LuxR-like proteins, respectively (Nealson et al., 1970; Ruby, 1996). The genes encoding these two proteins are often located adjacent to one another on the chromosome (Fuqua et al., 1996; Churchill and Chen, 2011; Gelencsér et al., 2012). LuxI-like proteins synthesize AHLs by cyclizing S-adenosyl methionine into a lactone ring and the addition of an acylated carbon chain from fatty acid biosynthesis pathways (Schafer et al., 1996). Chain length and modification at the third carbon (either -H, -OH, or -O) allow for species or group specificity (Schaefer et al., 1996; Fuqua et al., 2001). LuxR-like proteins are response regulators that mediate the expression of genes required for communal behavior in response to intracellular concentrations of cognate AHLs (Fuqua and Winans, 1994; Fuqua et al., 1996). Activated LuxR proteins often upregulate luxI transcription to enhance the rate of AHL synthesis, increasing AHL concentrations, and also modulate the expression of other genes (Fuqua et al., 1996, 2001; Case et al., 2008).

AHL-based QS is common in Proteobacteria, which are abundant in coastal marine systems (Dang and Lovell, 2002; Waters and Bassler, 2005; Ng and Bassler, 2009). One of the most abundant and biogeochemically active groups of marine α-proteobacteria is the Roseobacter clade (Gonzalez and Moran, 1997; Buchan et al., 2005). Roseobacters can comprise up to 30% of the total 16S rRNA genes in coastal environments and up to 15% in the open ocean (Buchan et al., 2005; Wagner-Dobler and Bibel, 2006). In coastal salt marshes, roseobacters are the primary colonizers of surfaces and mediate a wide range of biogeochemically relevant processes, including mineralization of plant-derived compounds and transformations of reduced inorganic and organic sulfur compounds (Gonzalez and Moran, 1997; Dang and Lovell, 2000; Buchan et al., 2005; Dang et al., 2008). Here, we describe some of the most compelling recent research that focuses on QS in the Roseobacter clade, provide a genomic perspective of QS systems in roseobacters, and highlight areas for further investigation.

ROSEOBACTERs AND QUORUM SENSING

QS was first reported in roseobacters associated with marine snow and hypothesized to contribute to the ability of group members to colonize particulate matter in the ocean (Gram et al., 2002). Subsequent studies further demonstrated that roseobacters are prolific colonizers of a variety of marine surfaces, both
inert and living, and the contribution of QS to this ability and other physiological is of growing interest (Dang and Lovell, 2002; Berger et al., 2011; Zan et al., 2012). Characterized Roseobacter isolates produce diverse AHL structures with acyl chains ranging from eight to eighteen carbons in length that display varying degrees of saturation as well as all three possible oxidation states (-H, -OH, or -O) at the third carbon (for structures see Gram et al., 2002; Wagner-Dobler et al., 2005; Cicirelli et al., 2008; Mohamed et al., 2008; Thiel et al., 2009; Berger et al., 2011; Zan et al., 2012). The production of AHLS has been detected by LuxR-LacZ fusion bioreporters and mass spectrometry for several isolates (Gram et al., 2002; Wagner-Dobler et al., 2005; Martens et al., 2007; Thiel et al., 2009; Berger et al., 2011; Zan et al., 2012). Of the 43 publicly available Roseobacter genomes, only five lack annotated luxl homologs: Oceanicola butzensis HTCC2597, Oceanicola sp. S124, Pelagibacter bernudensis HTCC2601, Rhodobacteraceae bacterium HTCC2255, and Ruegeria sp. TM1040. All except HTCC2255, however, have luxR homologs (Table A2). Thus far, experimental studies of QS have primarily focused on isolated representatives of the Ruegeria-Phaeobacter branch of the Roseobacter clade, with the exception of the description of a diunsaturated long chain AHL produced by Jannaschia helgolandensis (Thiel et al., 2009), a survey of 31 AHL producing isolates (Wagner-Dobler et al., 2005), and a recent analysis of QS in Dinoroseobacter shibae, where QS was shown to control motility, expression of a type IV secretion system, and whether the cells divided by binary fission or budding (Patzelt et al., 2013).

