ARTICLES

A new class of homoserine lactone quorum-sensing signals

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Quorum sensing is a term used to describe cell-to-cell communication that allows cell-density-dependent gene expression. Many bacteria use acyl-homoserine lactone (acyl-HSL) synthases to generate fatty acyl-HSL quorum-sensing signals, which function with signal receptors to control expression of specific genes. The fatty acyl group is derived from fatty acid biosynthesis and provides signal specificity, but the variety of signals is limited. Here we show that the photosynthetic bacterium *Rhodopseudomonas palustris* uses an acyl-HSL synthase to produce *p*-coumaroyl-HSL by using environmental *p*-coumaric acid rather than fatty acids from cellular pools. The bacterium has a signal receptor with homology to fatty acyl-HSL receptors that responds to *p*-coumaroyl-HSL to regulate global gene expression. We also found that *p*-coumaroyl-HSL is made by other bacteria including *Bradyrhizobium* sp. and *Silicibacter pomeroyi*. This discovery extends the range of possibilities for acyl-HSL quorum sensing and raises fundamental questions about quorum sensing within the context of environmental signalling.

Dozens of species of Proteobacteria use members of the LuxI family of signal generators to produce fatty acyl-HSL signals to which co-evolved members of the LuxR family of transcription factors respond. The lengths of the fatty acyl groups have been reported to vary from 4 carbons to 18 carbons depending on the specific system. LuxIR-type systems influence a variety of processes in bacteria including virulence in some animal and plant pathogens[1,2]. The anoxygenic phototrophic soil bacterium *Rhodopseudomonas palustris* CGA009 has a chromosomal *luxIR*-type pair, *rpaI* (*rpa0320*) and *rpaR* (*rpa0321*)[3] (Fig. 1a and Supplementary Fig. 1). Recently, we found that *rpaI* expression is activated specifically by growth of *R. palustris* on *p*-coumarate[4], a major aromatic monomer of lignin polymers, which comprise over 30% of all plant dry material. *R. palustris* degrades a wide variety of aromatic compounds[3]. To investigate the relationship between quorum sensing and *p*-coumarate in *R. palustris*, we sought to identify the product of RpaI activity. We first used a set of bioassays[5], which will detect all known acyl-HSLs. There was no detectable response to extracts of culture fluid from *R. palustris* grown on *p*-coumarate in any of the assays. Thus, we hypothesized that the *R. palustris* quorum-sensing signal has a unique structure, and only the cognate receptor RpaR will respond to the RpaI-generated signal.

A bioassay for the *R. palustris* signal

To test our hypothesis that *R. palustris* produces a unique acyl-HSL quorum-sensing signal, we required an RpaR-dependent promoter. Because *luxI*-type genes are often positively autoregulated by their cognate R protein[1,2] and the R proteins often bind to identifiable inverted repeat sequences called *lux* box-like sequences[6], we examined the DNA sequence upstream of the *rpaI* open reading frame and found two *lux* box-like elements centred at −76 and −35 bp upstream of the *rpaI* ATG start codon (Fig. 1a, b). We generated *R. palustris* CGA814, which has a chromosomal *rpaI::lacZ* mutation to test activation of the *rpaI* promoter by solvent extracts of *R. palustris* wild-type cultures grown on succinate in the presence and absence of *p*-coumarate. Extracts from cultures containing *p*-coumarate, but not succinate only, activated *rpaI::lacZ* expression (Fig. 1c). To assess whether *p*-coumarate was the most potent inducer of this system, we surveyed extracts of *R. palustris* grown on succinate plus each of 14 additional aromatic compounds (Supplementary Fig. 2) for *rpaI* promoter induction. Extracts from *p*-coumarate-grown cultures elicited the strongest response, although plant-derived aromatic compounds with modest hydroxylation differences on the phenyl ring exhibited small (<10%) amounts of induction (Fig. 1c). We also tested the ability of a number of synthetic fatty acyl-HSLs to activate the *rpaI* promoter and all results were negative (see Methods Summary).

