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Interkingdom Signaling Interference: The Effect of Plant-Derived Small Molecules on Quorum Sensing in Plant-Pathogenic Bacteria

Janak Raj Joshi,[1,4,*] Netaly Khazanov,[2,*] Amy Charkowski,[3] Adi Faigenboim,[1] Hanoch Senderowitz,[2] and Iris Yedidia[1]

[1]Department of Plant Sciences, Agricultural Research Organization, The Volcani Center, Rishon Lezion, Israel 7528809; email: irisy@volcani.agri.gov.il

[2]Department of Chemistry, Bar-Ilan University, Ramat-Gan, Israel 5290002; email: hsenderowitz@gmail.com

[3]Department of Agricultural Biology, Colorado State University, Fort Collins, Colorado 80523, USA

[4]Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, Colorado 80523, USA

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*These authors contributed equally to the manuscript.

Keyword

bacterial virulence, inhibitors, LuxI-LuxR, plant compounds, quorum sensing interference

Abstract

In the battle between bacteria and plants, bacteria often use a population density–dependent regulatory system known as quorum sensing (QS) to coordinate virulence gene expression. In response, plants use innate and induced defense mechanisms that include low-molecular-weight compounds, some of which serve as antivirulence agents by interfering with the QS machinery. The best-characterized QS system is driven by the autoinducer *N*-acyl-homoserine lactone (AHL), which is produced by AHL synthases (LuxI homologs) and perceived by response regulators (LuxR homologs). Several plant compounds have been shown to directly inhibit LuxI or LuxR. Gaining atomic-level insight into their mode of action and how they interfere with QS enzymes supports the identification and design of novel QS inhibitors.

Such information can be gained by combining experimental work with molecular modeling and docking simulations. The summary of these findings shows that plant-derived compounds act as interkingdom cues and that these allomones specifically target bacterial communication systems.

INTRODUCTION

Land plants and bacteria have coevolved for at least 400 million years and, consequently, there are many examples of beneficial plant–bacteria symbioses. However, concomitant with such favorable interactions, bacterial pathogenesis also emerged, requiring plants to defend themselves while still allowing colonization by beneficial bacterial partners. The resulting defense mechanisms that plants developed against bacterial pathogens rely, to a large extent, on an enormous variety of small-molecule antimicrobials. Conceivably, such compounds could act as antibiotics. However, the antimicrobial activity of most plant-derived compounds is orders of magnitude lower than that of common antibiotics. Indeed, attempts to isolate potent antibiotics from plants have not provided any broad-spectrum silver bullet compound, even though large-scale screening campaigns have been undertaken by both pharmaceutical and biotech firms (68). Moreover, resistance mechanisms against antibiotics spread quickly throughout bacterial populations. Therefore, it is unlikely that plants commonly resist pathogens through the production of antimicrobial compounds and instead have evolved more subtle and effective methods. Such methods involve the specific targeting of bacterial virulence traits rather than bacterial viability, thereby minimizing the risk of the development of bactericide resistance (135). A plausible target for such an inhibition strategy is the bacterial quorum sensing (QS)-based virulence gene regulation machinery. This is because, in some pathogens, QS regulates up to 25% of all genes, and it is responsible for the shift from saprophytic growth to pathogenic growth (72). Resistance to bacterial pathogens that rely on QS correlates with QS inhibition by plant metabolites in some plant species (53). In this review, we discuss the evolution of QS and the corresponding QS proteins within the top 10 most economically important plant-pathogenic bacteria and the plant-derived small molecules that inhibit them. We also provide insight into the mode of action of these inhibitors.

QUORUM SENSING: THE MASTER REGULATOR OF VIRULENCE IN PLANT-PATHOGENIC BACTERIA

The virulence activities of an individual bacterium are often ineffectual and, consequently, bacteria must synchronize bacterial gene expression among multiple cells. To coordinate gene expression, bacteria use a population density–dependent regulatory mechanism known as QS. This sophisticated cell-to-cell communication system allows bacteria to monitor the environment and modulate the expression of virulence genes via the use of diffusible extracellular signaling molecules known as autoinducers, which increase in concentration with increasing bacterial cell density (35). These systems are typically regulated by a positive feedback loop, allowing swift increases in QS expression once the system has been triggered.

Proteobacteria is a major phylum of Gram-negative bacteria that contain QS systems that utilize *N*-acyl-homoserine lactones (AHLs) as signaling molecules. These AHLs have a core invariant *N*-acylated homoserine lactone ring (HSL) attached to an acyl chain that varies in length from 4 to 18 carbon atoms. The acyl chain can differ in the degree of saturation and oxidation state. AHLs are synthesized from the precursors *S*-adenosyl-L-methionine (SAM) and a fatty acid by LuxI, and their signal is perceived upon binding to LuxR, which is a cytoplasmic transcriptional regulator (35–37, 126). The affinity of LuxR for DNA changes upon binding to

AHL, thus promoting the expression of LuxR-regulated genes. In plant-pathogenic bacteria, exceeding a threshold level of a signaling molecule results in the synchronized expression of virulence genes such as those leading to biofilm formation, plant-cell-wall-degrading enzyme (PCWDE) production, antibiotic production, and Hrp type III protein secretion (72).

The first and most studied QS machinery is in the marine bacterium *Vibrio fischeri*, where it controls bacterial bioluminescence through the signaling molecule *N*-(3-oxo-hexanoyl)-L-homoserine lactone (3-oxo-C6HSL) (78, 103). Among plant-pathogenic bacteria, early studies were of the QS-controlled transfer of the Ti plasmid in *Agrobacterium tumefaciens* (*Agt*), the QS systems of soft-rot Pectobacteriaceae, the hierarchic QS system of *Pseudomonas* species, including that of the opportunistic plant and animal pathogen *Pseudomonas aeruginosa* (*Psa*), and the QS system of *Xanthomonas*, which is based on fatty acid diffusible signaling factors and regulates several virulence factors and does not produce AHL (107, 126). A simple model of a complete QS circuit in plant-pathogenic bacteria is that of *Pectobacterium* spp. (formerly *Erwinia*) (74), which includes the AHL autoinducer synthase ExpI and two or more transcriptional regulators including ExpR1 and ExpR2 (72, 97, 110). ExpR, along with *Psa* LasI/LasR QS circuit components, was used in this review to further elucidate protein active sites and their potential interaction with plant compounds.

Evolutionary Perspectives of Quorum Sensing Proteins

One broadly accepted way to rank the importance of plant-pathogenic bacteria is based on their virulence and the economic risk they impose on cultivated crops (76). Six of the top 10 phytopathogenic species (76), as well as many other important pathogenic bacteria, rely on a LuxI→AHL→LuxR–based QS to mediate cell-to-cell communication and coordinate virulence. Because LuxI/LuxR-based QS systems dominate the virulence regulation in many pathogenic bacteria, LuxI/LuxR and their homologs have gained attention as potential targets for QS inhibition (13, 52, 91). To understand their evolution and distribution, we constructed phylogenetic trees based on protein sequences of AHL synthases (LuxI and homologs) and their cognate response regulators (LuxR and homologs) (67).

Maximum-likelihood trees of these evolutionarily conserved families and LuxR/I proteins from representative bacterial species of various families were built and analyzed (**Figure 1**). AHL is the signaling molecule for LuxI/LuxR-based QS systems and is synthesized by proteins in the LuxI family. LuxI is sporadically distributed within and among bacterial families, and the phylogeny of LuxI differs from that of the species themselves, suggesting that LuxI is horizontally acquired and subject to repeated insertion/deletion events. For example, *Dickeya* and *Pectobacterium* are closely related genera with similar virulence strategies (74), but their LuxI homologs are not monophyletic. Strains that harbor multiple LuxI homologs tend to encode distantly related LuxI sequences, suggesting that different AHLs are produced and/or the LuxI proteins would be susceptible to different host inhibitors. Comparisons of predicted functional residues among LuxI paralogs support this hypothesis. For example, one of the *Ralstonia solanacearum* LuxI homologs in strain GMI1000 encodes the conserved arginine shown as residue 31 in **Figure 2*a***, whereas the other LuxI homolog in this strain has a glutamine in this location.

Many species that lack *luxI* still encode LuxR homologs and species harboring *luxI* often encode one or more solo LuxR that are not adjacent to a *luxI* gene. Our phylogenetic analysis shows the same sporadic distribution of LuxR homologs as seen with LuxI, again suggesting that insertion/deletion of these genes is common. Solo *luxR* are often interspersed with *luxR* genes encoded adjacent to *luxI*, and the patterns suggest that *luxRI* partners may be acquired and the cognate *luxI* subsequently lost even though the *luxR* partner is retained. For example, the solo *Pectobacterium*