Culture-based studies of bacterial symbionts of marine sponges suggest that roseobacters are the primary producers of AHLS in these systems (Taylor et al., 2004). A model for sponge-associated roseobacters has been established using Ruegeria sp. KLH11 (Zan et al., 2011). Studies with this strain have been informative in providing insight into the contributions of QS to host-bacterial interactions. KLH11 contains two sets of luxRI homologs, designated ssaRI (RKLH11_1559 and RKLH11_2275) and ssbRI (RKLH11_1933 and RKLH11_260), and a recently discovered orphan luxl, designated sscl, that is not annotated in the publically available KLH11 genome. While orphan luxl have not been widely described in the literature, they are best described as luxl homologs that are not immediately adjacent to a corresponding luxR homolog on the chromosome. It has been proposed that sscl is a recent duplication of ssbl (Zan et al., 2012). Heterologous expression of SsaI, SsbI, and SscI in Escherichia coli showed that they predominantly produce long chain saturated and unsaturated AHLS (C12-16). More specifically, SsaI produces 3O-AHLS whereas SsbI and SscI produce 3OH-AHLS (Zan et al., 2012). The modification at the third carbon has been shown to affect the binding affinity of signaling molecules to LuxR homologs, and may allow KLH11 to finely tune its metabolism to cellular density and AHL diversity (Koch et al., 2005). KLH11 mutants deficient in QS display impaired motility, which corresponds to decreased transcription of genes encoding flagella biosynthesis machinery. The QS and motility impaired mutants form drastically thicker biofilms, suggesting when motility or QS is retarded, biofilm formation is increased (Zan et al., 2012). This may also suggest that biofilm formation may not be directly controlled by QS, but that when quorum is achieved, motility and biofilm dispersion are induced. Recent work has shown a phosphorelay system that controls motility in KLH11 is induced by QS (Zan et al., 2013). A similar phenotype has been observed in other roseobacters, and this trend may extend across the Ruegeria-Phaeobacter subgroup (Bruhn et al., 2006; Dobretsov et al., 2007).

QS-mediated physiologies have been implicated in one of the few examples of roseobacters demonstrating antagonistic behavior toward a eukaryotic host. Nautilia (formerly Ruegeria) sp. R11 readily colonizes the macroalga Delisea pulchra resulting in bleaching and subsequent death (Case et al., 2011; Fernandes et al., 2011). To combat infection, D. pulchra produces halogenated furanones, which have been shown to block AHL-based QS systems in many bacterial species. Active synthesis of furanones prevents macroalgal colonization by epiphytic bacteria, including Nautilia sp. R11. However, in the absence of halogen substrates required for furanone biosynthesis, colonization occurs rapidly (Manefield et al., 1999; Hentzer et al., 2002; Defoirdt et al., 2007). Further, it appears furanones may be effective against other potentially pathogenic Ruegeria spp. (Zhong et al., 2003).

QS is closely connected to antimicrobial production in several roseobacters. In Phaeobacter sp. strain Y4I, the regulatory controls dictating the production of the antimicrobial compound indigoidine are complex and include QS. Indigoidine production confers a competitive advantage to Y4I when grown in co-culture with Vibrio fischeri. Transposon insertions in either of two separate luxRI-like systems leads to an inability of Y4I mutants to produce wildtype levels of indigoidine and an inability to inhibit the growth of V. fischeri. This indicates a role for both QS systems in the synthesis of indigoidine (Cude et al., 2012). The presence of multiple QS systems in the genomes of many roseobacters suggests multi-layered control is a common feature to regulate energy intensive processes, including secondary metabolite production.