Signal purification and identification

Culture extracts were separated by C_{18}-reverse-phase high-performance liquid chromatography (HPLC). By using the *rpaI::lacZ* strain as a reporter we detected a single peak of activity in *p*-coumarate-grown cultures (Fig. 2a). By using high-resolution mass spectrometry we calculated a mass of 247.0844 for the compound in the active fraction, which is in agreement with a molecular formula of $C_{13}H_{13}NO_4$. This formula is inconsistent with any fatty acyl-HSL, but it corresponds to *p*-coumaroyl-HSL (hereafter called *p*C-HSL). Thus, we synthesized *p*C-HSL and monitored the ability of the synthetic compound to activate the *rpaI::lacZ* reporter (Fig. 2b). The expression of *rpaI::lacZ* was activated by synthetic *p*C-HSL and the half-maximal response was 5−10 nM, a sensitivity similar to those reported for fatty acyl-HSL signal receptors[7]. The response curve for the natural compound (Fig. 2c) was similar to that of synthetic *p*C-HSL. Stationary phase cultures of *R. palustris* grown on *p*-coumarate under either anaerobic photoheterotrophic or aerobic chemoheterotrophic conditions typically had activity equivalent to about 1 μM *p*C-HSL. These extracellular levels are comparable to signal concentrations produced by many bacteria with fatty acyl-HSL signalling systems[7].

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Tandem mass spectrometry of the natural product (Fig. 2d) showed a pattern indistinguishable from that of synthetic *p*C-HSL (Fig. 2e). Synthetic *p*C-HSL exhibited an HPLC elution profile similar to the natural product in both a methanol gradient (Fig. 2a) and in a 20:80 methanol:water isocratic separation, as well as a similar absorption spectrum (broad peak of maximum absorbance at 306 nm and a minor peak at 224 nm, data not shown). An NMR analysis of the natural and synthetic compounds (Supplementary Fig. 3) supports our conclusion that they are both *p*-coumaroyl-HSL (Fig. 2f). Furthermore, the NMR analysis indicates that the double bond in the aryl side chain is in the *trans* configuration for both the natural and synthetic compounds. We have not characterized the signal produced by *R. palustris* when we add *m*- or *o*-coumarate to cultures. As a rule, acyl-HSL synthases show some lack of specificity with respect to the acyl substrate and have limited, but measurable, abilities to use acyl substrates with similarity to the natural substrate[8–10]. Thus, it is reasonable to assume that when fed *m*- or *o*-coumarate, *R. palustris* synthesizes *m*- or *o*-coumaroyl-HSL, but this requires further investigation.