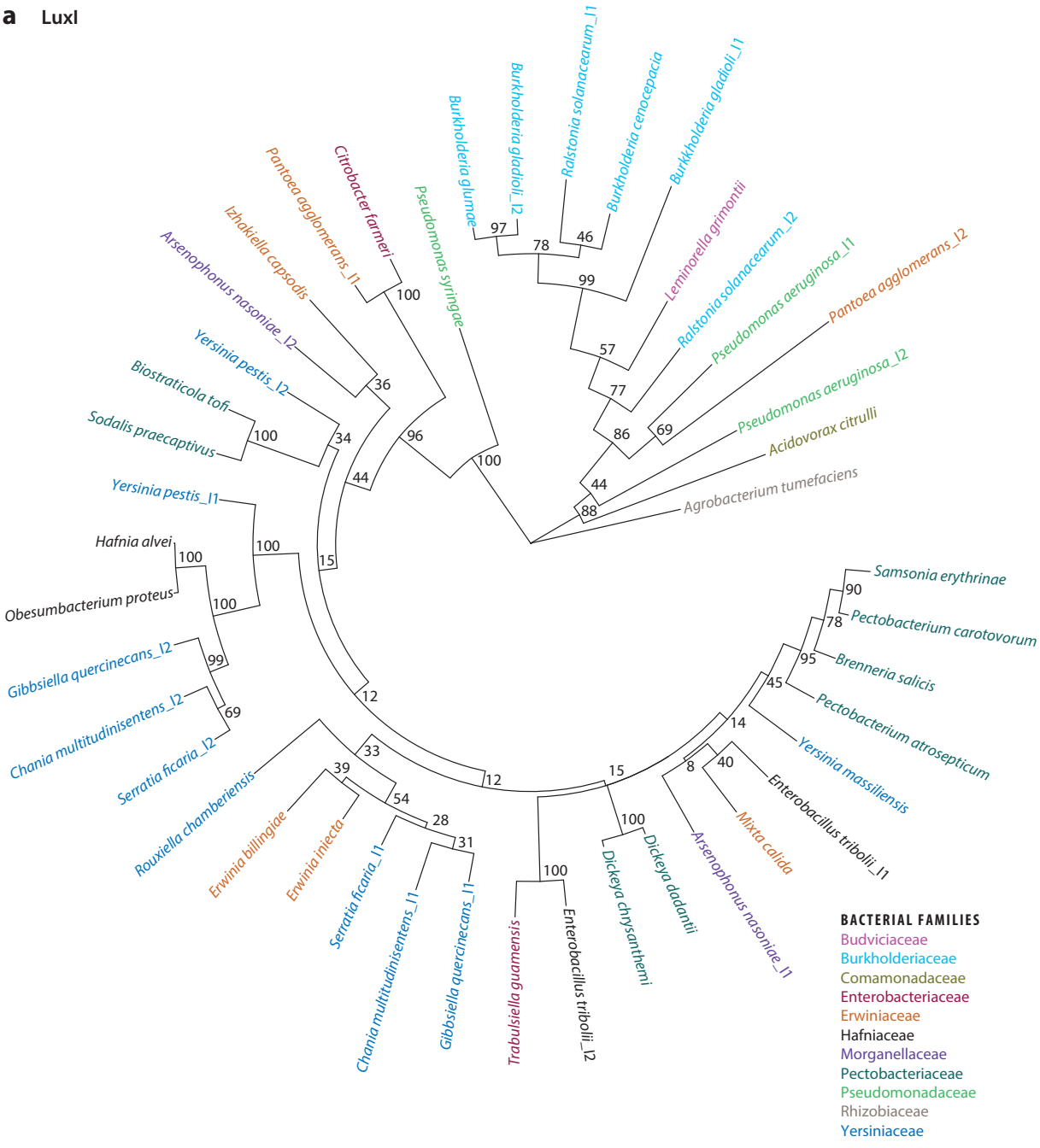


Figure 1

Continued

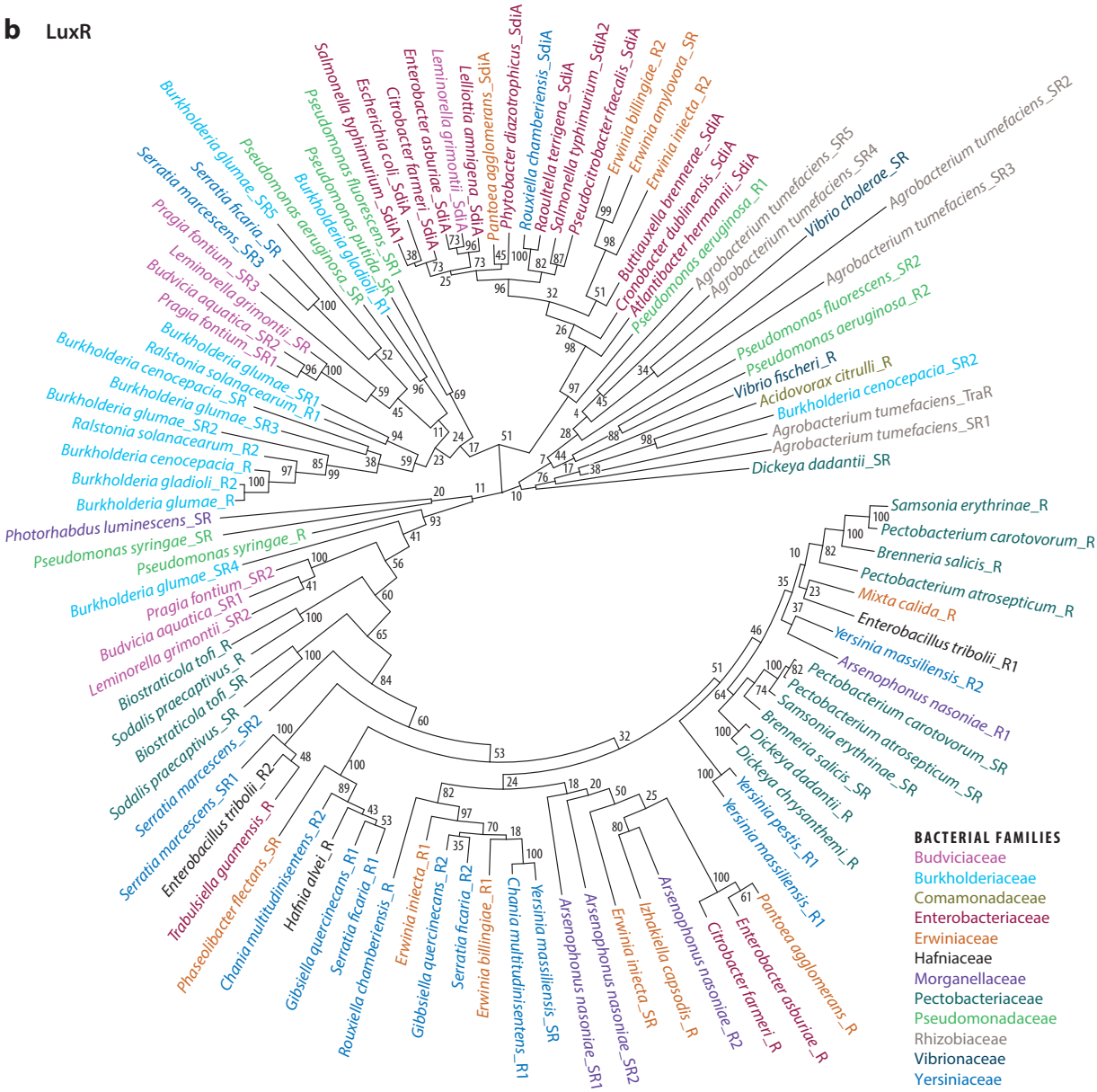


Figure 1

A maximum-likelihood tree with 500 bootstrap replicates using MEGA software (39) constructed based on the alignment of (*a*) LuxI and (*b*) LuxR protein sequences. Bootstrap values are shown as numbers at branch nodes. All sequences were retrieved from the ASAP database (**http://asap.genetics.wisc.edu/asap/home.php**), and type strains were used when possible. Different colored text indicates different bacterial families (Burkholderiaceae, Legionellales, Pseudomonadaceae, Erwiniaceae, Comamonadaceae, Rhizobiaceae, Yersiniaceae, Enterobacteriaceae, Pectobacteriaceae, Morganellaceae, Hafniaceae, Vibrionaceae, Budviciaceae, Listeraceae), and tip labels are written as genus and species. The LuxI or LuxR homolog number is indicated if there is more than one. The LuxI number does not necessarily correlate with the LuxR number in these two figures. In the LuxR tree, R indicates a LuxR homolog encoded adjacent to a LuxI homology and SR indicates solo LuxR. SdiA is a class of LuxR homologs found primarily in Enterobacterales that do not encode LuxI.

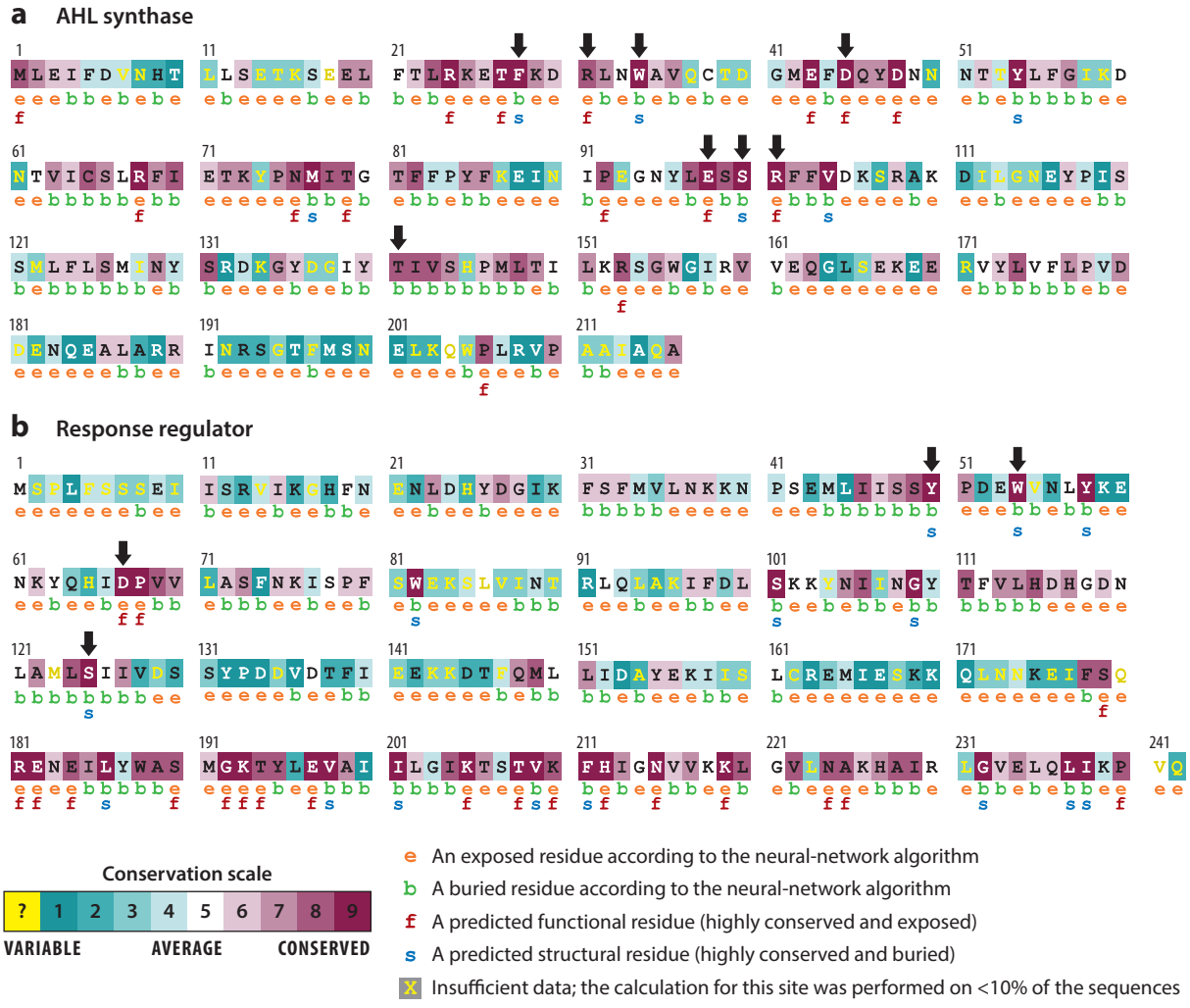


Figure 2

ConSurf server prediction of conserved residues according to the sequence alignment of (*a*) *N*-acyl-homoserine lactone (AHL) synthase and (*b*) response regulator (*luxR* homologs) in 9 of the top 10 plant-pathogenic bacteria (*Pectobacterium carotovorum*, *Erwinia amylovora*, *Pectobacterium atrosepticum*, *Pseudomonas aeruginosa*, *Pseudomonas syringae*, *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Dickeya solani*, and *Dickeya dadantii*) and other important plant pathogens (*Pectobacterium parmentieri*, *Erwinia carotovora*, *Pectobacterium aroidearum*, *Pectobacterium carotovorum* subsp. *betavascularum*, *Dickeya zeae*, *Pantoea stewartii*, *Acidovorax citrulli*, and the model bacterium *Vibrio fischeri*) that use AHLs as quorum sensing signaling molecules. The amino acid sequence presented is from *Pectobacterium parmentieri* SCC3193 (formerly *Pectobacterium wasabiae* SCC3193), and the amino acids interacting with the natural precursors (*S*-adenosyl-L-methionine and acyl carrier protein) are marked with arrows. Residue coloring is based on the conservation scale.

luxR clusters with the *Dickeya luxR* homolog encoded adjacent to a *luxI* gene, suggesting that the common ancestor of *Pectobacterium* and *Dickeya* acquired a *luxRI* cluster and that *Pectobacterium* later lost the *luxI* paralog but retained the now solo *luxR*. A closer examination of genomes shows that in some cases remnants of a *luxI* gene are evident next to the solo *luxR*, suggesting that at least some solo *luxR* may have previously had a cognate *luxI*. As with LuxI, variation in LuxR residues predicted to bind the AHL ligand is common. Altogether, the phylogeny of LuxR/LuxI

suggests that there is substantial pressure on bacterial pathogens to diversify LuxR/I systems, which is somewhat surprising because this bacterial signal usually regulates large numbers of genes in plant pathogens. This high level of variation that does not follow taxonomic relationships is not seen with other important regulators, such as sigma factors, two-component system proteins, and flagellar regulators such as FlhDC.