Tropodithietic acid (TDA) is a broad spectrum antimicrobial produced by multiple roseobacters in response to QS (Bruhn et al., 2005; Porsby et al., 2008; Berger et al., 2011). Genome analyses of Phaeobacter gallaeciensis strains isolated from geographically distant locations suggest they are capable of producing both AHLS and TDA (Thole et al., 2012). P. gallaeciensis 2.10 has been suggested to produce TDA in response to AHLS while colonizing the marine alga Ulva australis, thus protecting the alga from bacterial, fungal, and larval pathogens (Rao et al., 2007). A closely related strain, P. gallaeciensis DSM17395, which has also been shown to colonize U. australis (Thole et al., 2012), produces N-3-hydroxydecanoyl-homoserine lactone (3OHC10-HSL) using the Luxl homolog Pgal. 3OHC10-HSL activates the adjacent regulator, PgaR, in a concentration dependent manner, which leads to the upregulation of a TDA biosynthetic operon (Berger et al., 2011). Interestingly, in a Δpgal strain of DSM17395, addition of exogenous TDA is sufficient to upregulate TDA biosynthesis machinery, suggesting that regulation of TDA biosynthesis may involve multiple signals in some strains (Berger et al., 2011). The dual role of TDA as an autoinducer and an antimicrobial has also
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Quorum sensing in Roseobacters

FIGURE 1 | Continued
been demonstrated in Ruegeria sp. TM1040, which lacks AHL-based QS (Geng and Belas, 2010). Collectively, these data show that in addition to AHLs, roseobacters use novel autoinducers. In fact, recent investigations into novel non-fatty acyl-HSLs have shown that at least one Ruegeria, Ruegeria pomeroyi DSS-3, is capable of producing p-coumaroyl-homoserine lactone when grown in the presence of the aromatic lignin breakdown product p-coumaric acid (Schafer et al., 2008). This discovery raises the possibility that many novel signaling molecules could be produced by roseobacters in response to available local substrates, specifically plant-derived aromatics which are primary growth substrates for roseobacters (Buchan et al., 2000; Gulvik and Buchan, 2013). The production of specific signaling molecules in response to exogenously supplied substrates suggest a single signal interconverts unsaturated crotonyl-CoA to saturated butyryl-CoA as a precursor to fatty acid biosynthesis (Wallace et al., 1995). The helicase may be involved in DNA repair, protein degradation, or gene regulation (Snider et al., 2008). The B1 subgroup is the most abundant orientation within the B group, and contains a short-chain dehydrogenase following the helicase. This gene orientation is conserved in 14 Roseobacter genomes. Short-chain dehydrogenases are a large family of proteins that modify carbon chains of many substrates (Joerovall et al., 1995). The protein encoded by this gene may function to modify AHL biosynthesis substrates before or after AHL production.

Variations of the D topology are found in six Roseobacter genomes, all belonging to members of the Roseobacter subclade 4 (Figures 1A,B). These LuxI and LuxR proteins share >52 and >64% sequence similarity, respectively. The LuxI and LuxR of the D topology have been designated I₅ and R₅ (Figure 2). This topology shares two genes in common between the variations, fliG in the opposite orientation upstream of luxRI and an adenylsuccinate lyase encoding gene downstream. In E. coli, FliG is the flagellar motor switch that controls the spin direction of flagella (Roman et al., 1993). The characterized role of QS and motility in roseobacters was addressed previously (Zan et al., 2012), but none of the organisms containing the D topology have been investigated with respect to QS. The direct connection between QS and flagellar machinery may be an interesting avenue for future investigation. The other gene in this orientation putatively encodes an adenylsuccinate lyase, which is important in the de novo purine biosynthetic pathway and in controlling the levels of AMP and fumarate inside the cell (Tsai et al., 2007), suggesting purine biosynthesis may respond to QS.
The presence of orphan *luxI* genes appears common, especially in the *Sulfotobacter*, *Ruegeria*, and *Phaeobacter* genera (Table A1). The synteny of these *luxI* and their adjacent genes is conserved in the H, I, and J topologies. In organisms that have these three orientations, there is a *luxI*-like gene of the Iδ. The LuxI of these topologies share >52% sequence similarity. Shared among the H, I, and J topologies are different types of putative histidine kinase (HK) encoding genes upstream of the orphan *luxI*, suggesting the protein is part of a two-component phosphorelay (Dutta et al., 1999; Stock et al., 2000). These genes are in the same direction as the *luxI* in H and I and in the opposite in J (Figure 2). In *Vibrio harveyi*, the hybrid two-component HK LuxN has been shown to activate gene circuits that lead to coordinated behaviors, such as bioluminescence, in response to AHLs (Freeman and Bassler, 1999; Laub and Goulian, 2007). The HKs found these topologies share modest identity with the *Vibrio harveyi* LuxN (≤26%) suggesting similar regulatory systems may be present in roseobacters. While the similarity of gene sequence does not directly predict regulatory cascades or phenotypes, the development of model systems for each of these topologies will prove valuable for comparative studies across lineage members.

