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LuxR- and LuxI-Type Quorum-Sensing Circuits Are Prevalent in Members of the *Populus deltoides* Microbiome

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We are interested in the root microbiome of the fast-growing Eastern cottonwood tree, *Populus deltoides*. There is a large bank of bacterial isolates from *P. deltoides*, and there are 44 draft genomes of bacterial endophyte and rhizosphere isolates. As a first step in efforts to understand the roles of bacterial communication and plant-bacterial signaling in *P. deltoides*, we focused on the prevalence of acyl-homoserine lactone (AHL) quorum-sensing-signal production and reception in members of the *P. deltoides* microbiome. We screened 129 bacterial isolates for AHL production using a broad-spectrum bioassay that responds to many but not all AHLs, and we queried the available genome sequences of microbiome isolates for homologs of AHL synthase and receptor genes. AHL signal production was detected in 40% of 129 strains tested. Positive isolates included members of the *Alpha-*, *Beta-*, and *Gammaproteobacteria*. Members of the *luxI* family of AHL synthases were identified in 18 of 39 proteobacterial genomes, including genomes of some isolates that tested negative in the bioassay. Members of the *luxR* family of transcription factors, which includes AHL-responsive factors, were more abundant than *luxI* homologs. There were 72 in the 39 proteobacterial genomes. Some of the *luxR* homologs appear to be members of a subfamily of LuxRs that respond to as-yet-unknown plant signals rather than bacterial AHLs. Apparently, there is a substantial capacity for AHL cell-to-cell communication in proteobacteria of the *P. deltoides* microbiota, and there are also *Proteobacteria* with LuxR homologs of the type hypothesized to respond to plant signals or cues.

Many *Proteobacteria* use acyl-homoserine lactone (AHL) quorum-sensing (QS) signals for cell density-dependent gene regulation. Generally, AHL QS involves two regulatory genes, a member of the *luxI* family of AHL synthase genes and a member of the *luxR* family of AHL-responsive transcriptional regulatory genes. The *luxR-luxI* regulatory circuit in the marine bacterium *Vibrio fischeri* uses the freely diffusible *N*-3-oxohexanoyl-homoserine lactone ($3OC_6$-HSL) as a proxy for cell density to activate luminescence (*lux*) gene expression (1). Dozens of species of *Proteobacteria* possess homologs of *luxI* and *luxR* systems (2). The *luxI* homologs code for enzymes that collectively generate a variety of AHLs. Most of the described AHLs are fatty acyl AHLs with acyl groups of various lengths (C_4 to C_{18}). These AHLs possess either a hydroxyl, a carbonyl, or no substitution on the third carbon and can vary in the degree of side chain saturation (3, 4). More recently, LuxI homologs have been identified that synthesize aromatic (5, 6) and branched-chain AHLs (7). The *luxR* and *luxI* homologs are often but not always tightly linked, and AHL-responsive transcription factors show the greatest sensitivity to the signal produced by their cognate AHL synthase. Bacteria can contain multiple *luxI-luxR* homologs. For example, *Pseudomonas aeruginosa* possesses two AHL QS systems, the LasI-LasR system and the RhlI-RhlR system (8–10). *Proteobacteria* genomes can also possess orphan or solo *luxR* homologs. These are *luxR* homologs without a paired *luxI* homolog (2, 11). In fact, some bacteria have *luxR* homologs and do not have any *luxI* homologs. For example, *Escherichia coli* and *Salmonella enterica* serovar Typhimurium possess a *luxR* homolog called *sdiA*, which codes for a transcription factor responsive to AHLs produced by other bacteria (12). Some *luxR* homologs in plant-associated bacteria code for transcription factors that respond to plant-derived signals of unknown composition rather than AHLs (reviewed in reference 13).

Recently, we have undertaken a microbiome study in an effort to better understand the dynamic interface that exists between plant microbes and their hosts by using the woody perennial *Populus* as a model (14). *Populus* has intimate associations with arbuscular mycorrhizal and ectomycorrhizal fungi (15), as well as endophytic and rhizosphere bacteria (14, 16). It was the first tree to have its genome fully sequenced (17), and it has potential as a biofuel feedstock for cellulosic ethanol production (18). Because we know that AHL quorum sensing occurs in many plant pathogens, such as *Agrobacterium tumefaciens* (19), *Pantoea carotovora* (20), and *Pseudomonas syringae* (21), and in beneficial plant bacteria, such as *Bradyrhizobium japonicum* (7) and *Rhizobium leguminosarum* (22, 23), we were interested in assessing the prevalence of AHL QS systems and orphan *luxR* genes among members of the *Populus deltoides* (Eastern cottonwood) microbiota. rRNA sequence analysis revealed distinct bacterial endophyte and rhizosphere *P. deltoides* root microbiota (14). The rhizosphere is dominated by *Acidobacteria* (31%) and *Alphaproteobacteria* (30%), and endophytes are mainly *Gammaproteobacteria* (54%) and *Alphaproteobacteria* (23%) (14). Interestingly, nearly 35% of the endophytic bacterial sequences were comprised of a single *Pseudomonas fluorescens*-like operational taxonomic unit (OTU) (14). Approximately 1,100 bacteria have been isolated from *P. deltoides* tree roots collected from sites in Tennessee and North Carolina

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(24). Furthermore, draft genome sequences are available for over 40 different isolates, of which about half are members of the genus *Pseudomonas* (24, 25).