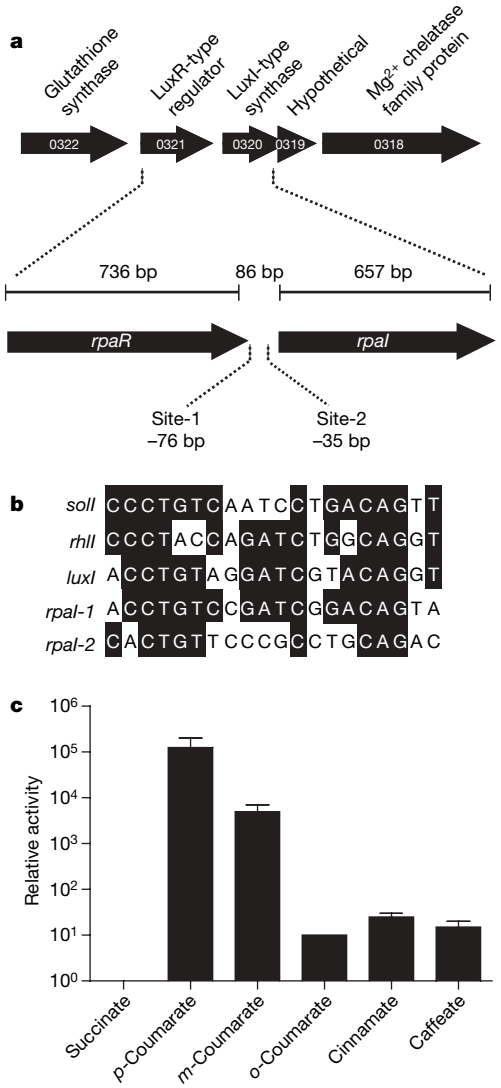

Figure 1 | The *rpaI* gene has potential RpaR binding sites in its promoter and plant-derived aromatic acids activate its expression. a, The *rpaR-rpaI* gene region with the locations of two potential *lux* box-like elements shown below. Annotations of genes are shown above. **b**, Comparison of the elements with known *lux* box-like elements from *Ralstonia solanacearum solI*, *Pseudomonas aeruginosa rhlI* and the *Vibrio fischeri luxI*. **c**, Relative signal activity extracted from cultures grown with 10 mM succinate or succinate plus 0.5 mM *p*-coumarate, *m*-coumarate, *o*-coumarate, cinnamate, or caffeate. Signal activity was measured with the *R. palustris rpaI::lacZ* reporter as described in the text. Relative β-galactosidase activity was calculated from duplicate cultures. Error bars indicate the range.

The *p*C-HSL-dependent regulon

A defining characteristic of fatty acyl-HSL signals is their ability to control expression of specific sets of genes[1,2]. We wanted to test whether the novel aryl-HSL produced by *R. palustris* served to regulate genes in addition to the gene for its own production. Thus, we performed transcriptome analyses of bacteria grown on succinate, with or without added *p*C-HSL. Expression of 17 genes was altered more than 2.5-fold ($P < 0.001$) by *p*C-HSL addition (Supplementary Table 1). As expected, expression of *rpaI* and the adjacent *rpa0319* (predicted to comprise an operon)[11] was induced by *p*C-HSL. Over one-half of the regulated genes have no known function, but several genes are annotated as encoding chemotaxis functions (*rpa1096*, *rpa1674*, *rpa3185* and *rpa4684*). None of the genes thought to be involved in *p*-coumarate degradation[4] is controlled by *p*C-HSL, consistent with our observation that the *R. palustris rpaI* mutant grows normally on *p*-coumarate (data not shown). As predicted, all of the *p*C-HSL-controlled genes were also regulated in *R. palustris* grown on *p*-coumarate (where *p*C-HSL is self generated) but not on succinate (a condition where *p*C-HSL is not made)[4]. These data suggest that *p*C-HSL is indeed a signal that regulates gene transcription in *R. palustris*.

RpaR–RpaI form a quorum-sensing circuit

To assess whether the *p*C-HSL signal functions in a quorum-sensing-dependent manner, we examined its accumulation during *R. palustris* growth in the presence of *p*-coumarate (Fig. 3a). Freshly inoculated