Having discussed the evolutionary aspects of bacterial QS, we now turn our attention to structural elements composing these machineries, namely the LuxI and LuxR proteins, with a special focus on important plant-pathogenic bacteria and a model bacterium for QS studies, *V. fischeri*.

LuxI-Type Proteins

LuxI family proteins are AHL synthases that catalyze the formation of AHL from SAM and acyl–acyl carrier protein (acyl-ACP) by acylation of SAM and lactonization of the methionine moiety (18). The mechanism driving the production of a certain AHL is mainly dependent on the LuxI-type protein and can be modulated by the external environment (130). A transcriptomic study based on a mutant in AHL synthase, ExpI of *Pectobacterium atrosepticum*, revealed that 26% of the transcriptome was under QS control, demonstrating that AHL is analogous to a major developmental hormone (72). These genes included those encoding major secretion systems such as the type I and II secretion systems (T1SS and T2SS), which are involved in the secretion of PCWDEs, the type VI secretion system (T6SS), and the Hrp type III secretion system (T3SS) and associated effector proteins, which play roles in the manipulation of plant defenses (41, 70, 95, 110). These studies also revealed effects of AHL signaling on secretion systems and overall virulence in *Vibrio harveyi*, *Psa*, and *Escherichia coli*.

In this review, we used the sequences of LuxI-type proteins of the top 10 plant-pathogenic bacteria, along with a few other noteworthy plant pathogens (76), as input for the ConSurf server (**http://consurf.tau.ac.il/2016/**). ConSurf performs a multiple sequence alignment of the input sequences followed by a conservation pattern analysis. ConSurf results are presented in **Figure 2*a*** and demonstrate the presence of conserved binding sites for both SAM and the ACP consisting of residues Phe27, Arg30, and Trp33 (SAM) and Asp45, Glu98, Ser100, Arg101, and Thr141 (ACP). These observations are later reinforced by an analysis of docking simulations (see below).

LuxR-Type Proteins

The LuxR protein family includes transcriptional regulators that upon binding of AHL modulate the transcription of their target genes. LuxR-type proteins have a DNA-binding domain and thus function as signal receptors and response regulators that typically activate transcription of virulence genes (97). Binding of AHL to LuxR-type proteins induces a conformational change that alters the protein's affinity for specific DNA sequences, or downstream proteins, acting as positive or negative regulators of gene expression (73). In several bacteria, such as *V. fischeri* and *Psa*, transcription is activated via an AHL-induced LuxR/LasR stable multimer (117).

The number of LuxR-type proteins in each bacterium is often higher than that of LuxI, a fact that has been linked to their capability to respond to the different signaling molecules found in mixed bacterial communities (123). These additional *luxR* genes are encoded elsewhere in the genome and are phylogenetically distant to LuxR that are paired with LuxI (**Figure 1**). Their phylogenetic distinctiveness provides further support that orphan LuxR play a different role in bacterial physiology than LuxR/LuxI pairs.

Molecular analyses of the family β-proteobacteria have shown that LuxR-type proteins such as *Pectobacterium carotovorum* (*Pc*) ExpR and VirR or *Erwinia stewartii* EsaR bind to the promoter region of their target DNA in the absence of the inducer molecule. In these cases, the protein is

shown to act as a repressor rather than a transcription activator (67, 14). Another example is ExpR, which binds to the promoter region of *rsmA*, thereby enhancing its transcription (14, 97). The Rsm system consists of RsmA, which is able to complex with mRNA of PCWDE genes to trigger their degradation by RNAses, and a small noncoding sRNA-*rsmB*, which regulates RsmA by preventing its activity by derepressing PCWDE transcription (23, 24). The link between ExpR and *rsmA* has been supported by mutational analysis of ExpR, where mutant *expR* yielded lower levels of RsmA and higher levels of exoenzymes (22). This confirmed *rsmA* as a negative regulator of the PCWDE genes *celV1*, *pehA*, *pelB*, *pel2*, and *prtW* (23, 24). The RNA fragments of the *rsmA* gene coupled with the RNA fragments from the PCWDE genes trigger decay by RNAses (61). Chatterjee et al. (14) demonstrated two types of structurally and functionally distinct ExpR proteins in *Pectobacterium*, with ExpR of class I strains mostly interacting with 3-oxo-C8-AHL, whereas those of class II strains mostly bind 3-oxo-C6-AHL.

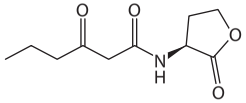
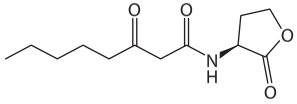
Binding of AHL forms a positive feedback loop that further promotes synchronization of population density (35, 85, 106). Consequently, downstream virulence activities such as PCWDE synthesis, biofilm formation, and antibiotic production are coordinated (97, 103, 131). These activities make the signal receptor the most attractive target for QS inhibition, as compounds that competitively block the binding of natural ligands are expected to considerably attenuate virulence.

ConSurf analysis was also performed on LuxR-type proteins of the top 10 pathogenic bacteria and a few other plant pathogens. The results are presented in **Figure 2*b*** and demonstrate a conversation pattern across residues. Many of these residues (e.g., Tyr50, Trp54, Asp67, Ser125) were implicated in ligand binding as predicted by docking simulations performed on ExpR from *Pc* [homology modeled after LasR (PDB code: 2uvo.pdb)] (52). These findings were reinforced by additional docking simulations (see **Table 1**).

PLANT COMPOUNDS WITH ANTIVIRULENCE ACTIVITIES

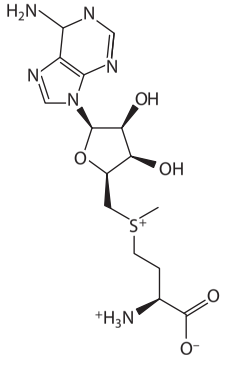
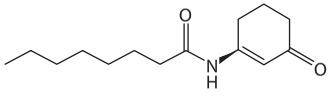
The sessile nature of plants, which exposes them to constant microbial pressure, might be the driving force behind their development of an array of active compounds with various degrees of antimicrobial activity. A vast number of bioactive metabolites have developed in plants through evolution. Of these, several hundreds of thousands are low-molecular-weight organic compounds that are not essential for basic metabolic processes and yet confer some degree of antimicrobial activity (32). Many such compounds have been associated with different roles in plant defense against pathogenic microbes or with beneficial or symbiotic microorganisms (123). Most of the defense-related molecules are derived from the isoprenoid, phenylpropanoid, alkaloid, or fatty acid/polyketide pathways (32). These pathways are responsible for the synthesis of different classes of antimicrobial molecules, including terpenes, organosulfur compounds, alkaloids, phenolics, coumarins, and saponins (32, 56). In addition, several plant species secrete AHL mimics that have been found to act as agonists or antagonists of QS systems. Most such AHL mimics remain unidentified, but their activities from secretions and extracts of plants such as pea, tomato, rice, soybean, crown vetch, carrot, water lily, habanero (chili), and garlic (60, 64, 99, 100, 115, 116) and marine algae such as *Delisea pulchra* and *Ulva lactuca* (64) have been reported. In the past several years, an increasing number of plant-derived compounds have been reported to have an inhibitory effect on QS or QS-related virulence factors in plant-pathogenic bacteria as well as on the opportunistic pathogen *Psa* (8, 15, 16, 88). Plant phenolics, more than any other group of molecules, are reported as potent QS inhibitors. Some have been shown to alter bacterial gene expression, whereas others use QS reporter strains or QS virulence determinants to document inhibition of QS. From a chemical perspective, phenolics share a phenol ring with one or more additional hydroxyl (−OH) groups, which are considered to contribute most of the antioxidant,

Table 1 Results of docking simulations for known plant-derived compounds that specifically interfere with quorum sensing (QS) and/or QS-dependent virulence determinants in Gram-negative bacteria[a]

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
C6-AHL  **O=C1[C@@H](NC(CC(CCC)=O)=O)CCO1**	**−6.4** T27, K106, R31, F103, F102, F124, F123, M147, L151, I150	**−7.2** T111, W54, S101, I106, V70, V69, W82, P68, D67, S125, Y50, M123, Q64, I46, M44, P41, S32, K30, Y58	**−6.0** F82, S98, S99, R100, F101, M77, T140, I141, V142, S143, M146, V67, F123, M126, W155, L150, L176	**−8.5** G143, V144, T145, F146, M149, F153, D114, W33, L31, L102, S103, R104, F105, A106, A107, F27, V26	**−7.9** I52, A50, Y47, S129, A127, A70, D73, P74, T75, V76, Y56, W88, T115, Y93, F101, A105, L110, W60, R61, Y64, L36, G38	Natural ligand or control	Proteo-bacteria	18
C8-AHL  **O=C1[C@@H](NC(CC(CCCCC)=O)=O)CCO1**	**−4.3** D45, Y47, R101, S100, T81, F82, I142, V143, E170, V172, S144, S166, W34, M147, L32, M77	**−7.0** I97, F98, S101, I106, Q64, W54, D67, P68, V69, V70, I46, M44, P41, M34, S32, K30, M123, S125, Y58, T111, Y50, W82	**−6.0** F82, M77, T140, I141, V142, S143, M146, L150, F123, M126, V67, L176, L174, W155, S98, S99, R100, F101	**−7.0** L31, W33, F27, V26, M149, F146, T145, V144, G143, I142, L141, F153, L126, I179, W178, C177, L102, S103, F105, A106	**−8.0** C79, V76, T75, D73, Y47, W60, R61, Y64, A105, F101, L125, G126, A127, S129, Y56, W88, Y93, L40, G38, L36, I52, T115	Natural ligand or control	Proteo-bacteria	17, 18

