So different and still so similar: The plant compound rosmarinic acid mimics bacterial homoserine lactone quorum sensing signals

Andrés Corral-Lugo, Abdelali Daddaoua, Alvaro Ortega, Manuel Espinosa-Urgel, and Tino Krell

Department of Environmental Protection, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, C/ Prof. Albareda, Granada, Spain

ABSTRACT
Apart from inter-bacteria communication quorum sensing (QS) mechanisms also enable inter-domain interactions. To interfere with bacterial QS, plants were found to secrete compounds; most of which of unknown identity. We have identified the plant compound rosmarinic acid (RA) to modulate Pseudomonas aeruginosa QS by binding to the RhlR QS regulator. RA was found to be a homoserine-lactone (HSL) mimic that caused agonistic effects on transcription, resulting ultimately in a stimulation of various RhlR controlled phenotypes like virulence factor synthesis or biofilm formation. Our study was initiated by in silico screening of an RhlR model with compound libraries, demonstrating that this approach is suitable to tackle a major bottleneck in signal transduction research, which is the identification of sensor protein ligands. Previous work has shown that plant compounds interfere with the function of orphan QS regulators. Our study demonstrates that this has not necessarily to be the case since RhlR forms a functional pair with the RhlI synthase. A wide range of structurally dissimilar compounds have been found to mimic HSLs suggesting that this class of QS regulators is characterized by a significant plasticity in the recognition of effector molecules. Further research will show to what extent RA impacts on QS mechanisms of other bacteria.

Bacteria have evolved an array of mechanisms to interact with each other. One of these mechanisms permits to monitor the bacterial cell density which is referred to as quorum sensing (QS). QS is based on the synthesis and detection of QS signals and homoserine lactones (HSL) are used for this purpose by a variety of bacteria. HSLs are synthesized by HSL synthases and detected by LuxR type transcriptional regulators. The study of QS is of particular relevance since these mechanisms control the expression of virulence related genes in many pathogens. Frequently, the genes encoding HSL synthases and the cognate canonical regulators are next to each other. However, a number of bacteria possess additional genes for LuxR paralogues that are not paired up with a synthase gene. These latter regulators are referred to as orphan regulators. The QS system of Pseudomonas aeruginosa has been extensively studied. It uses a multifunctional QS system that is based on the synthesis and detection of quinolone signals and HSLs. The HSL response is mediated by 2 synthase/regulator pairs, namely LasI/LasR and RhlI/RhlR, as well as by the orphan QscR regulator.

Apart from inter-bacterial communication, there is evidence that the modulation of QS mechanisms permits inter-domain communication. QS signals were found to interfere with eukaryotes and, in addition, signal molecules from eukaryotes interfere with bacterial QS mechanisms. For example, there is a significant amount of data showing that plants produce compounds that modulate bacterial QS mechanisms. However, most evidence is based on experiments with complex compound mixtures such as plant macerates or extracts. Interestingly, in most cases, these compounds stimulated QS mediated signaling processes, indicative of the presence of QS factors.
agonists in these plants. However, little is known on the molecular identity of these plant compounds and their bacterial targets. There is significant evidence that orphan QS regulators recognize plant derived products and this protein sub-family was found to play central roles in mediating interaction between plants and different plant associated bacteria like rhizobia, xanthomonads, and pseudomonads.

Our recent study has resulted in the identification of the molecular identity of a plant compound that has an agonistic effect on the bacterial QS system. We have shown that rosmarinic acid (RA) binds with high affinity to the RhlR QS regulator of P. aeruginosa. This binding enhanced transcription from RhlR dependent promoters in vitro and in vivo. We were also able to show that RA causes typical QS controlled and virulence associated phenotypes like an enhancement of the production of the pyocyanin and elastase virulence factors or a stimulation of biofilm formation (Fig. 1).

Our study was initiated by virtual docking experiments of a library containing all natural compounds to a homology model of the RhlR effector binding domain. These studies were conducted by our group and agreed partially with a previous study by Annapoorani et al. The output of this procedure is a docking score representing free energy changes upon binding (the more negative this score, the more likely the possibility of binding). From this analysis we have selected the compounds with lowest docking score, that were of plant origin and that were commercially available to conduct microcalorimetric binding studies using purified RhlR. We have carried out experiments with 9 selected compounds, which showed binding only in the case of RA, whereas the other compounds failed to interact. The docking output is thus characterized by a significant level of noise, which may partially be due to the fact that a RhlR homology model and not an experimentally determined structure was used for the in silico docking experiments. However, despite this noise our study

Figure 1. Schematic representation of the role of Rosmarinic acid in RhlR mediated quorum sensing mechanisms of P. aeruginosa.
shows that the approach chosen is a feasible alternative to the more obvious experimental strategy which would have consisted in the fractionation of complex plant-derived compound mixtures followed by bioassays and the identification of the active compound(s). Bacteria contain a high number of sensor proteins to monitor environmental signals and the lack of knowledge of their cognate signals is a major bottleneck in the field. Laboratory based high throughput ligand screening of recombinant purified protein may be a plausible approach to fill this gap of knowledge and this approach has been used successfully to functionally annotate bacterial chemoreceptors. However, the study by Corral-Lugo et al. demonstrates that in silico based high-throughput screening may be an alternative to laboratory based screening.