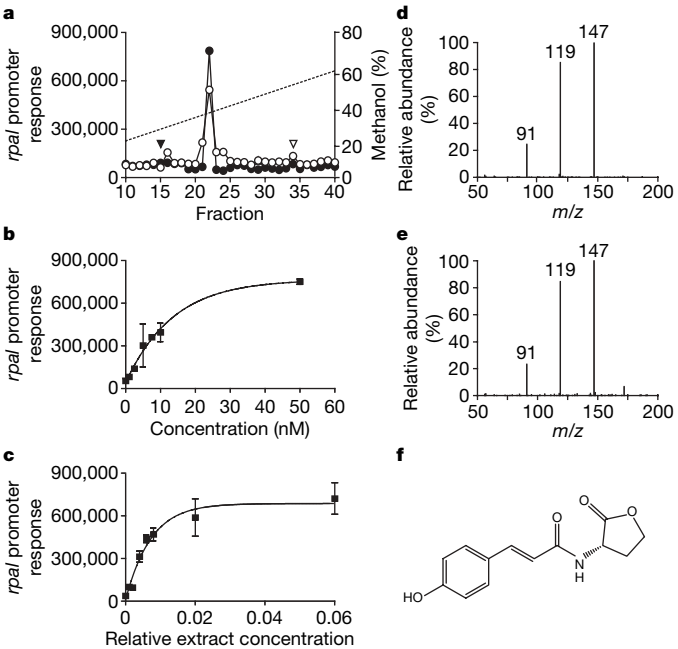

Figure 2 | Evidence indicating that the *R. palustris* quorum-sensing signal is *p*C-HSL. a, HPLC analysis of culture fluid extracts from *p*-coumarate-grown *R. palustris* (filled circles) and synthetic *p*C-HSL (open circles). 3OC6-HSL (filled triangle) and 3OC8-HSL (open triangle) were eluted in the indicated fractions. The dashed line shows the methanol gradient. **b**, **c**, The *rpaI* promoter response to synthetic *p*C-HSL (**b**) and *p*-coumarate-grown wild-type culture fluid extract (**c**). **d**, **e**, MS/MS daughter scans of the *m/z* 248 (M+H) ion from the natural product (**d**) and synthetic *p*C-HSL (**e**). **f**, The proposed *R. palustris* quorum-sensing signal, *p*C-HSL. Duplicate samples (**b**, **c**) were analysed. Error bars indicate the range.The *rpaI* promoter response was measured using the *R. palustris* CGA814 *rpaI::lacZ* chromosomal fusion. β-Galactosidase activity was measured with a luminescence reagent and is expressed as relative luminescence units.

cultures grew without lag, whereas *p*C-HSL production did not increase until later in growth, at which point aryl-HSL levels increased rapidly. This phenomenon is a characteristic of most quorum-sensing systems[12,13], which are positively autoregulated. By using real-time polymerase chain reaction (PCR), we examined *rpaI* expression during growth. Expression increased in concordance with *p*C-HSL concentration (89-fold increase over the initial 7 h of growth). These results are consistent with the conclusion that *p*C-HSL functions as a quorum-sensing signal.

With fatty acyl-HSL quorum-sensing systems, the *luxR* linked to the *luxI* homologue codes for the cognate signal-receptor transcription factor. Thus, one would assume that *rpaR* codes for a *p*C-HSL receptor. To test the assumption that RpaR is required for *p*C-HSL-dependent gene expression, we created reporter plasmids containing an *rpaI::lacZ* fusion with or without *rpaR* for use in the heterologous host, *Pseudomonas aeruginosa* MW1. When *rpaR* was present, *rpaI::lacZ* expression increased as a function of *p*C-HSL concentration (black bars, Fig. 3b). Without *rpaR*, *rpaI::lacZ* expression was high regardless of aryl-HSL levels (white bars, Fig. 3b). This is consistent with the hypothesis that RpaR functions as a *p*C-HSL-responsive repressor of the *rpaI* promoter. Thus, RpaR function is analogous to that of the LuxR homologue, EsaR, from *Pantoea stewartii*[14], which is a fatty acyl-HSL-responsive repressor protein.

Purified RpaI is an aryl-HSL synthase

We next investigated whether *p*C-HSL is generated by the *rpaI* gene product. We first showed that extracts from a *p*-coumarate-grown *rpaI* mutant contained no detectable *p*C-HSL (Fig. 3c). This is consistent with our hypothesis that *p*C-HSL is generated by RpaI. To obtain further evidence we expressed *rpaI* in both *Escherichia coli*, which cannot use *p*-coumarate as a carbon source, and *P. aeruginosa*, which can use *p*-coumarate as a carbon source. In either species *rpaI* directed the synthesis of *p*C-HSL, but only when exogenous *p*-coumarate was provided (Fig. 3c). Production of *p*C-HSL in recombinant *E. coli* and *P. aeruginosa* was low compared to production of *p*C-HSL by *R. palustris*. There are several possible explanations for this finding. For example, levels of active RpaI might be low in the recombinant bacteria or the recombinant bacteria might have a limited ability to activate *p*-coumarate.

To demonstrate definitively its role as a *p*C-HSL synthase we purified RpaI as a maltose binding protein (MBP) fusion for use in *in vitro* aryl-HSL synthesis experiments. Our results show that MBP–RpaI can catalyse the synthesis of *p*C-HSL from *S*-adenosylmethionine (SAM) and *p*-coumaroyl-coenzyme A (CoA) (Fig. 3d). This is similar to other LuxI-type enzymes that use SAM as the homoserine lactone ring donor and a thioesterified acyl group for the side-chain substrate[8–10]. Fatty acyl-HSL synthases prefer acyl–acyl carrier protein (ACP) intermediates from fatty acid biosynthesis, but can use acyl-CoA compounds to a lesser degree[8]. *p*-Coumaroyl-CoA formation has been detected in *R. palustris* crude cell extracts[15], consistent with reports from other bacteria suggesting that the initial step in *p*-coumarate degradation is aryl-CoA formation[16]. We are unaware of any report describing a *p*-coumaroyl-aryl carrier protein (ArCP) metabolite, although there is precedence for ArCPs in bacteria. For example, the *E. coli* EntB carboxy-terminal domain functions as an ArCP for 2,3-dihydroxybenzoic acid during enterobactin siderophore synthesis[17]. Interestingly, the *R. palustris* gene *rpa0319*, which is immediately downstream of *rpaI* and the expression of which is activated by *p*C-HSL addition (Supplementary Table 1), codes for a small ACP-sized polypeptide with a serine that could serve as a site for pantetheinylation. This polypeptide might function as an aryl donor for RpaI. We also note there are three genes in the *R. palustris* genome that are annotated as ACPs. Any one of these could function as an aryl carrier. Thus, it seems possible that a *p*-coumaroyl-ArCP might serve as the natural substrate for RpaI. However, regardless of which *p*-coumaroyl-thioester (-CoA or -ArCP) is the preferred substrate, our experiments demonstrate that RpaI is a *p*C-HSL synthase.