The rhizosphere and endophyte strain collection and available genomic sequence data (24, 25) have allowed us to take a census, or inventory, of the prevalence of AHL production and *luxI* and *luxR* homologs in members of the *P. deltoides* microbiome. We view this as a first step toward understanding the roles these factors play in the biology of this tree. Here, we describe a survey of 129 *P. deltoides* bacterial isolates for AHL production and an informatics investigation of the draft genomes of 44 isolates for *luxI* and *luxR* homologs. Our results suggest that AHL signaling is prevalent among members of the *P. deltoides* rhizosphere and endophyte microbiota, that solo or orphan *luxR* homologs are even more prevalent than complete AHL circuits, and that homologs of the putative plant signal-responsive LuxR enzymes exist in some members of the *P. deltoides* microbiota.

MATERIALS AND METHODS

Bacteria. Over 1,100 bacterial isolates from *P. deltoides* root samples taken near either the Caney Fork River (isolates with AP, GM, BT, or CF strain designations) in central Tennessee or the Yadkin River (isolates with YR strain designations) in North Carolina were available for our study (14, 26). Rhizosphere isolates were obtained as described elsewhere (14, 25). Isolates designated endophytes were obtained as follows: roots were surface sterilized via serial washes in sterile water, 3% H_2O_2, 6.15% NaOCl as described previously (14), and surface sterility was confirmed by touching the washed roots to LB plates and observing a lack of microbial growth. Fine roots were pulverized with a sterile mortar and pestle in 10 ml of $MgSO_4$ (10 mM) (14). The large debris was allowed to settle, and 100-μl samples were plated on R2A agar plates (Difco), which were incubated at 25°C. Colonies arising after several days of incubation were picked and restreaked a minimum of three times on R2A agar at 25°C. The isolation of *Rhizobium* sp. strain PDO1-076 has been described elsewhere (25).

Screening selected isolates for AHL production. We assessed the ability of 129 isolates representing several genera of *Proteobacteria* to produce fatty AHLs as follows: isolates were grown in 5 ml of buffered tryptone yeast extract broth [0.5% tryptone, 0.3% yeast extract, 0.13% $CaCl_2$, and 50 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS), pH 7] or DM medium [50 mM potassium phosphate, 0.1% $(NH_4)_2SO_4$, 0.2% NaCl, 7 mg/ml $MnSO_4{\cdot}4H_2O$, 0.02% $MgSO_4{\cdot}7H_2O$, 8 mg/ml $CaCl_2{\cdot}2H_2O$, 0.07% L-arginine, 0.01% L-glutamine, 1 mg/ml biotin, 1 mg/ml thiamine, 20% glucose] broth with shaking at 30°C. Cells were pelleted by centrifugation, and the culture supernatant fluid was extracted twice with equal volumes of acidified ethyl acetate and concentrated as described previously (27). Extracts were screened for AHL activity by using the *Agrobacterium tumefaciens* KYC55 (pJZ372, pJZ384, and pJZ410) bioassay method (28).

LuxI and LuxR phylogenies. We queried the 44 *P. deltoides* draft bacterial genome sequences for genes encoding LuxR or LuxI homologs by using the Integrated Microbial Genomes Database (http://img.jgi.doe.gov/cgi-bin/w/main.cgi). We aligned homologs by using Clustal W (http://www.genome.jp/tools/clustalw/). Phylogenetic trees were constructed by using the Jones-Taylor-Thornton protein distance algorithm, followed by the distance matrix-based Fitch algorithm in PHYLIP (http://evolution.genetics.washington.edu/phylip.html), and visualized with Phylodendron Treeprint (http://www.es.embnet.org/Doc/phylodendron/treeprint-form.html).