**FUTURE DIRECTIONS**

The repertoire of chemical signals in roseobacters is anticipated to be large and result in complex chemical signaling pathways in lineage members, some of which may contribute to interspecies interactions and should be investigated further. For example, uncharacterized roseobacters have been shown to be epibionts of the abundant cyanobacterial lineage *Trichodesmium*. While AHL-based interactions between *Trichodesmium* and select epibionts have been shown to stimulate mechanisms for phosphorus acquisition in this host (Hmelo et al., 2012; Van Mooy et al., 2012), a definitive role for roseobacters in this symbiosis has not yet been demonstrated. Similarly, it has been hypothesized that QS plays a role in the switch from mutualistic to antagonistic behavior proposed for *P. gallaeciensis* in its interactions with the phytoplankter *Emiliana huxleyi* (Seyedsayamdost et al., 2011). Finally, the relationships roseobacters have with vascular plants as they colonize plant material and transform plant-derived compounds.
(Buchan et al., 2000; Dang and Lovell, 2000; Buchan et al., 2001) is suggestive of inter-kingdom communication, such as that found in other α-proteobacteria [e.g., Agrobacterium tumefaciens and Sinorhizobium meliloti (Hughes and Sperando, 2008)]. Research in these areas would help elucidate the role of QS in the ability of roseobacters to colonize and interact with a diverse group of organisms.

The presence of orphan luxR-like genes in Proteobacterial genomes has been widely described, and their gene products have been shown to respond to AHLs and other molecules produced by other QS systems in the same organism or by other organisms (Malott et al., 2009; Patankar and González, 2009; Sabag-Daigle et al., 2012). Furthermore, it is possible that these LuxR family proteins bind structurally similar molecules that are not related to QS. In fact, it has been shown that cross-domain signaling can be mediated through LuxR homologs that bind non-AHL eukaryotic molecules (Subramoni and Venturi, 2009). In contrast, detailed studies of orphan lux-like gene products are rare and are an area ripe for study. Perhaps either novel non-LuxR-like proteins or proteins encoded by genes located in distal regions of the genome (Table A2) respond to the orphan LuxI-derived AHLs. Undoubtedly, more detailed characterization of such systems will lead to a better understanding of their biological roles in roseobacters as well as other lineages.

To date, experimental studies of QS in relatively few select roseobacters have revealed complex and multi-layered control mechanisms as well as novel signaling molecules. In addition to expanding our knowledge of these characterized systems, it is our hope that future studies also broaden our understanding of currently under investigated systems within the clade and their contribution to complex multi-species interactions.