The *p*-coumarate requirement for signal production, even in heterologous hosts, suggests that exogenous *p*-coumarate, most likely derived from plants, serves as the source of the coumaroyl moiety in

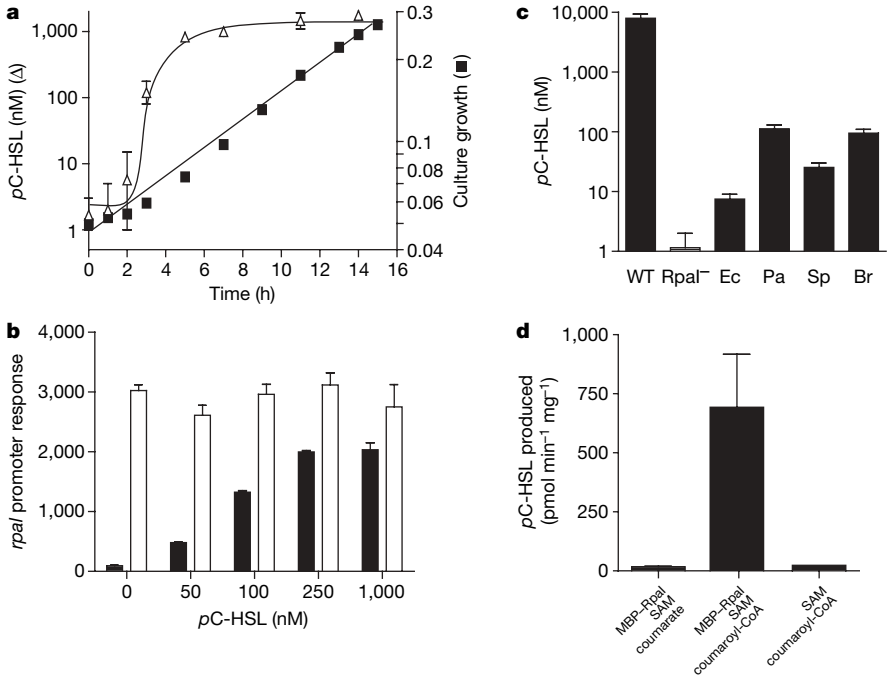

Figure 3 | *p*C-HSL is made during growth, requires RpaR for quorum-sensing-dependent activity, is synthesized by RpaI, and is made by other bacteria. **a**, *p*C-HSL production (open triangles) during growth (filled squares) of *R. palustris* with *p*-coumarate. Culture growth was measured as optical density at 660 nm. **b**, *p*C-HSL-dependent *rpaI* promoter activity requires RpaR. Expression of *rpaI::lacZ* from a plasmid with (black bars) and without (white bars) *rpaR* in *P. aeruginosa* MW1. **c**, *p*C-HSL production by *R. palustris* wild type (WT), an *rpaI* mutant (RpaI$^-$), *E. coli* DH5α (pRpaI) (Ec), *P. aeruginosa* MW1 (pRpaI) (Pa), *S. pomeroyi* DSS-3 (Sp) and *Bradyrhizobium* BTAi1 (Br) when grown with *p*-coumarate. **d**, *p*C-HSL synthesis by purified MBP–RpaI. Values are averages of triplicates (**a**) or duplicates (**b**–**d**). Error bars indicate the range.