RESULTS AND DISCUSSION

Production of AHLs by selected *P. deltoides* rhizosphere and endophytic *Proteobacteria*. As assessed by 16S rRNA gene sequence relationships, the collection of *P. deltoides* rhizosphere and endophytic isolates represented 85 bacterial genera. The most-abundant taxa were *Actinobacterium*, 14%, *Bacillus*, 17%, *Alphaproteobacteria*, 22%, *Betaproteobacteria*, 16%, and *Gammaproteobacteria*, 22% (24). We limited our screen to *Proteobacteria* because AHL QS is believed to be limited to this taxon. We screened 44 rhizosphere isolates and 85 endophytes from roots of mature *P. deltoides* trees for the production of AHLs that can be detected by a broad-range bioassay that responds to AHLs with fatty acyl chains ranging in length between 4 and 18 carbons (see Materials and Methods; also see references 28 and 29). The bioassay can also detect AHLs with the common substitutions that occur on carbon 3. The assay shows various sensitivities to different AHLs. Therefore, the strength of a response does not necessarily correlate directly with the level of an AHL. There will be a strong response to relatively low levels of the cognate AHL, *N*-3-oxooctanoyl-homoserine lactone ($3OC_8$-HSL) and a weak response to much higher concentrations of other AHLs, like *N*-butyryl-homoserine lactone (C_4-HSL). Furthermore, some AHLs are not detected at all by this assay, the aryl-AHLs being a case in point (5). Given these limitations, we nevertheless found that 51 isolates (40%) produced detectable AHLs. The production of AHLs was most prevalent in the *Alphaproteobacteria* (80% produced AHLs), whereas AHL production was detected for 20% of *Betaproteobacteria* and 19% of the *Gammaproteobacteria* (Table 1; also see Table S1 in the supplemental material). Among the endophytes, 34% produced detectable AHLs, and we detected AHL production by 50% of the rhizosphere isolates screened (see Table S1).

TABLE 1 Census of AHL-producing isolates and isolates with *luxI* and *luxR* homologs

Taxon	% of isolates (no. examined[a]) positive for AHL production[b]	% of genome sequences (no. examined[a]) positive for:	
		luxI homolog[c]	*luxR* homolog[c]
Alphaproteobacteria	80 (44)	100 (10)	100 (10)
Betaproteobacteria	20 (5)	17 (5)	60 (5)
Gammaproteobacteria	19 (80)	21 (24)	96 (24)

[a] A total of 129 isolates were screened for AHL production, and 44 draft genomes were analyzed.
[b] Determined as AHL activity in the *Agrobacterium* bioassay.
[c] As defined by COG3916 for *luxI* homologs and pfam03472 for the AHL-binding domain of *luxR* homologs in the IMG JGI database (http://img.jgi.doe.gov/cgi-bin/w/main.cgi).

As efforts to understand the role of the microbiome in the biology of *P. deltoides* continue, it is useful to obtain a sense of the potential for bacterial communication and signaling or cuing in this cottonwood host. We have learned that *Proteobacteria* are prevalent components of the microbiome (14) and that many genera of *Proteobacteria* utilize AHL quorum-sensing systems to control gene expression (3, 30). Furthermore, AHL quorum sensing has been shown to occur within members of many of the taxa represented in the collection of *P. deltoides* rhizosphere and endophyte isolates. Our analysis demonstrates that there is significant potential for AHL signaling in both the rhizosphere and endophytic environment of mature *P. deltoides* trees, as close to 50% of the isolates we screened produce AHLs (Table 1). Previous metagenomic analyses have revealed that there is capacity for AHL quorum sensing in soil, sewage sludge, the endophyte community of rice, and elsewhere (31–33). Furthermore, screens for AHL pro-

TABLE 2 Inventory of *luxI* and *luxR* homologs in draft genomes of *P. deltoides* rhizosphere and endosphere bacterial isolates

Proteobacteria taxon, bacterial genus[a] and isolate identifier	Origin of isolate	No. of:		Produces AHL[b]
		luxI-luxR homolog pairs	Orphan *luxR* homologs	
Alphaproteobacteria				
Bradyrhizobium, YR681	Endosphere	1	0	ND
Caulobacter, AP07	Rhizosphere	1	0	ND
Novosphingobium, AP12	Rhizosphere	1	0	Yes
Phyllobacterium, YR531	Endosphere	1	2	Yes
Rhizobium, AP16	Endosphere	1	4	Yes
Rhizobium, CF080	Endosphere	1	3	Yes
Rhizobium, CF122	Endosphere	1	6	ND
Rhizobium, CF142	Endosphere	2	6	Yes
Rhizobium, PD01-76	Rhizosphere	1	5	Yes
Sphingobium, AP49	Endosphere	1	1	Yes
Betaproteobacteria				
Acidovorax, CF316	Endosphere	0	2	ND
Burkholderia, BT03	Endosphere	1	2	Yes
Herbaspirillum, CF444	Endosphere	0	0	ND
Herbaspirillum, YR522	Endosphere	0	0	ND
Variovorax, CF313	Endosphere	0	1	ND
Gammaproteobacteria				
Pantoea, GM01	Rhizosphere	1	0	ND
Pantoea, YR343	Rhizosphere	1	0	ND
Polaromonas, CF318	Endosphere	0	0	ND
Pseudomonas, GM16	Endosphere	0	1	ND
Pseudomonas, GM17	Endosphere	2	0	Yes
Pseudomonas, GM18	Endosphere	1	1	Yes
Pseudomonas, GM21	Endosphere	0	1	ND
Pseudomonas, GM24	Endosphere	0	1	ND
Pseudomonas, GM25	Endosphere	0	2	ND
Pseudomonas, GM30	Endosphere	1	1	Yes
Pseudomonas, GM33	Endosphere	0	1	ND
Pseudomonas, GM41	Endosphere	0	1	ND
Pseudomonas, GM48	Endosphere	0	1	ND
Pseudomonas, GM49	Rhizosphere	0	1	ND
Pseudomonas, GM50	Endosphere	0	1	ND
Pseudomonas, GM55	Endosphere	0	1	ND
Pseudomonas, GM60	Endosphere	0	1	ND
Pseudomonas, GM67	Endosphere	0	1	ND
Pseudomonas, GM74	Rhizosphere	0	1	ND
Pseudomonas, GM78	Endosphere	0	1	ND
Pseudomonas, GM79	Endosphere	0	2	ND
Pseudomonas, GM80	Endosphere	0	1	ND
Pseudomonas, GM84	Rhizosphere	0	1	ND
Pseudomonas, GM102	Endosphere	0	1	ND