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APPENDIX

Maximum likelihood phylogenetic trees of LuxI-like and genetically linked LuxR-like sequences from 38 published roseobacter genomes were constructed. Protein alignments of the LuxI and LuxR homologs were done using the MUSCLE algorithm with default parameters (Edgar, 2004), and manually curated. The phylogenetic trees were generated using MEGA 5.2 following published methods (Hall, 2013). The Maximum Likelihood statistical method was used with the WAG model of amino acid substitution and gamma distribution with invariant sites (G+I) selected. Gaps were handled with a 95% partial deletion data treatment, and the phylogeny was tested with 1000 bootstrap replications (Tamura et al., 2011). Bootstrap values are reported in percentages and shown at nodes where values are >50%. Groups were divided and defined by natural divisions in the trees and gene topology in the genome (Figure 2). The LuxRI protein sequences of Vibrio fischeri (Accession: AAQ90231.1 and AAP22376.1) were used to root the trees. LuxR and LuxI homologs of six proteobacterial species with sequence similarity to at least one roseobacter sequence in each subgroup (>30% identity) were included in the alignments to assess the validity of the groupings. The non-roseobacter LuxRI included were: Sinorhizobium meliloti (Accession: ABC88593.1 and CAC46417.1), Bradyrhizobium elkanii (Accession: WP_018273827.1 and WP_018272735), Rhizobium leguminosarum (Accession: YP_002281222.1 and CAD20929.1), Agrobacterium tumefaciens (Accession: WP_003501811.1 and AAZ50597.1), Pseudomonas putida (Accession: CAO85746.1 and CAO85747), Pseudomonas aeruginosa (CAO85753.1 and CAO85754.1).

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Table A1 | Paired LuxRI and orphan LuxI homologs identified in the 38 sequenced roseobacters.

| Strains | Gene orientation | luxR gene locus | luxI gene locus |
|---------|------------------|----------------|----------------|
| Rhodobacterales bacterium HTCC2083 | A | RB2083_3272 | RB2083_3256 |
| Ruegeria sp. KLH11 | A | RKLH11_1559 | RKLH11_2275 |
| Roseovarius sp. 217 | A | ROS217_18272 | ROS217_18267 |
| Roseovarius sp. TM1035 | A | RTM1035_10475 | RTM1035_10485 |
| Roseovarius lacuscaerulensis ITI-1157 | A | SL1157_2477 | SL1157_2476 |
| Roseovarius sp. DSS-3 | A | SPO2286 | SPO2287 |
| Roseovarius sp. TV15 | A | RTV15_010100013877 | RTV15_010100013872 |
| Citreicella sp. 357 | B | C357_10197 | C357_10192 |
| Citreicella SE45 | B | CSE45_4055 | CSE45_4054 |
| Roseobacter denitrificans OCh 114 | B | RD1_1638 | RD1_1639 |
| Sagittula stellata E-37 | B | SSE37_11169 | SSE37_11164 |
| Ruegeria sp. KLH11 | B | RKLH11_1933 | RKLH11_260 |
| Dinoroseobacter shibae DFL 12 | B1 | DSHI_2852 | DSHI_2851 |
| Loktanella sp. SE62 | B1 | LSE62_0618 | LSE62_0617 |
| Phaeobacter galleaeciensis 2.10 | B1 | PGA2_c03430 | PGA2_c03440 |
| Phaeobacter galleaeciensis DSM 17395 | B1 | PGA1_c03880 | PGA1_c03890 |
| Phaeobacter galleaeciensis ANG1 | B1 | ANG1_1316 | ANG1_1315 |
| Phaeobacter sp. Y41 | B1 | RBY41_1689 | RBY41_3631 |
| Ruegeria sp. KLH11 | B1 | RKLH11_1933 | RKLH11_260 |
| Rhodobacterales bacterium HTCC2150 | B1 | RB2150_14426 | RB2150_14421 |
| Roseobacter sp. AzwK-3b | B1 | RAZWK3B_04270 | RAZWK3B_04275 |
| Roseobacter sp. GAI101 | B1 | RGA101_376 | RGA101_3396 |
| Roseobacter sp. MED193 | B1 | MED193_10428 | MED193_10423 |
| Roseovarius lacuscaerulensis ITI-1157 | B1 | SL1157_0613 | SL1157_0612 |
| Ruegeria sp. R11 | B1 | RR11_2850 | RR11_2620 |
| Ruegeria sp. TV15 | B1 | RTV15_010100017779 | RTV15_010100017784 |
| Roseobacter sp. R2A57 | B2 | R2A57_2403 | R2A57_2404 |
| Thalassiobium R2A620 | B2 | TR2A62_3165 | TR2A62_3166 |
| Maritimibacter alkaliphilus HTCC2654 | B3 | RB2654_09024 | RB2654_09014 |
| Rhodobacterales bacterium HTCC2083 | B4 | RB2083_3265 | RB2083_730 |
| Roseobacter litoralis Och 149 | B4 | RLO149_c030690 | RLO149_c030680 |
| Dinoroseobacter shibae DFL 12 | C | DSHI_0312 | DSHI_0312 |
| Jannaschia sp. CCS1 | C | JANN_0619 | JANN_0620 |
| Loktanella vestfoldensis SKA53 | D | SKA53_05835 | SKA53_05830 |
| Loktanella sp. SE62 | D1 | LSE62_3230 | LSE62_3231 |
| Oceanicola granulosus HTCC2516 | D1 | OG2516_02284 | OG2516_02294 |
| Octadecabacter antarcticus 307 | D1 | QA307_2044 | QA307_4586 |
| Roseobacter sp. CCS2 | D1 | RCCS2_02083 | RCCS2_02078 |
| Octadecabacter arcticus 238 | D2 | QA238_4151 | QA238_2886 |
| Roseobacter sp. SK209-2-6 | E | RSK20926_22079 | RSK20926_22084 |