There is a significant body of evidence demonstrating that orphan LuxR regulators are responsible for the recognition of plant-derived compounds. It was proposed that orphan LuxR have evolved from canonical HSL-responsive QS LuxRs and to play a major role in plant–bacteria interactions. Here we show that a canonical LuxR regulator that forms a pair with the Rhl synthase, and not an orphan regulator, is the target for a plant derived compound. This implies that canonical as well as orphan LuxR regulators have to be considered as candidates to identify target receptors for plant derived compounds.

A number of HSL-mimics have been reported in the literature. These compounds are either structurally related to HSLs or share no obvious structural similarities with HSLs like the triphenyl compounds as identified by Muh et al. (2006) or riboflavin and lumichrome as reported by Rajamani et al. (2004). These compounds have either agonistic or antagonistic effects on QS mediated signaling processes. With RA we identify another structurally unrelated agonist. Data taken together suggest that the LuxR family of QS regulators is characterized by a significant molecular plasticity in the recognition of structurally dissimilar compounds.

Our work raises a number of questions that will need to be answered to get a more complete picture on the effects observed for RA. We have shown that RA modifies P. aeruginosa QS by binding to the Rhl receptor and it remains to be established whether RA also interferes with the QS of other bacteria. The other set of questions concerns the biological significance of our observations. Since RA secretion from plant roots occurred upon infection we hypothesize that the action of RA may correspond to a plant defense mechanism. However, experiments need to be conducted to verify this hypothesis; for example to establish whether plant mutants unable to secrete RA are more susceptible to infection that the wt plant.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by FEDER funds and Fondo Social Europeo through grants from the Junta de Andalucía (grants CVI-7335 to T.K. and CVI-7391 to M.EU) and the Spanish Ministry for Economy and Competitiveness (grants BIO2010-16937 and BIO2013-42297 to T.K; grant BFU2010-17946 to M.EU).

References

[1] Rutherford ST, Bassler BL. Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med 2012; 2:a012427; PMID:23125205; http://dx.doi.org/10.1101/cshperspect.a012427
[2] Subramoni S, Venturi V. LuxR-family ‘solos’: bachelor sensors/regulated signalling molecules. Microbiol 2009; 155:1377-85; http://dx.doi.org/10.1099/mic.0.026849-0
[3] Schuster M, Sexton DJ, Diggle SP, Greenberg EP. Acylhomoserine lactone quorum sensing: from evolution to application. Annu Rev Microbiol 2013; 67:43-63; PMID:23682605; http://dx.doi.org/10.1146/annurev-micro-092412-155635
[4] Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, Greenberg EP, Iglewski BH. Quinolone signaling in the cell-to-cell communication system of Pseudomonas aeruginosa. Proc Natl Acad Sci USA 1999; 96:11229-34; PMID:10500159.
[5] Venturi V, Fuqua C. Chemical signaling between plants and plant-pathogenic bacteria. Annu Rev Phytopathol 2013; 51:17-37; http://dx.doi.org/10.1146/annurev-phyto-082712-102239
[6] Mathesius U, Mulders S, Gao M, Teplitski M, Caetano-Anolles G, Rolfe BG, Bauer WD. Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. Proc Natl Acad Sci USA 2003; 100:1444-9; PMID:12511600.
[7] Venturi V, Keel C. Signaling in the Rhizosphere. Trends Plant Sci 2016; S1360-1385(16):00006-6; In press; PMID:26832945.
[8] Degrassi G, Devescovi G, Solis R, Steindler L, Venturi V. Oryza sativa rice plants contain molecules that activate different quorum-sensing N-acyl homoserine lactone biosensors and are sensitive to the specific AiiA lactonase. FEMS Microbiol Lett 2007; 269:213-20; PMID:17227455.
[9] Subramoni S, Gonzalez JE, Johnson A, Pechy-Tarr M, Rochat L, Pausen I, Loper JE, Keel C, Venturi V. Bacterial subfamily of LuxR regulators that respond to plant compounds. Appl Environ Microbiol 2011; 77:4579-88;
[10] Gonzalez JF, Venturi V. A novel widespread interkingdom signaling circuit. Trends Plant Sci 2013; 18:167-74; PMID:23089307; http://dx.doi.org/10.1016/j.plants.2012.09.007