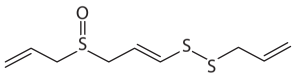
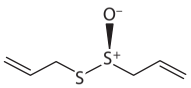
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
***S*-adenosyl methionine**  **NC1=NC=NC2=C1N=CN2[C@@H]3O[C@H](C[S+](C)CC[C@H]([NH3+])C([O-])=O)[C@H](O)[C@@H]3O**	**−6.2** Y47, F82, T81, D45, W34, M77, R101, S100, F102, V143, I142, S144, M147, L32, E170, E43, F28	**−1.8** I46, M44, P41, Y58, W54, K30, S32, F33, M34, H115, L114, V113, T111, L121, A122, M123, S125, F74, S73, V70, V69, Y50, D67, Q64, I106	**−4.0** M77, F82, T81, F47, E46, D45, R68, M42, S99, R100, F101, T140, I141, V142, M146	**−6.0** I169, D170, R172, W33, L31, L102, S103, R104, F105, A106, V144, T145, F146, M149, M152, D114, F27, V26, L28, F83	**−3.8** W60, R61, Y64, A70, D73, T75, V76, C79, I52, A50, Y47, L125, G126, A127, S129, L40, L39, G38, L36, T115, Y56, Y93, W88, F101, L110, A105	Natural ligand or control	Proteo-bacteria	18
J8-C8  **O=C1C=C(NC(CCCCCCC)=O)CCC1**	**−3.3** V172, E170, S166, L32, F82, W34, T81, D45, Y47, M77, R101, F28, F102, S100, I142, V143, M147, S144	**−5.4** Q64, D67, V69, V70, P41, M44, I46, M34, S32, K30, M123, S125, Y50, Y58, W54, S101, I106, W82, I111	**−5.0** T140, I141, V142, S143, M146, L150, V67, F123, M126, W155, L176, S98, S99, R100, F101, M77, F82	**−6.6** L102, S103, F105, A106, A107, D114, F153, M152, M149, S148, F146, T145, V144, G143, I142, L141, L126, C177, W178, I179	**−7.0** A105, L110, F101, V76, T75, D73, A70, W60, R61, Y64, I52, A50, Y47, L40, G38, L36, A127, S129, T115, Y56, Y93, W55	Known inhibitor of acyl-HSL synthase TofI	*Burkholderia glumae*	17

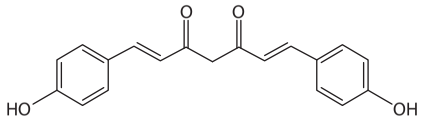
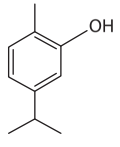
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
Ajoene  **C=CCS(C/C=C/SSCC=C)=O**	**−4.3** T27, F28, L32, F102, F103, V104, K106, S120, F123, F124, T141, V143, M147, I150, L151, W156, L177	**−0.2** K30, S32, M34, P41, M44, I46, Y50, W54, Q64, D67, V69, V70, S73, S101, I106, T111, L121, A122, M123, S125	**−3.4** V67, M77, S98, S99, R100, F101, F123, M126, T140, I141, V142, S143, M146, L150, W155, L176	**−4.1** F27, L31, W33, L102, S103, R104, F105, L141, I142, G143, V144, T145, F146, M149, F153, C177, W178, I179	**−3.3** L36, G38, L39, L40, Y47, A50, I52, R61, Y64, A70, D73, T75, V76, C79, T80, T115, L125, G126, A127, S129	Elastase activity, entero-toxin synthesis QS, LuxR family	*Psa*, *Escherichia coli*	71
Allicin  **C=CCS[S@@+]([O-])CC=C**	**−4.4** F102, F103, V104, S120, F123, F124, T141, V143, M147, I150, L151, W156, L177	**−3.6** K30, S32, M34, P41, M44, I46, Y58, K59, Q64, V70, S73, F74, V113, H115, L121, A122, M123	**−3.1** L12, E18, E19, G41, M42, S44, D48, Y54, L56, L63, S66, V67, R68, S99, R100, F102	**−4.2** V26, F27, L31, W33, L102, S103, R104, F105, A106, A107, V144, T145, F146, M149, F153	**−3.3** L36, G38, Y56, W60, Y64, D73, T75, V76, W88, Y93, F101, A105, L110, T115, A127, S129	Biofilm formation, EPS secretion, QS	*Psa*	71

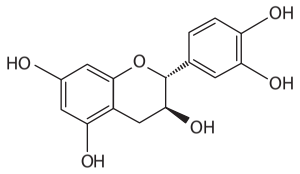
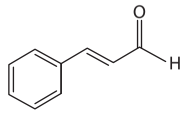
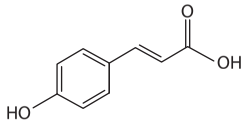
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
Bisdesmethoxycurcumin  **OC1=CC=C(/C=C/C(CC(/C=C/C2=CC=C(O)C=C2)=O)=O)C=C1**	**−6.4** M147, P146, S144, I142, V143, F28, F102, V172, F82, T81, D45, Y47, R101, M77, S100, L32, W34	**−8.7** I106, W82, S101, W54, Q64, D67, V69, V70, S73, S135, M123, A122, L121, M34, F33, S32, K30, Y58, M44, I46, Y50, T111	**−6.5** V67, W155, F123, M126, L176, L150, S98, S99, R100, F101, F102, V103, M42, M146, S143, V142, I141, T140	**−11.6** M149, S148, F146, T145, V144, G143, I141, I142, A107, A106, F105, R104, S103, L102, C177, I179, L126, F153, W33, L31, Q30, F27, V26, G112, D114	**−11.0** Y47, A50, I52, L40, L39, G38, L36, T115, L125, G126, A127, S129, Y64, R61, W60, W88, Y56, A105, V111, L110, Y93, I92, F101, D73, T75, V76, C79	QS, infection QS system inhibition	*Agt*	29
Carvacrol  **OC1=C(C)C=CC(C(C)C)=C1**	**−6.1** W34, D45, Y47, M77, T81, F82, S100, R101, F102, I142, V143, S144, M147, V172	**−6.2** K30, S32, F33, M34, P41, S42, M44, I46, V55, Y58, K59, Q64, V70, S73, L121, A122, M123	**−4.7** L12, E18, E19, G41, M42, E43, S44, D48, Y54, L56, L63, S66, V67, R68, R100, F102	**−6.5** W33, L78, F83, S103, R104, V144, T145, F146, I169, R172, V174	**−6.6** L36, G38, Y47, L39, L40, E48, A50, I52, R61, Y64, D65, A70, V76, A127, G126, L125	QS, biofilm, exoenzyme secretion AHL synthase	*Pectobacterium* spp., *Chv*	11, 52

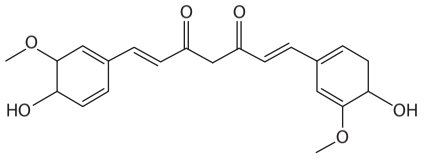
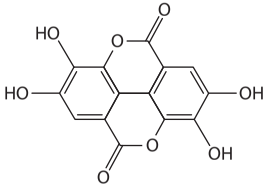
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected		
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action	Pathogens	Reference(s)
Catechin  **O[C@H]1CC2=C(O)C=C(O)C=C2O[C@@H]1C3=CC(O)=C(O)C=C3**	**−5.4** L32, W34, M77, T81, F82, S100, R101, F102, I142, V143, S144, S166, E167, E170, V172	**−6.9** K30, S32, M34, P41, M44, I46, Y50, W54, Y58, K59, Q64, D67, V69, V70, I106, T111, L121, A122, M123, S125	**−6.6** Y9, L12, Q13, S17, E18, E19, G41, M42, E43, S44, D45, D48, Y54, L56, L63, S66, V67, R68, R100	**−7.2** V26, F27, L31, W33, L78, L102, S103, R104, F105, A106, A107, D114, L118, G143, V144, T145, F146, S148, M149, M152, F153	**−8.2** L36, G38, Y47, E48, A50, I52, Y56, W60, R61, Y64, D65, A70, D73, T75, V76, W88, Y93, F101, A105, L110, T115, A127, S129	Violacein production, biofilm, pyocyanin and elastase activity, QS genes, QS response regulator	*Chv*, *Burkholderia* spp., *Psa* PAO1	10, 120
Cinnamaldehyde  **[H]C(/C=C/C1=CC=CC=C1)=O**	**−5.5** T27, F102, F103, V104, K106, S120, F123, F124, V143, M147, I150, L151	**−5.6** K30, S32, M34, P41, M44, I46, Y50, Y58, K59, Q64, V70, M123, S125	**−5.1** S98, S99, R100, F101, F123, M126, T140, I141, V142, M146, L150, W155, L176	**−6.8** W33, L78, S103, R104, V144, F146, I169, R172, V174	**−4.3** L36, Y56, W60, Y64, D73, T75, V76, W88, Y93, F101, A105, L110, T115, A127, S129	QS, biofilm, T3SS, QS genes, AHL synthase	*Psa*, *Dickeya dadantii*	13, 50, 118
Coumaric acid  **OC1=CC=C(/C=C/C(O)=O)C=C1**	**−4.5** W34, D45, Y47, M77, T81, F82, S100, R101, F102, I142, V143, M147	**−5.7** K30, S32, M34, P41, S42, M44, I46, Y50, W54, Y58, K59, Q64, V70, T111, M123, S125	**−5.2** S98, S99, R100, F101, F123, M126, T140, I141, V142, M146, L150, W155, L176	**−6.0** W33, L78, F83, S103, R104, V144, T145, F146, I169, R172, V174	**−6.1** L36, I52, Y56, W60, R61, Y64, D73, T75, W88, I92, Y93, F101, A105, L110, S129	Biofilm, T3SS, QS, AHL synthesis	*D. dadantii*	70

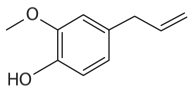
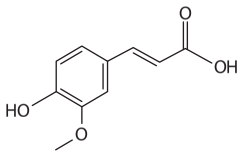
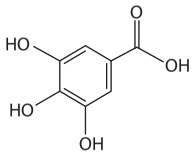
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected		
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action	Pathogens	Reference(s)
Curcumin  **OC1=C(OC)C=C(/C=C/C(CC(/C=C/C2=CC(OC)=C(O)C=C2)=O)=O)C=C1**	**−4.0** L32, W34, D45, Y47, M77, T81, F82, S100, R101, I142, S144, P146, M147, S166, E170	**−9.5** K30, S32, F33, M34, P41, M44, I46, Y50, W54, Y58, Q64, D67, V69, V70, S73, F74, W82, F98, S101, I106, G109, Y110, T111, L121, A122, M123, S125	**−4.3** Q40, G41, M42, M77, S99, R100, F101, F102, V103, T140, I141, V142, S143, F146	**−7.2** V26, F27, L31, W33, L78, F83, S103, R104, F105, A106, A107, D114, V144, T145, F146, S148, M149, M152, I169, D170, R172, V174	**−9.9** L36, G38, L39, L40, Y47, A50, F51, I52, Y56, W60, R61, Y64, A70, D73, P74, T75, V76, C79, T80, W88, I92, Y93, F101, A105, L110, T115, L125, G126, A127, S129	AHL synthesis, biofilm, EPS production, QS gene expression, swimming motility	*Vibrio* spp., *E. coli*, *Psa* PAO1, *Serratia marcescens*	88, 89
Ellagic acid  **O=C(OC1=C(O)C(O)=C2)C3=CC(O)=C(O)C4=C3C1=C2C(O4)=O**	**−10.5** W34, D45, Y47, M77, T81, F82, S100, R101, I142, V143, S144, M147, E170, V172	**−1.7** K30, Y50, W54, Y58, D67, V69, V70, W82, S101, I106, T111, M123, S125	**−4.2** M77, F82, S98, S99, R100, F101, T140, I141, V142, S143, M146	**−8.5** W33, L78, F83, R104, V144, T145, F146, I169, D170, R172, V174	**−7.8** L36, G38, Y47, A50, I52, Y56, W60, R61, Y64, A70, D73, T75, V76, C79, W88, F101, L110, A127, S129	Violacein production, biofilm, AHL synthesis QS response regulator	*Chv*, *Psa*, *E. coli*	5, 45, 105