[a] Bacterial genus designations are derived from analysis of 16S rRNA gene sequence information (24, 25).
[b] As detected using the *Agrobacterium* bioassay described in Materials and Methods. ND, not detected.

duction by *Proteobacteria* isolated from rice and oat rhizospheres are similar to the results we report for the Eastern cottonwood isolates. Thirty-five percent of rice rhizosphere isolates (33) and 23% of wild oat rhizosphere (34) isolates showed AHL production. Forty percent of the isolates we screened showed AHL production. Our results support an emerging view that AHL quorum sensing is prevalent in the rhizosphere and endophyte communities of a variety of plants.

An inventory of *luxI* and *luxR* homologs in the sequenced genomes of *P. deltoides*-associated bacteria. There are draft genome sequences of 44 bacterial isolates from *P. deltoides* (24, 25). We identified *luxI* and *luxR* homologs in these genomes by searching for AHL synthase COG category COG3916 and the transcription factor pfam AHL-binding region motif pfam03472, respectively. As expected, no AHL QS genes were found in the five available genomes from members of the *Firmicutes* or *Bacteriodetes*. Among the 39 sequenced *Proteobacteria* genomes, we identified 18 *luxI* homologs and 72 *luxR* homologs (Table 2; also see Table S2 in the supplemental material). One hundred percent of the *Alphaproteobacteria* genomes had at least one *luxI* and one *luxR* homolog. Twenty percent of the *Betaproteobacteria* genomes had a *luxI* homolog, and 60% had a *luxR* homolog. Of the *Gam-*

maproteobacteria genomes, 21% had a *luxI* homolog and 96% had a *luxR* homolog (Table 1). We found that nearly half of the isolates screened produce AHLs (Table 1), and these data appear to underestimate the potential for AHL production among the screened isolates, as some isolates have homologs of AHL synthases but failed to produce AHLs that could be detected with the bioassay we employed (Table 2). The potential to detect AHLs appears to be even more prevalent than the potential to produce AHLs, with the prevalence of at least one *luxR* homolog in about 90% of the draft genome sequences (Table 1).

From the perspective of AHL quorum-sensing researchers, it is of interest that *luxI* homologs were identified in the genomes of three isolates that did not produce detectable AHLs (Table 2). These isolates included bacteria that were identified as *Bradyrhizobium* sp. strain YR681 and *Caulobacter* sp. strain AP07 (see Table S1 in the supplemental material). There are several possible explanations for the lack of AHL detection in cultures of these three isolates. The conditions we used to grow the isolates may not be appropriate for AHL synthesis or the encoded LuxI-type enzyme may not be functionally active, but another intriguing possibility is that these strains could produce novel AHLs that are not detected by using the *Agrobacterium* bioassay. We note that our phylogenetic analysis places the LuxI homologs of the three isolates within a subgroup of LuxI homologs known to catalyze the synthesis of noncanonical AHLs (AHLs with acid side chains that are not straight-chain fatty acids), such as RpaI and BjaI (Fig. 1A). Because of its relatively high homology with BjaI from *B. japonicum* strain USDA110 (7), we predict that the LuxI from *Bradyrhizobium* YR681 synthesizes isovaleryl-HSL. Although the *Agrobacterium* reporter responds to long-chain AHLs, it does not respond to this branched-chain acyl-HSL (7).

