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ARTICLE ADDENDUM

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

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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 several 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.

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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).[1] 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.[1] 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.[2] The QS system of Pseudomonas aeruginosa has been extensively studied. It uses a multi-signal QS system that is based on the synthesis and detection of quinolone signals and HSLs.[3,4] 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.[5] QS signals were found to interfere with eukaryotes[6] and, in addition, signal molecules from eukaryotes interfere with bacterial QS mechanisms.[5] For example, there is a significant amount of data showing that plants produce compounds that modulate bacterial QS mechanisms.[7] However, most evidence is based on experiments with complex compound mixtures such as plant macerates or extracts.[8,9] Interestingly, in most cases, these compounds stimulated QS mediated signaling processes, indicative of the presence of QS

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agonists in these plants.[5] 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.[10]

Our recent study[11] 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.[12] 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

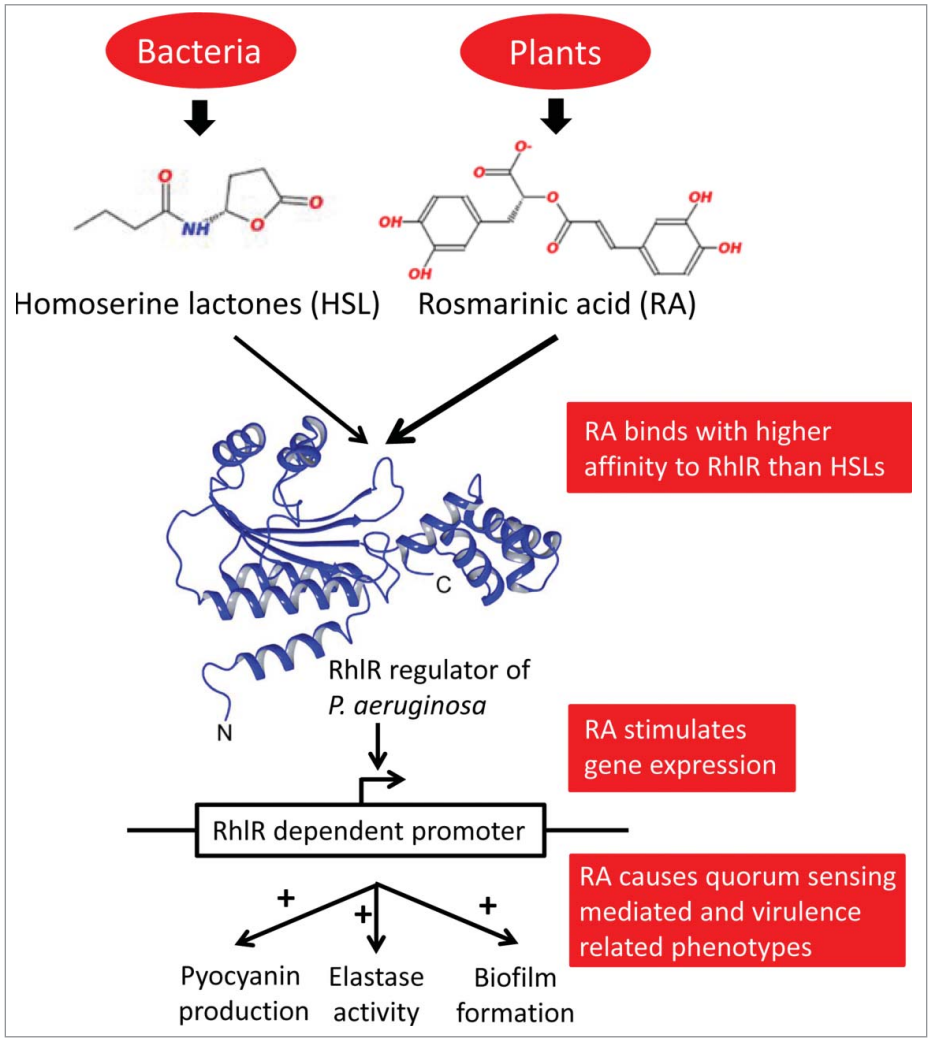

Figure 1. Schematic representation of the role of Rosmarinic acid in RhlR mediated quorum sensing mechanisms of P. aeruginosa.

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[13,14] and this approach has been used successfully to functionally annotate bacterial chemoreceptors.[15,16] 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.[9,17,18] It was proposed that orphan LuxR have evolved from canonical HSL-responsive QS LuxRs and to play a major role in plant–bacteria interactions.[10] Here we show that a canonical LuxR regulator that forms a pair with the RhlI 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[19-22] are share no obvious structural similarities with HSLs like the triphenyl compounds as identified by Muh et al. (2006)[23] or riboflavin and lumichrome as reported by Rajamani et al. (2004).[24] These compounds have either agonistic[19,23,24] or antagonistic[20-22] 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 modulates P. aeruginosa QS by binding to the RhlR 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[25] 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.

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