(Continued)
Table A1 | Continued

| Strains                  | Gene orientation | luxR gene locus   | luxI gene locus   |
|--------------------------|------------------|-------------------|-------------------|
| Sulfitobacter NAS-14.1   | E                | NAS141_01141      | NAS141_01136      |
| Maritimibacter alkaliphilus HTCC2654 | F | RB2654_20053 | RB2654_20048 |
| Roseovarius sp. 217      | G                | ROS217_01405      | ROS217_01410      |
| Roseobacter litoralis Och 149 | G1 | RLO149_c036220 | RLO149_c038210 |
| Sulfitobacter NAS-14.1   | H                | NAS141_00695      |                   |
| Sulfitobacter sp. EE-36  | H                | EE36_01635        |                   |
| Roseovarius nubinhibens ISM | I      | ISM_03755        |                   |
| Oceanibulbus indolifex HEL45 | I | OIHEL45_00965 |                   |
| Ruegeria sp. R11         | J                | RR11_2017         |                   |
| Roseobacter sp. MED193   | J                | MED193_08053      |                   |
| Ruegeria sp. TV15        | J                | RTW15_00100005486|                   |
| Dinoroseobacter shibae DFL 12 | K | DSHL_4152       |                   |
| Phaeobacter gallaeciensis 2.10 | L | PGA2_c18970 | PGA2_c18960 |
| Phaeobacter sp. Y4I      | L1               | RBY4I_1027        | RBY4I_3464        |
| Phaeobacter gallaeciensis 2.10 | M | PGA2_c07460 |                   |
| Phaeobacter gallaeciensis DSM 17395 | M | PGA1_c07680 |                   |
| Rhodobacterales bacterium HTCC2150 | N | RB2150_11281 | RB2150_11291 |
| Roseobacter litoralis Och 149 | O | RLO149_c036590 |                   |
| Roseobacter sp. AzwK-3b  | P                | RAZWK3B_19371     |                   |
| Roseobacter sp. SK209-2-6 | Q          | RSK20926_15126    | RSK20926_15131    |
| Roseobacter sp. GAI101   | Q1               | RGA101_1101       |                   |
| Ruegeria lacuscaerulensis ITI-1157 | R | SL1157_1706 |                   |
| Ruegeria sp. TrichCH4B   | S                | SCH4B_1938        |                   |

Homologs of LuxI encoding genes were determined using BlastP to characterized proteins (E-value < e^−3) on Roseobase (www.roseobase.org) and are consistent with the genome annotations. The LuxR gene loci listed do not represent all homologs within the genomes, but were determined based using BlastP with the autoinducer binding domain sequence from Pfam (PF03472) on Roseobase, and proximity to luxI homologs. These were also consistent with genome annotations. Gene orientations are represented in Figure 2.