Cognate pairs of *luxI-luxR* homologs. Generally, *luxI* genes and their cognate *luxR* genes are in close proximity. This was true in the case of almost all of the genomes with *luxI* homologs. There were three exceptions, *Caulobacter* sp. AP07, *Phyllobacterium* sp. strain YR531, and *Rhizobium* sp. strain CF080. For *Caulobacter* sp. AP07, the *luxI* (PMI01_00487) homolog-containing contig is small, and the *luxI* homolog is close to the end of the contig. There is only one *luxR* homolog in the AP07 genome (PMI01_02945), and it is also near the end of a small contig. It is possible that these genes are in close proximity in the genome, and it is equally possible that they are at a considerable distance from each other. Both *Phyllobacterium* sp. YR531 and *Rhizobium* sp. CF080 have multiple *luxR* homologs and a single *luxI* homolog. For both of these genomes, the *luxI* homolog (PMI41_00059 and PMI07_05253) and a *luxR* homolog (PMI41_00575 and PMI07_01442) are adjacent to clusters of conjugal transfer (*tra* and *trb*) genes but on separate contigs. We believe the QS gene organization in these genomes might be similar to that of *A. tumefaciens*, where *traI* and *traR*, which are encoded on the same Ti conjugal plasmid, are separated by a large stretch of DNA encoding opine catabolism genes and are adjacent to conjugal transfer genes (19). In fact, the *Phyllobacterium* sp. YR531 and *Rhizobium* sp. CF080 LuxI homologs are phylogenetically close to the *A. tumefaciens* TraI (Fig. 1A).

Phylogeny of the AHL QS genes. We constructed phylogenetic trees of the polypeptides encoded by the *luxI* homologs and *luxR* homologs that constitute apparent AHL QS systems (Fig. 1A and B). Several of the LuxI homologs are very closely related to well-characterized AHL synthases from other bacteria. The LuxI homologs in *Rhizobium* sp. strain CF142 and PDO1-076 show 88% or higher amino acid identity to CinI in *Rhizobium leguminosarum* biovar viciae strain 3841 and SinI in *Ensifer* (formerly *Sinorhizobium*) *meliloti* strain 1021. The LuxI homolog in *Bradyrhizobium* sp. YR681 (PMI42_07398) shares 74% amino acid identity with BjaI from *Bradyrhizobium japonicum* strain USDA110. *Pseudomonas chloroaphis* strain 30-84 PhzI and CsaI are 99% identical to those in isolate GM17 (PMI20_01270 and PMI20_01418, respectively). This is not surprising, because multilocus sequence typing places isolate GM17 as a *Pseudomonas* sp. that is most closely related to *P. chloroaphis* (D. Pelletier, unpublished data). We find the phylogenies of the LuxI homologs from *Bradyrhizobium* sp. YR681 and *Caulobacter* sp. AP07 particularly interesting. These LuxI homologs are most closely related to a subclass of LuxI homologs that produce non-straight-chain fatty acyl-HSLs. Two of the known members of this subclass, RpaI and BraI, catalyze synthesis of the aryl-HSLs *p*-coumaroyl- and cinnamoyl-HSL (5, 6), and the third, BjaI, synthesizes isovaleryl-HSL (7). For several reasons, stated above, the production of these signals would escape detection by the *A. tumefaciens* bioassay, and both *Bradyrhizobium* sp. YR681 and *Caulobacter* sp. AP07 were negative in this bioassay. The predicted cognate LuxR homologs' phylogenies are shown in Fig. 1B. Consistent with the idea that these LuxR homologs coevolved with their predicted LuxI cognates, the tree is similar, although not identical, to the tree of LuxI homologs.

Orphan or solo *luxR* homologs. Many of the genomes showed an excess of *luxR* homologs over *luxI* homologs or possessed one or more *luxR* homologs and no discernible *luxI* homolog (Table 2). The excess *luxR* homologs are orphans or solos (11). There is a limited literature on orphans, but we know that some orphan LuxR homologs respond to AHLs produced by the bacteria in which they occur (35), some respond to AHLs produced by other bacteria (12, 36), and some do not respond to AHLs, instead responding to plant-derived elicitors (37–40). All three categories are likely represented in the *P. deltoides* isolates for which genomic sequence information is available. There are examples of isolates, like CF122 and others, with a cognate *luxI-luxR* homolog pair and several additional *luxR* homologs. We speculate that in at least some cases, one or more of the additional *luxR* homologs codes for a transcription factor that interacts with a self-produced AHL. There are examples of isolates with multiple *luxR* homologs and without *luxI* homologs. The proteins these *luxR* homologs encode might respond to AHLs produced by other bacteria (Fig. 2), or they might respond to plant-derived compounds (Fig. 3).

We have constructed phylogenetic trees of those polypeptides encoded by solo *luxR* homologs (Fig. 2) thought not to be responsive to plant compounds because they do not have the sequence signatures and genomic context typical of the hypothesized plant-responsive class (this class is discussed below and in the legend to Fig. 3). Presumably, these solo LuxRs respond to AHLs produced either by other bacteria or by the bacteria themselves (possible in those cases in which an isolate possesses a *luxI* homolog). There were four subfamilies of solo LuxRs that were present among multiple isolates of *Rhizobium* species, including homologs of ExpR (Fig. 2, turquoise lines) from *Rhizobium leguminosarum* and *E. meliloti* (41), indicating that each of these LuxR homologs is common among the *Rhizobiaceae*. There was also a *Pseudomonas* subfamily of solo LuxRs, which includes PpoR from *Pseudomonas putida* (42), which was conserved in 20 of 21 *Pseudomonas* isolates (Fig. 2, red lines). Although these pseudomonad solo LuxRs do