[11] Corral-Lugo A, Daddaoua A, Ortega A, Espinosa-Urgel M, Krell T. Rosmarinic acid is a homoserine lactone mimic produced by plants that activates a bacterial quorum-sensing regulator. Sci Signal 2016; 9:ra1; PMID:22986632; http://dx.doi.org/10.1126/scisignal.aaa8271

[12] Annapoorani A, Umamageswaran V, Parameswari R, Pandian SK, Ravi AV. Computational discovery of putative quorum sensing inhibitors against LasR and RhlR receptor proteins of Pseudomonas aeruginosa. J Comput Aided Mol Des 2012; 26:1067-77; PMID:22986632; http://dx.doi.org/10.1007/s10822-012-9599-1

[13] McKellar JL, Minnell JJ, Gerth ML. A high-throughput screen for ligand binding reveals the specificities of three amino acid chemoreceptors from Pseudomonas syringae pv. actinidiae. Mol Microbiol 2015; 96:694-707; PMID:25656450; http://dx.doi.org/10.1111/mmi.12964

[14] Krell T. Tackling the bottleneck in bacterial signal transduction research: high-throughput identification of signal molecules. Mol Microbiol 2015; 96:685-8; PMID:25708679; http://dx.doi.org/10.1111/mmi.12975

[15] Fernandez M, Morel B, Corral-Lugo A, Krell T. Identification of a chemoreceptor that specifically mediates chemotaxis toward metabolizable purine derivatives. Mol Microbiol 2016; 99:34-42; PMID:26355499; http://dx.doi.org/10.1111/mmi.13215

[16] Corral-Lugo A, de la Torre J, Matilla MA, Fernandez M, Morel B, Espinosa-Urgel M, Krell T. Assessment of the contribution of chemoreceptor-based signaling to biofilm formation. Environ Microbiol 2015; http://dx.doi.org/10.1111/1462-2920.13170

[17] Furlaga S, Venturi V. OryR is a LuxR-family protein involved in interkingdom signaling between pathogenic Xanthomonas oryzae pv. oryzae and rice. J Bacteriol 2009; 191:890-7; PMID:19028884; http://dx.doi.org/10.1128/JB.01507-08

[18] Chatnaparat T, Prathuangwong S, Ionescu M, Lindow SE. XagR, a LuxR homolog, contributes to the virulence of Xanthomonas axonopodis pv. glycines to soybean. Mol Plant Microbe Interact 2012; 25:1104-17; PMID:22746827; http://dx.doi.org/10.1094/MPMI-01-12-0008-R

[19] Rabin N, Delago A, Inbal B, Krief P, Meijler MM. Tailormade LasR agonists modulate quorum sensing in Pseudomonas aeruginosa. Org Biomol Chem 2013; 11:7155-63; PMID:24057196.

[20] Ishida T, Ikeda T, Takiguchi N, Kuroda A, Ohtake H, Kato J. Inhibition of quorum sensing in Pseudomonas aeruginosa by N-acyl cyclopentylamides. Appl Environ Microbiol 2007; 73:3183-8; PMID:17369333.

[21] Kaufmann GF, Sartorio R, Lee SH, Rogers CJ, Meijler MM, Moss JA, Clapham B, Brogan AP, Dickerson TJ, Janda KD. Revisiting quorum sensing: Discovery of additional chemical and biological functions for 3-oxo-N-acylhomoserine lactones. Proc Natl Acad Sci USA 2005; 102:309-14; PMID:15623555.

[22] Bottomley MJ, Muraglia E, Bazzo R, Carfi A. Molecular insights into quorum sensing in the human pathogen Pseudomonas aeruginosa from the structure of the virulence regulator LasR bound to its autoinducer. J Biol Chem 2007; 282:13592-600; PMID:17363638.

[23] Muh U, Hare BJ, Duerkop BA, Schuster M, Hanzelka BL, Heim R, Olson ER, Greenberg EP. A structurally unrelated mimic of a Pseudomonas aeruginosa acyl-homoserine lactone quorum-sensing signal. Proc Natl Acad Sci USA 2006; 103:16948-52; PMID:17075036.

[24] Rajamani S, Bauer WD, Robinson JB, Farrow JM, 3rd, Pesci EC, Teplitski M, Gao M, Sayre RT, Phillips DA. The vitamin riboflavin and its derivative lumichrome activate the LasR bacterial quorum-sensing receptor. Mol Plant Microbe Interact 2008; 21:1184-92; PMID:18700823; http://dx.doi.org/10.1094/MPMI-21-9-1184

[25] Walker TS, Bais HP, Deziel E, Schweizer HP, Rahme LG, Fall R, Vivanco JM. Pseudomonas aeruginosa-plant root interactions. Pathogenicity, biofilm formation, and root exudation. Plant Physiol 2004; 134:320-31; PMID:14701912.