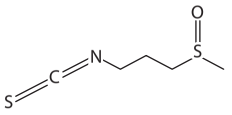
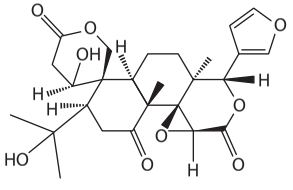
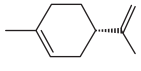
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
Eugenol  **OC1=C(OC)C=C(CC=C)C=C1**	**−6.1** T27, F102, F103,V104, K106, S12, F123, F124, V143, M147, I150, L151	**−6.7** K30, S32, F33, M34, P41, S42, M44, I46, Y58, K59, Q64, V70, S73, L121, A122, M123, S125	**−4.8** Y9, L12, E18, E19, C38, G41, M42, E43, S44, D48, Y54, L56, L63, S66, V67, R68, S99, R100, F102	**−6.5** W33, L78, F83, V144, T145, M146, I169, D170, R172, V174	**−5.8** L36, G38, L39, L40, Y47, E48, A50, I52, Y56, R61, Y64, D65, A70, D73, V76, C79, T80, L125, G126, A127, S129	QS, biofilm, elastase, pyocyanin synthesis, AHL synthase QS response regulator	*Pecto-bacterium* spp., *Psa*, *Chv*	52, 137
Ferulic acid  **OC1=CC=C(/C=C/C(O)=O)C=C1OC**	**−6.2** W34, D45, Y47, M77, T81, F82, S99, S100, R101, F102, T141, I142, V143, S144, M147	**−6.6** K30, S32, M34, P41, S42, M44, I46, Y50, W54, Y58, K59, V69, V70, Q64, A122, M123, S125	**−4.8** Y9, L12, E18, E19, L20, G41, M42, S44, Y54, L56, L63, S66, V67, R68, R100	**−5.5** W33, D45, L78, V82, F83, R104, V144, F146, I169, D170, R172, V174	**−7.1** L36, I52, Y56, W60, R61, Y64, D73, T75, W88, I92, Y93, F101, F102, A105, L110, T115, S129	QS, AHL synthesis	*Chv*	8
Gallic acid  **OC(C1=CC(O)=C(O)C(O)=C1)=O**	**−6.5** F28, L32, W34, M77, P82, S100, R101, F102, I142, S144, M147, V172	**−4.7** K30, Y50, W54, Y58, D67, V69, W82, I97, S101, I106, T111, M123, S125	**−5.5** L12, E18, E19, G41, M42, E43, S44, D48, Y54, L56, S66, V67, R68, R100	**−5.1** W33, L78, F83, V144, F146, I169, D170, R172, V174	**−7.2** L36, I52, Y56, W60, R61, Y64, D73, T75, V76, C79, W88, F101, L110, T115, A127, S129	Violacein produc-tion, biofilm, AHL synthesis	*Agt*, *Psa* PAO1, *Chv*	8, 96

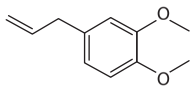
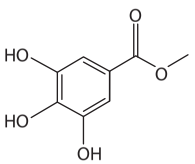
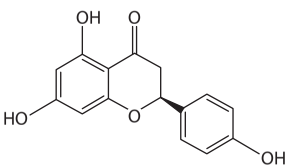
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
Iberin  **S=C=NCCCS(C)=O**	**−4.1** W34, I142, V143, R101, S100, F102, M147, F28, S144, F82, M77	**−4.2** V70, S73, Q64, L121, A122, M123, M34, S32, K30, I46, M44, P41, Y58, K59	**−4.5** L12, Y9, E18, E19, V67, S66, L63, L56, Y54, G41, M42, S44, D48	**−4.5** I179, C177, F153, W33, M149, F27, F146, T145, V144, G143, F105, S103, L102	**−4.1** A127, S129, L36, Y64, T115, Y56, W60, L110, F101, W88, A105, Y93, D73, T75, V76	Biofilm formation, rhamno-lipid biosynthe-sis, downreg-ulation of Gac/Rsm, LasR, RhlR	*Psa*	48, 114
Ichangin  **O=C1C[C@@]([H])(O)[C@]2([C@@](C(C)(O)C)([H])CC([C@]3(C)[C@]2([H])CC[C@]4(C)[C@]35[C@@](O5)([H])C(O[C@@]4([H])C6=COC=C6)=O)=O)CO1**	**−3.8** T27, R31, L32, F102, F103, V104, K106, S120, F123, F124, P146, M147, I150	**−2.8** K17, N20, M44, I45, I46, I47, S48, S49, Y50, D52, V55, N56, K59	**−4.1** M42, R68, M77, F82, S99, R100, F101, F102, V103, I141, V142, S143, M146, L165, I171	**−5.8** W33, Q34, D45, Q46, Y47, L78, V82, F83, R104, V144, I169, R172	**−3.2** K16, L17, S20, Q24, E48, N49, A50, F51, I52, V53, G54, N55, Y56, A58, R61, E62, D65	QS response regulator *luxO*	*Vibrio harveyi*	124
Limonene  **C=C(C)[C@H]1CCC(C)=CC1**	**−5.6** K106, S120, V104, F103, F123, F102, M147, L151, V143, F124, I150	**−5.8** I46, M44, P41, Q64, K30, S32, F33, M34, M123, A122, L121, F74, S73, V113, Y58	**−3.5** S44, E43, M42, G41, C38, E18, E19, R68, V67, S66, S99, R100, F102, R68, L63, L56, Y54, D48, L12	**−1.4** L102, S103, R104, F105, F153, F27, W33, L31, M149, F146, T145, V144, G143	**−5.1** V76, C79, A127, G126, L125, L36, G38, L39, L40, I52, A50, Y47, A70, R61, Y64, D65	Motility, biofilm, auto-inducer-2	*E. coli* O157:H7	128

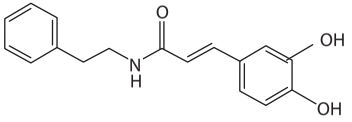
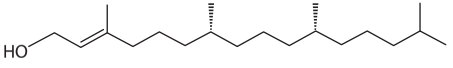
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
Methyl eugenol  **C=CCC1=CC=C(OC)C(OC)=C1**	**−4.7** T81, W34, R101, M77, D45, Y47, S99, S100, V143, T141, M147, F102, I142, F82	**−6.0** Y50, I46, M44, S42, P41, K59, Y58, V55, K30, S32, F33, M34, Q64, L121, A122, M123, S125, V70, V69	**−4.4** W155, L176, M126, F123, M146, S143, V142, I141, T140, L150, M77, F101, R100, S99, S98	**-4.5** F27, W33, F105, R104, S103, L102, C177, I179, I142, G143, V144, T145, F146, M149, F153	**−5.2** S129, A127, T115, L36, Y64, I52, R61, W60, A105, W88, I92, Y93, D73, P74, T75, V76, F101, L110, Y56	Biofilm, QS, EPS production, QS inhibition	Gram-negative bacteria	87
Methyl gallate  **OC1=CC(C(OC)=O)=CC(O)=C1O**	**−6.4** T81, M77, R101, S100, I142, M147, F102, V143, V172, S144, F82, W34	**−5.5** W54, Y50, Y58, K30, S32, M123, S125, T111, V69, D67, I106, W82, S101	**−5.7** D48, D45, S44, M42, G41, R100, R68, S66, L63, L12, V7, Y54, L56, E19, E18	**−5.8** F83, L78, V174, R172, D170, I169, W33, V144, T145, F146	**−6.4** T115, D73, T75, V76, S129, Y56, W88, A105, F102, F101, Y93, L110, Y64, R61, W60, L36	QS, biofilm AHL synthase	*Chv*, *Psa*	44
Naringenin  **OC(C=C1)=CC=C1[C@H](C2)OC3=CC(O)=CC(O)=C3C2=O**	**−5.4** R101, S100, M77, Y47, D45, T81, F82, W34, E170, S166	**−7.3** P41, S42, M44, I46, Y58, K59, W54, Q64, D67, V69, V70, T111, S125, M123, A122, M34, S32, Y50, K30, I106	**−5.3** M77, F101, R100, S99, S98, M146, L150, V142, I141, T140, M126, F123, W155, L176, V67, M42	**−8.0** I169, D170, R172, V174, F83, L78, R104, S103, V144, T145, F146, W33, Q34	**−8.1** Y64, R61, W60, I52, A50, Y47, L36, G38, L39, L40, L125, G126, A127, S129, C79, V76, T75, W88, D73, A70, T115, F101, L110, Y56	QS, biofilm, T3SS, AHL production and signaling, QS-controlled genes	*V. harveyi*, *E. coli* (O157:H7), *Psa*	4, 121

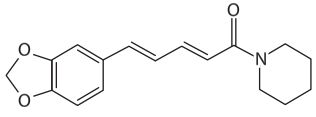
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
Phenylethylcinnamamide  **OC1=CC(/C=C/C(NCCC2=CC=CC=C2)=O)=CC=C1O**	**−7.1** F102, K106, T27, R108, D105, E26, R31, A107, F103, V104, F123, S120, I150, M147, F124, L151, F102	**−8.9** M34, F33, S32, K30, Y58, Y50, I106, S101, W54, Q64, D67, V69, V70, S73, F74, L121, A122, M123, S125, T111, P41, M44, I46	**−8.2** L165, I171, M146, S143, V142, I141, T140, W155, L150, L176, F123, M126, S98, S99, R100, F101, F82, M77	**−6.5** F153, G143, V144, T145, F146, M149, W33, L31, D114, A113, G112, L118, P109, A107, A106, F105, R104, S103, L102, F27	**−10.4** A70, T115, D73, T75, V76, F101, W88, I92, Y93, A105, Y56, L110, W60, R61, Y64, Y47, A50, I52, L40, L39, G38, L36, L125, G126, A127, S129	QS, virulence QS system inhibition	*Agt*	29
Phytol  **OC/C=C(C)/CCC[C@H](C)CCC[C@H](C)CCCC(C)C**	**−5.6** F103, L32, F28, F102, V143, M147, L151, I150, F124, F123, S120, K106, V104, D105, A107, T27, R31	**−5.9** S101, K30, S32, M34, I126, S125, M123, A122, L121, Y50, F74, S73, V70, V69, D67, Q64, P41, M44, I46, K59, Y58, W54, W82, T111, Y110, G109, I106	**−3.6** F101, R100, S99, S98, W155, L150, L176, F123, M126, V67, T140, I141, V142, S143, M146, F82, M77, I171, M42	**−2.1** V174, R172, I169, W33, L31, F83, L78, S103, R104, F105, A106, A107, P109, V26, F27, L18, M152, M149, S148, F146, T145, V144, D114	**−2.8** A70, D73, T75, V76, T115, Y93, I92, A105, F102, F101, W88, L110, V111, W60, R61, Y64, Y56, I52, A50, Y47, L36, G38, L39, L40, S129, A127, G126, L125	Biofilm, motility, pyocyanin	*Psa* PAO1	94