*p*C-HSL. We found that *p*-coumarate at concentrations as low as 1 μM supported *p*C-HSL production in *R. palustris* cultures grown with succinate as the main carbon source. Production of the *Agrobacterium tumefaciens* fatty acyl-HSL also requires chemicals provided by another organism. However, for *A. tumefaciens* the chemical is a plant-produced opine required to activate expression of the LuxR-type regulator[18], not to serve as part of the actual quorum-sensing signal.

Other bacteria produce *p*C-HSL

Is *p*C-HSL synthesis unique to *R. palustris*? To address this question we screened for *p*C-HSL activity in extracts of bacteria that can use *p*-coumarate as a carbon source and that are known from genomic sequence analyses to have *luxI* homologues. When grown in the presence of *p*-coumarate, two bacteria, *Bradyrhizobium* sp. BTAi1 and *Silicibacter pomeroyi* DSS-3, produced relatively small amounts of material that behaved biologically and chemically like *p*C-HSL (Fig. 3c). *Bradyrhizobium* BTAi1 is a photosynthetic, nitrogen-fixing symbiont that forms root and stem nodules on *Aeschynomene* plant species[19]. *S. pomeroyi* is a coastal bacterioplankton member of the *Roseobacter* clade, an abundant marine group of importance in global sulphur and carbon cycles[20]. Thus, *p*C-HSL production does not appear to be unique to *R. palustris*. That *Bradyrhizobium* and *S. pomeroyi* produce low levels of *R. palustris* signal activity suggests that the growth conditions we have used are not optimal for signal production, or the more interesting possibility that *p*-coumarate is similar to but not the natural substrate for their aryl-HSL synthases.

Discussion

Our description of a non-fatty acyl-HSL (aryl-HSL) quorum-sensing signal that is derived from an exogenously supplied substrate expands the range of potential acyl-HSLs enormously. The concept that only straight-chain fatty acids in metabolic pools can serve as substrates for quorum-sensing signal production no longer holds and one can imagine any number of organic acids from the environment serving as the acyl group on a quorum-sensing signal. In the case we describe here the acyl group is derived from a plant metabolite, *p*-coumarate. The use of *p*-coumarate for quorum-sensing signal production results in a single signal that integrates two distinct cues: sufficiently high bacterial population densities and the availability of a particular exogenous substrate. This could be beneficial to an organism that controls functions via quorum sensing that are useful only under a particular set of conditions. *p*-Coumarate is produced by plants as a constituent of the major plant polymer lignin and *p*-coumarate synthesis is also stimulated by tissue damage and other stresses[21,22]. Because *p*C-HSL production requires a source of *p*-coumarate we speculate that there is an intimate relationship between *p*C-HSL-producing bacteria and specific plants and that this relationship involves *p*C-HSL signalling. It is distinctly possible that *p*C-HSL serves not only as an intraspecies bacterial signal but also as an inter-kingdom signal to a host plant. The discovery of *p*C-HSL opens up this question.