*Orphan luxI homologs are defined as those that do not have an immediately adjacent luxR gene. All reported orphan luxI genes are located and at least 100 kb from the end of the draft genome contig.

*Vibrio fischeri LuxI (AAP22378), Agrobacterium tumefaciens TraR (AAZ50697) and Phaeobacter gallaeciensis PgaI (YP_006571842).*
**Table A2 | Putative orphan LuxR encoding genes that do not have an adjacent luxI on the chromosome.**

| Strains                       | luxR gene locus          |
|-------------------------------|--------------------------|
| Citreicella sp. 357           | C357_03001               |
| Citreicella sp. SE45          | CSE45_1818, CSE45_4969   |
| Dinoroseobacter shibae DFL 12| Dshi_1560, Dshi_1819     |
| Jannaschia sp. CCS1           | Jann_1153, Jann_2301, Jann_3193 |
| Loktanella sp. SE62           | LSE62_3779               |
| Martimibacter alkaliphilus HTCC2654 | RB2654_10983, RB2654_03619 |
| Oceanibulbus indolifex HEL-45 | OIHEL45_01695, OIHEL45_02625, OIHEL45_13145 |
| Oceanicola batsensis HTCC2597 | OB2597_03302             |
| Oceanicola granulosus HTCC2516| OG2516_08027             |
| Oceanicola sp. S124           | OS124_01010007942, OS124_01010007975 |
| Octadecabacter antarcticus 238| OA238_3367, OA238_3623   |
| Octadecabacter antarcticus 307| OA307_2044               |
| Pelagibaca bermudensis HTCC2601| R2601_24964, R2601_10664 |
| Phaeobacter galleciensis 2.10 | PGA2_c15480, PGA2_c18970 |
| Phaeobacter galleciensis DSM 17395 | PGA1_c15590             |
| Phaeobacter galleciensis ANG1 | ANG1_869                 |
| Phaeobacter sp. Y41           | RBY41_896                |
| Rhodobacterales bacterium HTCC2083 | RB2083_1776           |
| Rhodobacterales bacterium HTCC2150 | RB2150_02239            |
| Roseobacter denitrificans Och 114 | RD1_3967               |
| Roseobacter litoralis Och 149 | RLO149_c004710, RLO149_c036470 |
| Roseobacter sp. AzwK-3b       | RAZWK3B_15865           |
| Roseobacter sp. CC52          | RCCS2_00422              |
| Roseobacter sp. GAI101        | RGA101_670               |
| Roseobacter sp. MED193        | MED193_03932             |
| Roseobacter sp. R2A57         | R2A57_3570               |
| Roseobacter sp. SK209-2-6     | RSK20926_03972, RSK20926_18892 |
| Roseovarius nubinhibens ISM  | ISM_09921, ISM_15660    |
| Roseovarius sp. TM1035        | RTM1035_08219            |
| Roseovarius sp. 217           | ROS217_20327             |
| Ruegeria pomeroyi DSS-3       | SPO197_4                 |
| Ruegeria sp. KLH11            | RKLH11_1390              |
| Ruegeria sp. R11              | RR11_2316                |
| Ruegeria sp. TM1040           | TM1040_3102, TM1040_1212 |

(Continued)

**Table A2 | Continued**

| Strains                       | luxR gene locus          |
|-------------------------------|--------------------------|
| Ruegeria sp. TrichCH4B        | SCH4B_0463, SCH4B_4179, SCH4B_4368, SCH4B_4682 |
| Ruegeria lacuscaerulensis ITI-1157 | SL1157_2844           |
| Sagittula stellata E-37      | SSE37_06082              |
| Sulfitobacter sp. EE-36       | EE36_03628               |
| Sulfitobacter sp. NAS-14.1    | NAS141_08556             |
| Thalassibium sp. R2A62        | TR2A62_0664              |

Homologs of LuxR encoding genes were determined using BlastP with the autoinducer binding domain sequence from Pfam (PF03472) on Roseobase (www.roseobase.org).