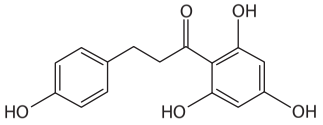
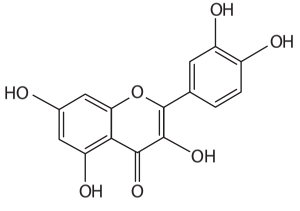
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
Pinene **CC1=CC[C@@H]2C(C)(C)[C@H]1C2**	**−5.0** K106, M147, L151, I150, F124, F102, S120, F123, F103, V104	**−4.1** Y58, K59, K30, S32, F33, M34, L121, A122, M123, F74, K39, V70, P41, M44, I46, Q64	**−3.3** R100, D48, L12, E19, E18, Y54, L56, C38, G41, M42, E43, S44, L63, S66, V67, R68	**−5.9** L78, F83, R104, L86, V144, F146, V174, W33, R172, I169	**−5.6** V76, C79, L125, G126, A127, Y47, A50, F51, I52, L36, G38, L39, L40, D65, Y64, R61, A70	Coloniza-tion, QS signaling	*Campylo-bacter jejuni*	108
Piperine  **O=C(N1CCCCC1)/C=C/C=C/C2=CC3=C(C=C2)OCO3**	**−3.4** Y47, Q46, D45, W34, F82, I142, V172, V143, S144, L32, M147, F102, R101, S100, F28, M77, T81	**−7.3** S125, M123, A122, L121, I46, M44, P41, S73, V70, V69, D67, K30, Q64, M34, F33, S32, S101, Y58, W54, I106, W82, T111, Y50	**−7.1** F82, M77, I171, L165, F101, R100, S99, S98, W155, L150, F123, M126, L176, V67, M146, S143, V142, I141, T140	**−5.3** W33, V144, T145, V144, F146, F27, M149, F153, F105, R104, S103, L102, L78, F83, V174, R172, D170, I169	**−9.0** I52, A50, Y47, L36, G38, L39, L40, C79, V76, T75, D73, A70, W88, T115, F101, Y56, W60, R61, Y64, S129, A127, G126, L125	Violacein produc-tion	*Cbv*	122

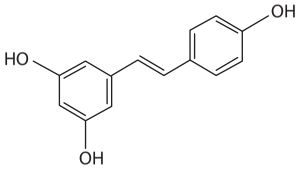
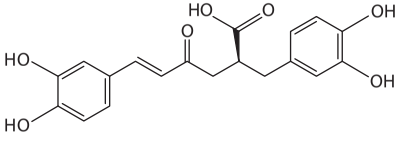
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
Phloretin  **OC1=CC=C(CCC(C2=C(O)C=C(O)C=C2O)=O)C=C1**	**−5.4** L32, W34, D45, Y47, M77, T81, F82, S100, R101, I142, V143, S144, M147, S166, E170, V172	**−7.1** K30, S32, M34, P41, M44, I46, Y50, W54, Y58, K59, Q64, D67, V69, V70, W82, F98, S101, I106, T111, M123, S125	**−8.0** Y9, L12, Q13, S17, E18, E19, C38, G41, M42, E43, S44, D48, Y54, I55, L56, S66, V67, R68, S99, R100	**−6.1** V26, F27, L31, W33, F102, S103, R104, F105, A106, A107, G112, D114, L118, G143, V144, T145, F146, M149, M152, F153	**−9.4** L36, G38, L40, Y47, A50, I52, Y56, W60, R61, Y64, D65, A70, D73, T75, V76, W88, F101, A105, L110, T115, A127, S129	Biofilm	*E. coli* (O157:H7)	65
Quercetin  **OC(C(O)=C1)=CC=C1C2=C(O)C(C3=C(O)C=C(O)C=C3O2)=O**	**−10.1** E170, V172, S144, V143, I142, F82, W34, T81, Y47, D45, M77, R101, S100	**−7.7** P41, M44, I46, K59, Y58, M34, S32, K30, Y50, W54, I106, V70, V69, D67, Q64, L121, A122, M123, S125, T111	**−7.2** Y9, L12, L56, Y54, S17, E18, E19, D48, L63, S66, V67, R68, R100, S44, M42, G41, S39, C38	**−5.7** G143, V144, T145, F146, S148, M149, M152, F153, F27, V26, D114, W33, L31, A106, F105, R104, S103, L102	**−9.3** Y47, A50, I52, L40, L39, G38, L36, C79, V76, T75, D73, T115, W88, W60, R61, Y64, Y56, F101, L125, G126, A127, S129	EPS production, QS gene expression, biofilm QS response regulator	*Psa*	86

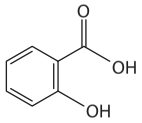
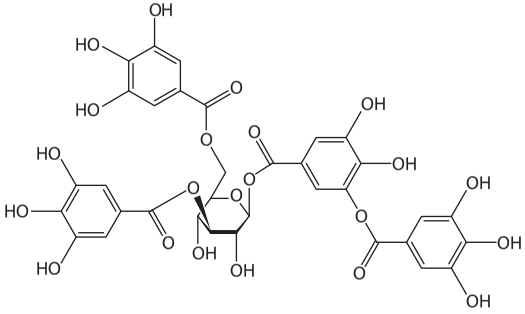
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected	Pathogens	Reference(s)
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action		
Resveratrol  **OC1=CC(/C=C/C2=CC=C(O)C=C2)=CC(O)=C1**	**−5.7** W34, D45, Y47, M77, T81, F82, S100, R101, I142, V143, S144, E170, V172	**−6.2** K30, S32, M34, P41, S42, M44, I46, Y50, W54, Y58, K59, Q64, D67, V69, V70, I106, T111, M123, S125	**−6.0** Y9, L12, Q13, S17, E18, E19, G41, M42, E43, S44, D48, Y54, L56, S66, V67, R68, S99, R100, F102	**−4.8** F27, Q30, L31, W33, S103, R104, F105, A106, A107, T108, P109, G112, A113, D114, L118, V144, T145, F146, M149, M152	**−5.9** L36, G38, L39, L40, Y47, E48, A50, I52, Y56, W60, R61, Y64, D65, A70, D73, T75, V76, W88, Y93, F101, A105, L110, T115, G126, A127, S129	Biofilm	*Psa*, *E. coli* O157:H7	15
Rosmarinic acid  **OC1=CC(/C=C/C(C[C@@H](C(O)=O)CC2=CC(O)=C(O)C=C2)=O)=CC=C1O**	**−6.7** F28, L32, W34, D45, Y47, M77, T81, F82, S100, R101, F102, I142, V143, S144, M147, E170, V172	**−9.7** K30, S32, F33, M34, P41, M44, I46, Y50, W54, Y58, K59, Q64, D67, V69, V70, S73, W82, S101, I106, T111, L121, A122, M123, S125	**−7.2** M42, M77, S98, S99, R100, F101, F102, V103, D104, K105, T140, I141, V142, S143, M146	**−7.1** V26, F27, L31, W33, L78, F83, S103, R104, F105, A106, D114, V144, T145, F146, S148, M149, M152, I169, R172, V174	**−10.7** L36, G38, L39, Y47, A50, I52, Y56, W60, R61, Y64, A70, D73, T75, V76, C79, W88, I92, Y93, F101, A105, L110, T115, L125, G126, A127, S129	Biofilm formation, pyocyanin production and elastase, QS gene expression, QS response regulator 'RhlR,' HSL mimic	*Psa* PAO1	19

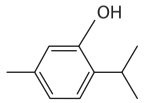
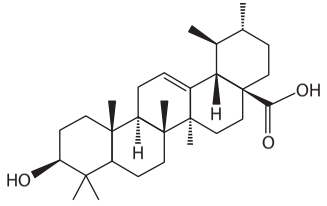
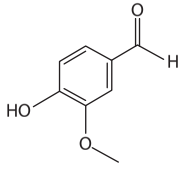
(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected		
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action	Pathogens	Reference(s)
Salicylic acid  **OC(C1=C(O)C=CC=C1)=O**	**−5.3** W34, M77, F82, S100, R101, F102, T141, I142, V143, S144, V172	**−5.9** K30, Y50, W54, Y58, D67, V69, V70, W82, S101, I106, T111, M123	**−4.6** L12, E18, E19, G41, M42, S44, D48, Y54, L56, L63, S66, V67, R68, R100	**−6.4** W33, L78, F83, S103, R104, F105, V144, T145, F146, I169, V174	**−1.1** Y56, W60, R61, Y64, D73, T75, W88, Y93, F101, A105, L110, T115, S129	QS, biofilm, exoenzyme secretion, AHL synthase 'ExpI'	*Pectobacterium* spp., *Psa*, *Agt*	13, 49, 51, 136
Tannic acid  **O[C@H]1[C@H](OC(C2=CC(OC(C3=CC(O)=C(O)C(O)=C3)=O)=C(O)C(O)=C2)=O)O[C@@H]([C@H](C1OC(C4=CC(O)=C(O)C(O)=C4)=O)O)COC(C5=CC(O)=C(O)C(O)=C5)=O**	**−13.5** T27, D30, R31, L32, N33, W34, K37, F102, F103, V104, D105, K106, A107, K110, G114, N115, S116, Y117, P118, V119, S120, S144, P146, M147, E170	**−10.4** R13, V14, I15, K16, K17, N20, K21, S42, Q43, I45, I46, I47, S48, S49, Y50, P51, D52, V55, N56, K59, E60, S160, L161, R163, E164	**−10.0** S98, S99, R100, F101, K105, R109, E114, T140, I141, V142, S143, R144, A145, M146, K148, I149, R152, L165, K168, E169, R170, I171	**−10.9** E7, A8, G9, D51, P72, T74, R75, P76, Q80, E81, E90, A93, P94, R95, S96, A97, H98, W100, R136, G137	**−11.1** D43, S44, Q45, D46, Y47, E48, N49, H63, A67, G68, Y69, R71, V72, P74, V76, S77, T80, Q81, I92, K97, Q98, E100, L125	QS, biofilm AHL synthesis	*Psa*, *Salmonella enterica*, *E. coli* O157:H7, *Pseudomonas putida*, *Chv*	13, 109

(*Continued*)

Table 1 (*Continued*)