METHODS SUMMARY

Rhodopseudomonas palustris strains CGA009[3] and CGA814 (*rpaI::lacZ*) were grown photoheterotrophically in photosynthetic medium (PM) with succinate (10 mM) plus *p*-coumarate (0.5–3 mM, where indicated) as described elsewhere[23]. When indicated, plant-derived aromatic acids (0.5 mM, Supplementary Fig. 2) were added to PM succinate cultures. *P. aeruginosa* MW-1 (*lasI*$^-$, *rhlI*$^-$)[24], *E. coli* DH5α and XL1-blue were grown in Luria broth (LB) plus 1 mM *p*-coumarate where indicated. *Bradyrhizobium* BTAi1 and *Silicibacter pomeroyi* DSS-3 were grown aerobically in PM medium and basal medium[20], respectively, with 10 mM succinate and 1 mM *p*-coumarate. Bacteria were grown at 30 °C. The following synthetic fatty acyl-HSLs (1 μM) were tested for their ability to activate the *rpaI* promoter as measured by β-galactosidase activity expressed from the *R. palustris* CGA814 *rpaI::lacZ* reporter strain: C4-HSL, 3OHC4-HSL, 3OHC6-HSL, 3OC6-HSL, C7-HSL, C8-HSL, 3OHC8-HSL, C12-HSL, 3OC12-HSL and C16-HSL. Plasmid and strain constructions are described in the Methods. To monitor *p*C-HSL we mixed test samples (culture extracts, HPLC fractions, or synthetic compounds) with strain CGA814, incubated for 16 h in light, and then measured β-galactosidase activity[24]. Microarray analyses were a comparison of wild-type *R. palustris* CGA009 grown photoheterotrophically with succinate as the carbon source with and without 10 μM synthetic *p*C-HSL. For purification of *p*-coumaroyl-HSL we grew cultures of *R. palustris* CGA009 with *p*-coumarate to early stationary phase, removed cells by centrifugation and then extracted signal from the culture fluid with ethyl acetate. The active material was purified by gradient and isocratic HPLC[25]. For synthesis of *p*C-HSL we developed a novel method involving microwave irradiation as described in detail in the Methods.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions A.L.S. performed all experiments except those described below. C.M.O. and J.R.S. performed and interpreted NMR analyses and synthesized *p*C-HSL. Y.O. assisted in substrate survey and growth curve experiments and performed RT–PCR analyses. J.J.H. constructed strains and performed experiments analysing RpaR-dependent gene activation. G.B.-B. and C.M.P. constructed strains. S.S. purified MBP–RpaI protein. K.J. synthesized *p*-coumaroyl-CoA. A.L.S., E.P.G. and C.S.H. designed experiments, analysed data and wrote the paper.

Author Information The primary microarray data have been deposited in the NCBI's Gene Expression Omnibus (GEO, www.ncbi.nlm.nih.gov/geo) under the accession number GSE10642. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to C.S.H. (csh5@u.washington.edu).

METHODS

Plasmid and strain constructions. The *rpaI::lacZ* reporter strain, *R. palustris* CGA814, was constructed by replacing a 357-bp fragment of *rpaI* with a *lacZ*::Km^r cassette[26] in pJQ200KS[27] and mating this construct into *R. palustris* CGA009. To investigate the role of *rpaR* in quorum-sensing regulation of *rpaI* expression, *lacZ* (with ribosomal binding site) from pHRP309[26] was cloned into pBBR1MCS-5[28] to create pBBR1MCS-5lacZ. The *rpaR*-containing *rpaI::lacZ* reporter plasmid, pRpaR-PrpaI, was constructed by amplifying a 1,040-bp DNA fragment containing 222-bp upstream of *rpaR*, *rpaR*, and the 86-bp intergenic region between *rpaR* and *rpaI* (Fig. 1a) from *R. palustris* and cloning it into pBBR1MCS-5lacZ. An *rpaI::lacZ* plasmid lacking *rpaR*, pPrpaI, was constructed by amplifying the 86-bp DNA region upstream of *rpaI* and cloning it into pBBR1MCS-5lacZ. To express RpaI in the heterologous hosts *E. coli* DH5α and *P. aeruginosa* MW1, pRpaI was constructed by amplifying the *rpaI* gene and cloning it into pBBR1MCS-5[28].

Detection of *p*-coumaroyl-HSL. Cultures to be tested for *p*C-HSL were extracted twice with equal volumes of acidified ethyl acetate (EtAc, 0.1 ml glacial acetic acid per litre of solvent). Levels of *p*C-HSL were followed by using either the *R. palustris* strain CGA814 *rpaI::lacZ* reporter or *P. aeruginosa* MW1 (pRpaR-PrpaI). Test samples (culture extracts, HPLC fractions, or synthetic compounds) were added to 1.5 ml Eppendorf tubes (*R. palustris* CGA814 assay) or 13-mm glass vials (*P. aeruginosa* pRpaR-PrpaI assay) and the solvent was removed by evaporation under N_2. *R. palustris* CGA814 in PM succinate (0.5 ml culture) was added to each test sample and incubated in light for 16 h. Cells were lysed with chloroform (10% vol/vol) and β-galactosidase activity was measured with a Tropix Galacto-Light Plus Kit (Applied Biosystems) as described previously[24]. Logarithmic-phase *P. aeruginosa* MW1 (pRpaR-PrpaI) cultures (0.5 ml) were added to each test sample. After 4 h shaking at 30 °C cells were lysed and β-galactosidase activity measured. For *p*C-HSL measurement we used a standard curve generated with synthetic *p*C-HSL. Results were similar regardless of whether the reporter strains were grown in the presence or absence of *p*-coumarate.