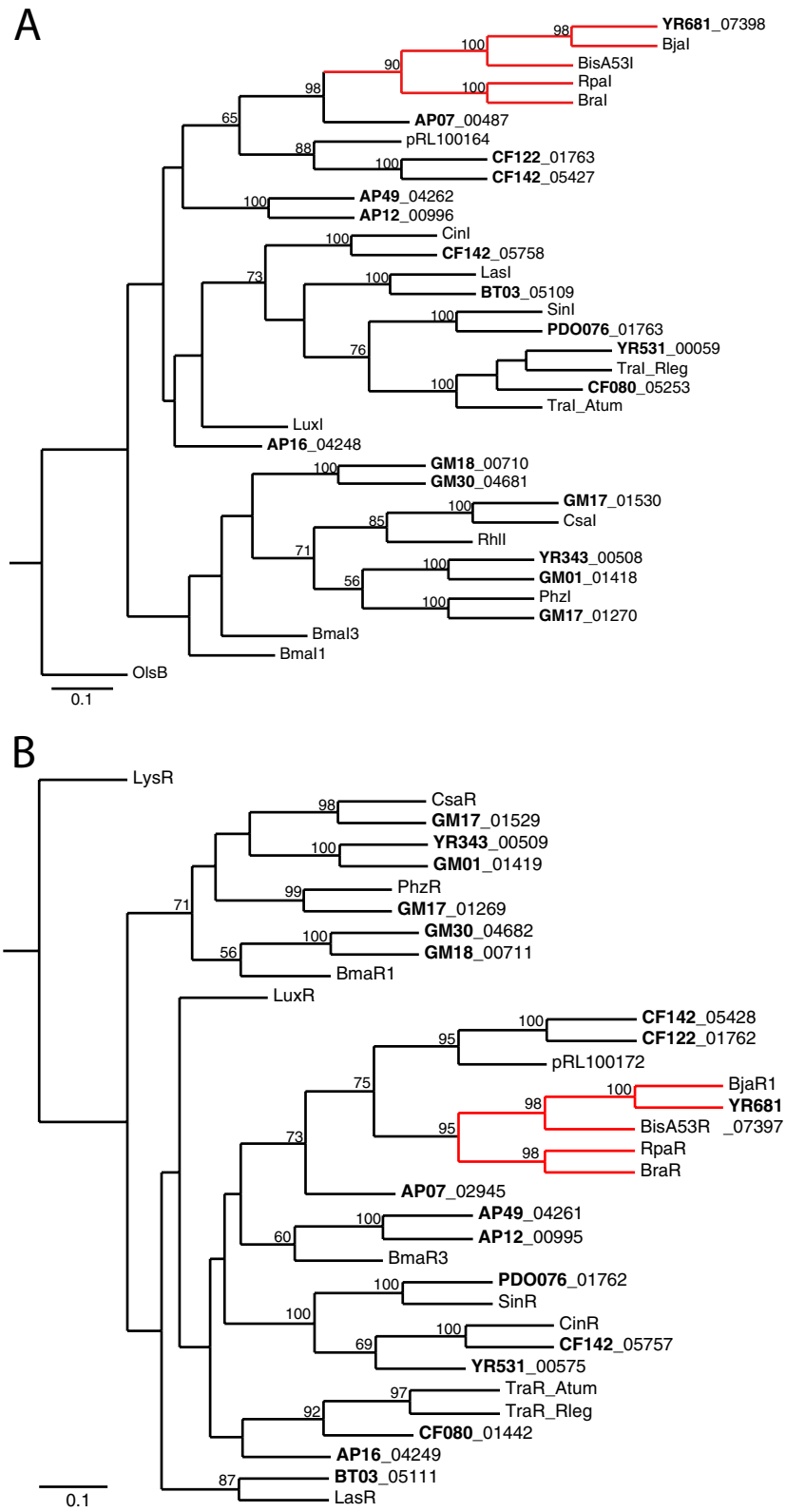

FIG 1 Phylogenetic trees of LuxI-LuxR family members from *Populus* bacterial isolates (bold lettering) and select *Proteobacteria*. The scale indicates the number of substitutions per residue, and bootstrap values as the percentage of 100 samples are shown for nodes with values of 50% or greater. (A) Phylogenetic tree of LuxI AHL synthases from members of the *Populus* bacterial isolates and select *Proteobacteria*. The subfamily tree of AHL synthases that synthesize atypical QS signals is highlighted in red. OlsB, an ornithine acyltransferase, is included as an outgroup. (B) Phylogenetic tree of LuxR-type receptors from members of the *Populus* bacterial isolates and select *Proteobacteria*. The subfamily tree of LuxRs that responds to atypical QS signals is highlighted in red. LysR, a transcriptional regulator containing a helix-turn-helix DNA-binding motif, is included as an outgroup. Detailed information for each LuxI and LuxR homolog is given in Table S2 in the supplemental material.