Compounds and chemical structure	Binding score (kcal/mol) and interacting amino acids					Activity affected		
	ExpI (HM) aa	ExpR (HM) aa	LasI 1kzf.pdb aa	TofI 3p2h.pdb aa	LasR 2uvo.pdb aa	Effect and mode of action	Pathogens	Reference(s)
Thymol  **CC1=CC=C(C(C)C)C(O)=C1**	**−5.8** F28, L32, W34, D45, Y47, M77, T81, F82, S100, R101, F102, I142, V143, S144, M147, V172	**−4.6** K30, S32, Y50, W54, Y58, D67, P68, V69, V70, W82, I97, S101, I106, T111, M123, S125	**−4.2** L12, E18, E19, G41, M42, S44, D48, Y54, L56, L63, S66, V67, R68, S99, R100, F102	**−4.6** W33, L78, F83, V144, F146, I169, D170, R172, V174	**−5.7** L36, Y56, W60, R61, Y64, D73, T75, V76, W88, Y93, F101, A105, L110, T115, S129	Violacein production	*Chv*, *E. coli*	112
Ursolic acid  **CC1(C)[C@@H](O)CC[C@]2(C)[C@@]3([H])CC=C4[C@]5([H])[C@@H](C)[C@H](C)CC[C@]5(C(O)=O)CC[C@@]4(C)[C@]3(C)CCC21**	**−4.1** T27, R31, L32, F102, F103, V104, K106, S120, P123, F124, S144, P146, M147, I150	**−2.1** M1, L4, R25, Y26, D27, V128, D129, S130, F139, K143, T146, L150	**−3.0** M77, T81, F82, S99, R100, F101, T140, I141, V142, S143, M146, L165, I171	**−3.9** W33, Q34, D45, Q46, Y47, L78, V82, F83, R104, V144, F146, I169, D170, R172, V174	**−3.0** L36, G38, Y47, A50, I52, Y56, W60, R61, Y64, D65, A70, D73, T75, V76, W88, Y93, F101, A105, L110, T115, A127, S129	Biofilm	*E. coli*, *V. harveyi*	4, 101
Vanillin  **[H]C(C1=CC(OC)=C(O)C=C1)=O**	**−5.8** F28, L32, W34, M77, T81, F82, S100, R101, F102, I142, S144, M147	**−6.0** K30, S32, M34, P41, S42, M44, I46, Y58, K59, Q64, V70, L121, A122, M123, S125	**−5.3** L12, E18, E19, L20, G41, M42, S44, D48, Y54, L56, L63, S66, R68, F102, R100	**−5.9** W33, L78, F83, V144, F146, I169, D170, R172, V174	**−4.7** L36, Y56, W60, R61, Y64, D73, T75, V76, W88, Y93, F101, A105, L110, T115, A127, S129	Violacein production, biofilm AHL synthesis	*Chv*, *Psa* PAO1, *Agt*, bacterial mixture in cartridge filters	55, 118

[a]Key protein–ligand interactions and docking scores as identified/calculated by the Glide program are provided for two homology models (ExpI and ExpR) and three crystal structures (LasI, TofI, and LasR).

Abbreviations: *Agt*, *Agrobacterium tumefaciens*; AHL, *N*-acyl-homoserine lactone; *Chv*, *Chromobacterium violaceum*; EPS, exopolysaccharide; HM, homology model; HSL, homoserine lactone ring; *Psa*, *Pseudomonas aeruginosa*; QS, quorum sensing; T3SS, type III secretion system.

anti-inflammatory, anticarcinogenic, and antimicrobial activities of these compounds (27, 62, 93). More than 8,000 phenolic structures have been reported, ranging from single-ring phenolics such as low-molecular-weight phenolic acids to highly polymerized substances such as tannins, which are synthesized via the shikimic acid or phenylpropanoid pathway (28, 90). Comprehensive screening of phenolic compounds has yielded several molecules with pharmacological properties and medical applications (75). Some of these phenolics and other plant-produced secondary metabolites have been reported to affect bacterial virulence and are summarized in **Table 1**. In the subsequent section, we briefly discuss the most important mechanisms by which plant-derived compounds interfere with bacterial virulence.

EFFECT OF PLANT MOLECULES ON VIRULENCE DETERMINANTS

Effect on Quorum Sensing Signaling Molecules

Because of the linkage of QS and bacterial virulence (3, 30, 103), interfering with bacterial communication by degrading the QS signaling molecules or inhibiting their synthesis has received considerable attention (33). The development of bacterial reporter strains that provide measurable outputs (e.g., bioluminescence, pigments, enzymes) in response to a QS signal allowed screening of large numbers of potential inhibitors that affect the QS process. Such strategies have been comprehensively reviewed in the past several years (25, 41, 64).

Plants' natural products, including extracts from several edible plants, reduce the levels of AHL in *Pseudomonas*, *Chromobacterium*, and *Salmonella* (41, 59, 101, 115). Among phenolic compounds, salicylic acid (SA), cinnamic acid (CA), carvacrol, and eugenol were frequently reported to reduce the synthesis of AHL in different bacterial models, including *Psa*, *Pectobacterium* spp., *Chromobacterium* spp. and *Vibrio* spp. (13, 49, 52, 63, 83). Other single-ring phenolics like vanillin and thymol were also reported to affect the synthesis of QS molecules in several bacterial species (16, 80, 81). Multiple-ring polyphenols, including flavonoids and stilbenes like curcumin (87), resveratrol (65), ellagic acid (105), naringenin (4), and ichangin (124), have been shown to interfere with AHL signaling in *Chromobacterium violaceum* (*Chv*), *Psa*, and *Vibrio* spp.

Biofilm Formation

Biofilms are assemblages of microorganisms attached to a substratum embedded within self-produced polysaccharides or a glycocalyx matrix that function as a cooperative consortium that confers an advantage to the community (77). Biofilms benefit bacteria by improving nutrient acquisition and protection from environmental conditions and antimicrobials, thereby facilitating their undesirable presence in industrial, medicinal, and agricultural settings (11, 20). Biofilm formation is influenced by several environmental factors, including the topography and hydrophobicity of plant surfaces, which necessitates the role of bacterial flagellar motility, fimbriae type I and IV, and curly to provide access to the target surfaces. Another important factor is the synthesis and secretion of extracellular polymeric substances (EPSs) that are regulated by QS (79). The involvement of QS in EPS biosynthesis has been demonstrated for several plant-pathogenic bacteria, including *Pantoea stewartii*, *E. amylovora*, and *R. solanacearum* (40). Promising anti-biofilm activities were demonstrated with extracts of ginger (58), oregano (84), tea (129), and other medicinal plants (132). The active constituents of such extracts have been identified as simple phenolics (33), polyphenols, terpenes, and flavonoids (see **Table 1**).

Essential oils, the active components of aromatic plants, including compounds such as carvacrol, eugenol, methyl eugenol, and thymol, were reported to reduce biofilm formation of several Gram-negative bacteria such as *Pc*, *Psa*, and *Chv* at nonlethal concentrations (11, 52, 82, 137). The

volatiles carvacrol and thymol also inhibited the biofilm formation of *Pectobacterium* spp. (52, 127). Other active compounds are SA and vanillin as well as phenylpropanoids such as cinnamaldehyde, which all inhibit biofilm formation of *Psa*, *Pc*, *Chv*, *Vibrio* spp., and *Agt* (50, 118). These compounds not only inhibit biofilm formation but also, in some cases, remediate biofouling (55). Polyphenols such as curcumin (a constituent of *Curcuma longa*) inhibit biofilm formation of *Psa* and *V. harveyi* as well as their adherence to polypropylene surfaces (89, 102). Tannic acid and catechin (abundant compounds in tea) inhibit biofilm formation of *Psa* and *E. coli* (7, 47). Polyphenols like resveratrol, ellagic acid, phloretin, iberin, ursolic acid, and phytol inhibit biofilms of *Psa*, *E. coli*, *Vibrio* spp., and *Fusobacterium nucleatum* (59, 65, 66). Reduced production of EPSs was demonstrated in the presence of specific phenolics and made the biofilm more susceptible to antibiotics or other antimicrobial agents. Allicin and ajoene, sulfur-containing compounds isolated from garlic, inhibit adhesion as well as EPS secretion of *Psa*, the integral component of biofilms (71).

Bacterial Secretion Systems

Secretion systems are a major trait of plant pathogens, dedicated to secreting virulence factors into the host (38). Pathogenic bacteria rely on different classes of secretion systems: Sec, Tat, T1SS–T7SS, SecA2, sortase, and injectosome (38). The T3SS is a highly conserved virulence determinant of many Gram-negative pathogens and is largely regulated by QS (6, 42, 104). The basic model of soft-rot *Erwinia* suggests that QS controls the production of PCWDEs, which are secreted into the extracellular environment through T1SS (protease) and T2SS (pectinases and cellulases). Transcriptomic analysis of *P. atrosepticum* SCRI1043 revealed a set of essential virulence factors regulated by QS, including T1SS and T2SS but more importantly T3SS, which translocates effector proteins to host cells, thereby interfering with the host's defense response (72). The QS-dependent expression of T3SS includes the *hrp* cluster with components of the structural apparatus, the effector DspA/E, helpers HrpN and HrpW, and regulators *hrpL*, *hrpS*, and *hrpY*. This makes T3SS an attractive target to control pathogenic bacteria and affect virulence. Plant molecules such as naringenin, coumaric acid, and CA and their derivatives are capable of inhibiting the expression of T3SS components and helper genes (*hrpS*, *hrpL*, *hrpN*, *hrpA*, and *rpoN*) in *D. dadantii* (69, 70) and *Psa* (4, 135). The ability to react to plant phenolic compounds requires the GacA/GacS system, which positively regulates the levels of *rsmB*, which then neutralizes the activity of RsmA, the post-transcription negative regulator that affects expression of target virulence genes (70). These findings were supported by the inhibitory effects of CA derivatives, SA, and benzoic acid on the promoter activity of *hrpA* and transcript levels of *hrpN* and *hrpL* in *E. amylovora* (57). The compounds did not affect bacterial growth but significantly decreased the hypersensitive response (HR) of tobacco leaves after *E. amylovora* inoculation. Recently, these compounds were reported to inhibit T3SS in *Xanthomonas oryzae* (34). Phenolic compounds also abolished or reduced the HR response caused by *X. oryzae* in tobacco leaves without affecting cell multiplication. These findings suggest that phenolic compounds affect T3SS in several phytopathogenic bacteria, preventing the suppression of the plant defense system during bacterial invasion. Contrary to these results, eugenol, SA, chlorogenic acid, resveratrol, CA, and fumaric acid were reported as inducers of T3SS in *R. solanacearum* (134). In such cases, the host compounds may play a role in promoting compatible interactions between susceptible hosts and pathogens by favoring pathogen invasion.

Extracellular Enzymes and Other Virulence Factors

To successfully infect plants, pathogenic bacteria are dependent on synchronized production of extracellular enzymes capable of degrading cell walls of different plant tissues. Such enzymatic

complexes mostly contain pectin-degrading enzymes such as pectate lyase, polygalacturonase, pectin methylesterase, cellulases, and others, which are secreted to the extracellular milieu by T2SS and proteases that are secreted by T1SS. The activities of PCWDEs are of central importance for virulence in soft-rot bacteria, including the genera *Pectobacterium* and *Dickeya*. These pathogens rely on QS for regulation of PCWDE where the presence and concentration of AHL largely determine their transcription via regulation by ExpR. Upon binding, the AHL-ExpR complex prevents transcription of *rsm*A, encoding the negative regulator RsmA, thereby releasing transcription of PCWDEs (2, 14, 23, 97). Plant molecules implicated as inhibitors of exoenzyme secretion are mostly phenolic compounds that reduce the secretion of exoenzymes in *Pectobacterium* (49, 50, 52) and pyocyanin toxin in *Pseudomonas* (118). In *Pc* ATCC39048, the genes for carbapenem antibiotic synthesis are clustered in the operon CarA-H, which is under QS control (97). In this strain, AHL is synthesized by the LuxI family member CarI and the response regulator CarR, which operates as a transcription activator of the CarA-H operon in the presence of AHL. The QS system of this strain was inhibited by compounds extracted from *Scorzonera sandrasica*, which inhibited not only violacein production in *Chv* CV026 but also carbapenem production (9). Other reports supported the interference by SA, tannin, and catechin production of pyocyanin toxins, elastase, and protease of *Pseudomonas* (98, 120). Similarly, eugenol suppressed the production of exotoxins by *Staphylococcus aureus*, and the phenolic volatiles thymol, carvacrol, and eugenol modulated the expression of genes responsible for production of toxins in *Vibrio cholerae* (119).