Purification and identification of *p*-coumaroyl-HSL. *R. palustris* CGA009 (4-l culture) was grown with 10 mM succinate and 3 mM *p*-coumarate to early stationary phase. Cell-free supernatant fluid was extracted twice with equal volumes of EtAc. The quorum-sensing signal was purified for MS from extracts as described previously[25] except that the final isocratic elution was in 20% methanol in water. In the 20% methanol gradient the natural product was eluted with a characteristic skewed absorbance peak (305 nm) and the synthetic *p*C-HSL showed a peak with the same shape and elution profile. MS/MS was performed with a Waters Micromass Quatro II (collision energy 30 eV, collision gas argon, cone 35 V). To obtain sufficient purified signal for NMR analysis, we extracted 13 l of cell culture. Before HPLC separation, the extracts were dried, dissolved in 50% methanol in water, and passed through a C_{18} Sep-Pak cartridge (Waters Corp.) from which activity was eluted in 20–50% methanol.

Chemical synthesis of *p*-coumaroyl-HSL. PS-carbodiimide resin (0.5 g), *p*-coumarate (0.0382 g, predominantly trans, Sigma C9008), (*S*)-(-)-α-amino-γ-butyrolactone HBr (0.0275 g) and 5 ml of dimethylformamide were added to a 10-ml reactivial (Pierce). After microwave irradiation (1,000 W, 30 s, Milestone START labstation), PS-carbodiimide resin was removed by vacuum filtration and solvents were removed *in vacuo*. The resulting residue was dissolved in acetonitrile and purified by preparative TLC (EtAc:Hex, 80:20 v/v). Product band ($R_F = 0.14$) was eluted (ACN:MeOH, 80:20 v/v, 60 min) to yield a white solid (0.0095 g, 0.038 mmol. 24.8%). The final structure was confirmed by TLC (EtOAc:Hex, 80:20 v/v) $R_F = 0.14$, MS/MS (Fig. 2e) and ^{1}H-NMR (Supplementary Fig. 3).

Gene expression analyses. For transcriptome studies, wild-type *R. palustris* CGA009 was grown with succinate (conditions under which *p*C-HSL is not produced) to an absorbance of 0.1 at 660 nm. We then split the culture in half and added 10 μM synthetic *p*C-HSL to one half. The amended and unamended cultures were further incubated until the density reached 0.5. Cellular RNA was isolated[29], cDNA was prepared and transcriptome analysis was carried out with an Affymetrix *R. palustris* Custom GeneChip as previously described[30]. Real-time PCR reactions included 1 ng cDNA and 200 nM primers in 25 μl of SYBR green PCR amplification master mix (Applied Biosystems). PCR conditions were 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, and 1 min at 60 °C. Genomic DNA was used as a standard and *fixJ* (*rpa4248*) constitutive expression was used as an internal control.

Purification of MBP–RpaI fusion protein. To obtain purified RpaI fusion protein, the *rpaI* gene was cloned into pMalc2 (New England Biolabs) creating pMBPrpaI. *E. coli* XL1-blue (pMBPrpaI) was grown at 30 °C in 1 l LB plus 2% glucose and ampicillin to an absorbance of 0.4 at 600 nm. MBP–RpaI expression was then induced (0.3 mM IPTG) and incubation continued at 16 °C overnight. Cells were pelleted, resuspended in a buffer[9] and broken by sonication. The MBP–RpaI fusion protein was purified from clarified cell extracts by amylose column chromatography (New England Biolabs protocol). We were unable to cleave MBP from RpaI using the factor X_A cleavage site.

***In vitro* *p*-coumaroyl-HSL synthesis assays.** Aryl-HSL synthesis assays were performed as reported previously[8] except that reaction mixtures (0.1 ml) contained 20 mM Tris pH 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol, 4 μg MBP–RpaI, 36 nCi carboxy-^{14}C-labelled SAM (60 mCi mmol^{-1}, final SAM concentration 60 μM, American Radiochemical Company) and 40 μM *p*-coumaroyl-CoA or *p*-coumarate and were incubated for 75 min at 30 °C. We synthesized *p*-coumaroyl-CoA as described previously[15].

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