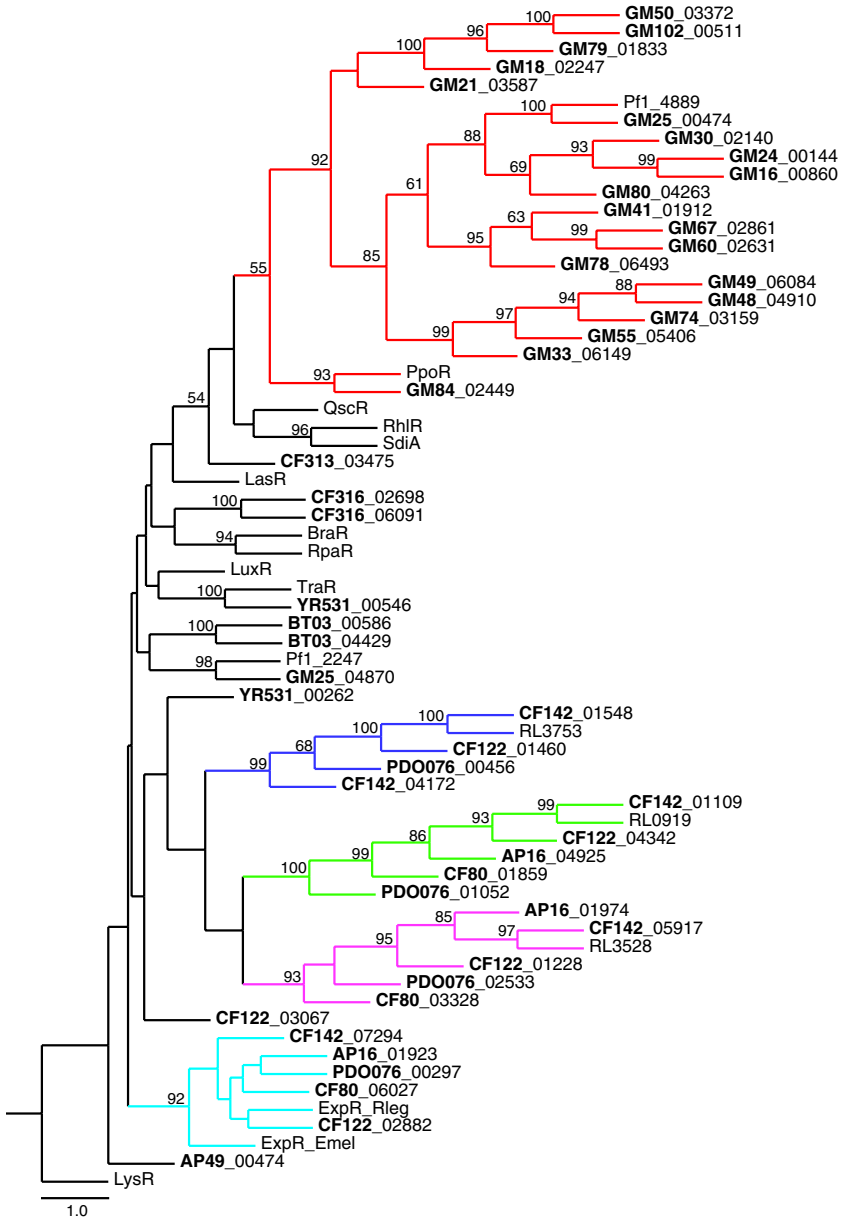

FIG 2 Phylogenetic tree of likely non-plant-responsive solo LuxR polypeptides from *Populus* bacterial isolates (bold lettering) and select *Proteobacteria*. The scale indicates the number of substitutions per residue, and bootstrap values as the percentage of 100 samples are shown for nodes with values of 50% or greater. Each subfamily tree of solo LuxR receptors present in multiple isolates is highlighted in a separate color. The *Pseudomonas* subfamily PpoR homologs are highlighted in red. The *Rhizobium* subfamily ExpR homologs are highlighted in turquoise. *Rhizobium* subfamily LuxR members without a described homolog are highlighted in blue, green, and magenta. LysR, a transcriptional regulator containing a helix-turn-helix DNA-binding motif, is included as an outgroup. Detailed information for each LuxR homolog is given in Table S2 in the supplemental material.

not share high levels of amino acid identity (e.g., PpoR and Pf1_4889 are 29% identical and 60% similar), their genes are flanked by the same genes on the chromosome, suggesting that a *ppoR* homolog was present in a common ancestor of these strains. The prevalence and conservation of solo LuxR subfamilies among nearly all our isolates suggest that they play a role in the biology of these bacteria.

Orphan LuxR proteins predicted to respond to plant-derived compounds. Our analysis not only shows a significant potential for AHL signaling in the rhizosphere and endophytic populations of *P. deltoides*, it also suggests a potential for a transcriptional response to specific plant-produced chemical elicitors through one of the subgroups of proteins encoded by *luxR* homologs. There is relatively little known about the LuxR homologs believed to respond to plant-derived elicitors; the most-studied examples are from plant-pathogenic members of the genus *Xanthomonas* (37, 39, 40). The LuxR homologs in these bacteria activate the expression of adjacent proline iminopeptidase (*pip*) genes, which are virulence factors (13). The plant metabolites that serve as ligands for these LuxR homologs have yet to be elucidated. Other orphan LuxR homologs predicted to respond to plant metabolites have been identified on the basis of protein sequence and the

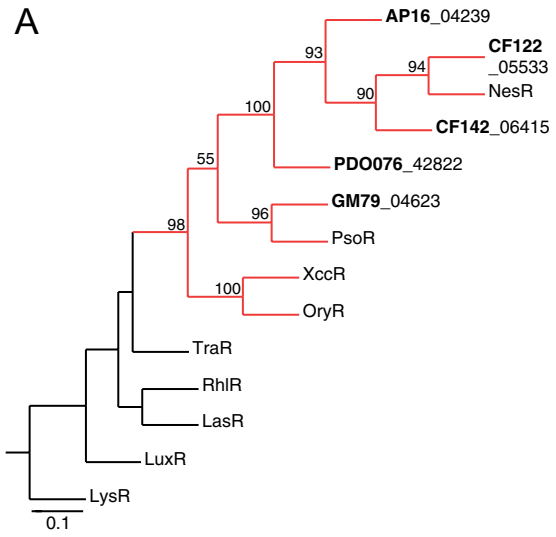

FIG 3 Likely plant-responsive LuxR homologs are present in *Populus* isolates. (A) Phylogenetic tree of selected probable plant-responsive LuxR family members from *Populus* bacterial isolates (bold lettering) and selected *Proteobacteria*. The scale indicates the number of substitutions per residue, and bootstrap values as the percentage of 100 samples are shown for nodes with values of 50% or greater. The subfamily tree of LuxR receptors that respond to a plant compound is highlighted in red. LysR, a transcriptional regulator containing a helix-turn-helix DNA-binding motif, is included as an outgroup. Amino acid sequence information for each LuxR homolog is detailed in Table S2 in the supplemental material. (B) Gene organization map of the plant-responsive *luxR* and *pip* genes in *Pseudomonas* sp. strain GM79 and *Rhizobium* sp. strains AP16, CF122, CF142, and PD01-076 (similar to *nesR* in *E. meliloti* strain 1021). (C) Sequence alignment of putative R-binding sites found upstream from the *pip2* and *pip3* genes. Site positions in which there is a majority agreement are colored red. The coordinates on the right indicate where the inverted repeat is centered relative to the ATG start site (in bp). A single asterisk indicates that the binding site overlaps the 3′ coding region of the *luxR* gene, and a double asterisk indicates that the binding site overlaps the 5′ coding region of the *luxR* gene. The *oryR* gene is adjacent to only a single downstream *pip* gene (analogous to *pip2*); the DNA sequence of the OryR-binding site is shown for comparison.

context of neighboring genes (13). The genes that code for these predicted plant-responsive LuxR homologs are flanked by *pip* homologs. Members of this family also code for polypeptides with substitutions in one or two of the conserved residues in the AHL-binding region (Y61W [Y mutated to W at position 61] and/or W57M) (reviewed in reference 13). We identified five *luxR* homologs with the above-mentioned characteristics (Fig. 3A), four of which are in *Rhizobium* species genomes and one in a pseudomonad. All five of these genes are embedded between *pip2* and *pip3* homologs in an organization similar to that of *E. meliloti nesR* (Fig. 3B), a gene that has been reported to be involved in stress adaptation and competition during root nodule development (43). There are inverted repeat DNA elements found in the regions upstream from *pip2* and *pip3* (Fig. 3C). Similar inverted repeats occur in the *pip* regions of plant-pathogenic *Xanthomonas* sp. Plant-beneficial *Pseudomonas fluorescens* strains have similarly organized *pip* loci except that they lack the inverted repeat sequences. Evidence suggests that the inverted repeats are binding sites for LuxR homologs (13).

Taken as a whole, our results support an emerging view that AHL quorum sensing is prevalent in the rhizosphere and endophyte communities of a variety of plants. Our work extends the knowledge base not only to include Eastern cottonwood but also to show that there is a great capacity for AHL signal production, which was universal among the *Alphaproteobacteria* isolates we examined and lower in the *Beta-* and *Gammaproteobacteria* isolates. Most of the isolates we examined showed a capacity for AHL recognition by orphan or solo LuxR homologs even if there was not a capacity for AHL production. Our analysis also supports the view that some *Proteobacteria* possess LuxR homologs that may be capable of responding to plant metabolites. We are now poised to begin to understand what roles AHL quorum sensing plays in the biology of the Eastern cottonwood and other plants.

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