MECHANISMS UNDERLYING QUORUM SENSING INHIBITION BY PLANT MOLECULES

The antimicrobial properties of plant-derived molecules, including phenolics, are well documented; however, in most cases, no specific targets have been suggested (21, 27). At lethal concentrations, many hydrophobic phenolics perturb membrane integrity and bind to proteins, DNA, and/or ribosomes, thereby affecting ADP-ribose transferase activity. However, at sublethal concentrations that do not interfere with bacterial growth or membrane properties, the antimicrobial targets of plant-derived QS inhibitors are poorly characterized. The mechanisms underlying the promising potential of various plant compounds as means to control bacterial virulence are, however, starting to accumulate (41, 64, 81). These include inhibition of the synthesis of QS signaling molecules by phenolic compounds (13) or the inhibition of specific virulence determinants that are known to be associated with QS in *Psa* and *Pectobacterium* (14, 44, 49, 52). Only a few studies present evidence for direct interference with AHL synthesis. SA, tannic acid, *trans*-cinnamaldehyde, carvacrol, and eugenol have been reported to act directly on QS systems (13, 51, 52, 70). These compounds also modulate the expression of many QS-related genes, including *expI/expR* in *Pectobacterium* (49, 51) and *lasI/lasR*, *rhlI/rhlR*, and *pqsA* in *Psa*, in response to methyl gallate (44). This may support a direct effect on QS system genes. To further explore the sites of action, the compounds were applied to a QS-negative *E. coli* strain expressing only AHL synthase or receptor (13, 51, 52). The results showed a direct effect on the AHL synthase as the main target for the plant molecules (13, 52). Exposure of *Pectobacterium* strains to SA, CA, carvacrol, or eugenol inhibited QS proteins and impaired virulence. The strong interference with virulence was almost completely recovered upon exogenous application of AHL, suggesting that the translation of QS central proteins (ExpI and ExpR in *Pectobacterium*) was normal in the presence of the phenolic compounds, whereas their activity was blocked (13, 49, 52). Apparently, in the presence of the above phenolics, AHL synthesis was inhibited in a dose-dependent response (52). This fits well with the QS model of brute force virulence that allows full activation of QS-dependent genes only when conditions are favorable for successful infection (72, 97).

Both LuxI- and LuxR-type proteins may serve as targets for plant-derived inhibitors of virulence. In pectobacteria, mutational analysis of *expI* or *expR* of *Pectobacterium parmentieri* SCC3193 revealed the dominance of ExpI over ExpR for virulence inhibition, mainly because ExpR is a negative regulator of PCWDEs and its absence does not impair virulence (2, 95, 110). This system presents a different model than that of *Vibrio* or *Pseudomonas* in which mutants in LuxR-type proteins display significantly decreased virulence (43, 125). Accordingly, LuxR proteins acting as positive regulators of virulence make a promising target for virulence inhibition. In some cases, as was demonstrated for rosmarinic acid, the plant molecule acts as a stimulator of the response regulator RhlR in *Psa* PAO1. The formation of rosmarinic acid is induced in plant roots in response to PAO1 and competes with the bacterial ligand *N*-butanoyl-homoserine lactone, stimulating an increase in RhlR-mediated transcription that results in higher QS-dependent gene expression, biofilm formation, and production of the virulence factors pyocyanin and elastase. In this case, rosmarinic acid serves as both a homoserine lactone mimic and a component of the plant defense mechanism (19). The direct interaction between the RhlR protein and the ligand rosmarinic acid was supported by docking simulations (a plausible binding mode and a favorable docking score for the interaction between Rh1R and rosmarinic acid were predicted by Schrödinger's Glide) and further validated by direct binding assays using isothermal titration calorimetry using purified RhlR (19). The stimulating activity was explained as a plant strategy in which rosmarinic acid is produced as an integral plant defense compound, which stimulates a premature QS response in *Psa* upon binding, thereby preventing a synchronized attack of the plant tissue by this opportunistic bacterium (19).

Another potential mechanism by which plant-derived compounds may interfere with bacterial QS is through inhibition of the AHL synthase, although it is a relatively less explored strategy. Recently, the phenolic acid SA and the essential oil carvacrol were shown to inhibit the activity of ExpI in *Pectobacterium* (51, 52). Methyl gallate, another phenolic compound, exerted profound anti-QS effects on the synthesis of AHL and biofilm formation, motility, proteolytic and elastase activity, and the expression of QS-related genes in *Psa* (44). A few studies have highlighted the structure of AHL synthase with respect to its inhibition by plant molecules (12, 13, 17, 92). The resolved 3D structure of AHL synthase LasI from *Psa* revealed two binding sites that revealed shared conserved residues with many other plant-pathogenic bacteria, including *Acidovorax citrulli*, *Agt*, *E. amylovora*, *Pseudomonas syringae*, *Pectobacterium* spp., *P. stewartii*, and *Dickeya* spp. (**Figure 2**). These highly conserved residues are potential targets for natural compounds as well as for synthetic inhibitors that would compete with the natural ligands at the active site. This approach has already yielded synthetic analogs such as holo-ACP, sinefungin, D/L-*S*-adenosylhomocysteine, L-*S*-adenosylcysteine, butyryl-SAM, and *N*-(3-oxocyclohex-1-enyl) octanamide ($C_{14}H_{23}NO_2$-J8-C8), which inhibited AHL synthesis in *Psa* and *Burkholderia glumae* (17, 35, 92, 111). Among these compounds, J8-C8 was confirmed as a potent inhibitor of AHL synthesis upon binding to AHL synthase from *B. glumae*, as demonstrated by both functional and X-ray crystal structure analyses (17). Blocking autoinducer synthases opens new prospects for interference with bacterial communication systems in a wide range of bacteria (13, 91).

To provide a more comprehensive atomic-scale insight into the interactions of some phenolic compounds with QS proteins, we employed molecular docking. We assembled a small database of plant-derived compounds from the literature that specifically interfere with QS and/or QS-dependent virulence determinants in Gram-negative bacteria (see **Table 1**). Next, we docked these compounds onto binding sites identified on homology models of ExpI/ExpR from *Pectobacterium* spp. that we had previously constructed based on the known crystal structures of LasI/TofI/LasR. Finally, the compounds were docked to all targets, i.e., the homology models and the binding sites of the crystallographic structures (51, 52). For each compound, we identified

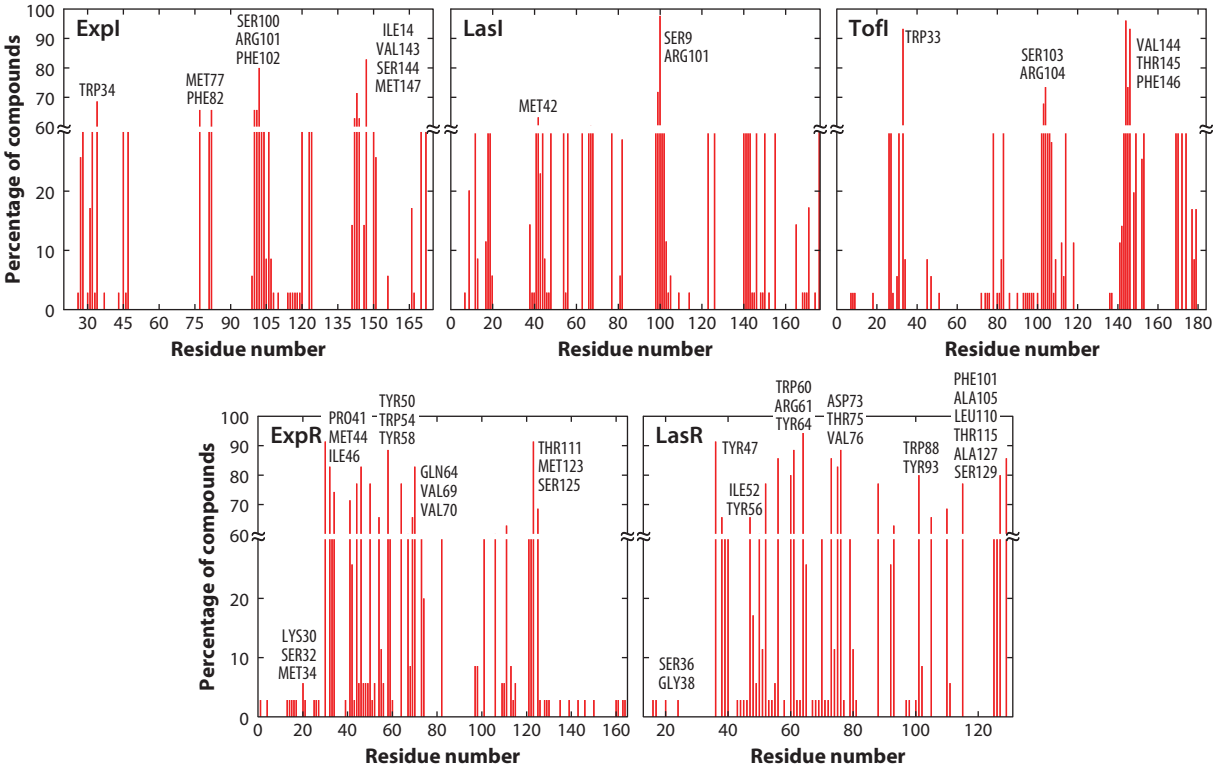


Figure 3

The percentage of compounds interacting with (i.e., found within 4 Å of) each binding site residue in the five protein targets considered in this review. Residues that interact with >60% of the compounds are marked.

a plausible binding mode and characterized its strength with a docking score. Finally, for each ligand and binding mode, we identified all protein residues that reside within 4 Å of any of the ligand atoms and defined them as interacting residues. The results of this analysis are summarized in **Table 1** and **Figure 3**.

An in-depth analysis of the binding modes and docking scores of all the ligands with all the target proteins is beyond the scope of this review. Moreover, although the resulting binding modes are likely reliable and could provide insights into the interactions between ligands and binding site residues (see below), the docking scores could only be used to rank the relative affinities of the ligands to a given target but not to different targets. Thus, it is not possible to conclude from these results the relative preferences of ligands to specific targets. Finally, it is important to note that a docking score provides information only about the strength of ligand–protein interactions and does not take into account additional factors that may affect ligand activity such as solubility, permeation, and metabolic stability. Thus, a clear relationship between docking scores and inhibitory activity could not always be formed. Still, several observations can be made. Tannic acid, ellagic acid, and quercetin are predicted to be the best binders to ExpI, with scores significantly higher than for any other ligand. All of these compounds feature multiple aromatic rings. Tannic acid, together with rosmarinic acid, bisdesmethoxycurcumin, curcumin, and phenylethylcinnamamide, is predicted to be the best binder to ExpR. Except for tannic acid, all these compounds

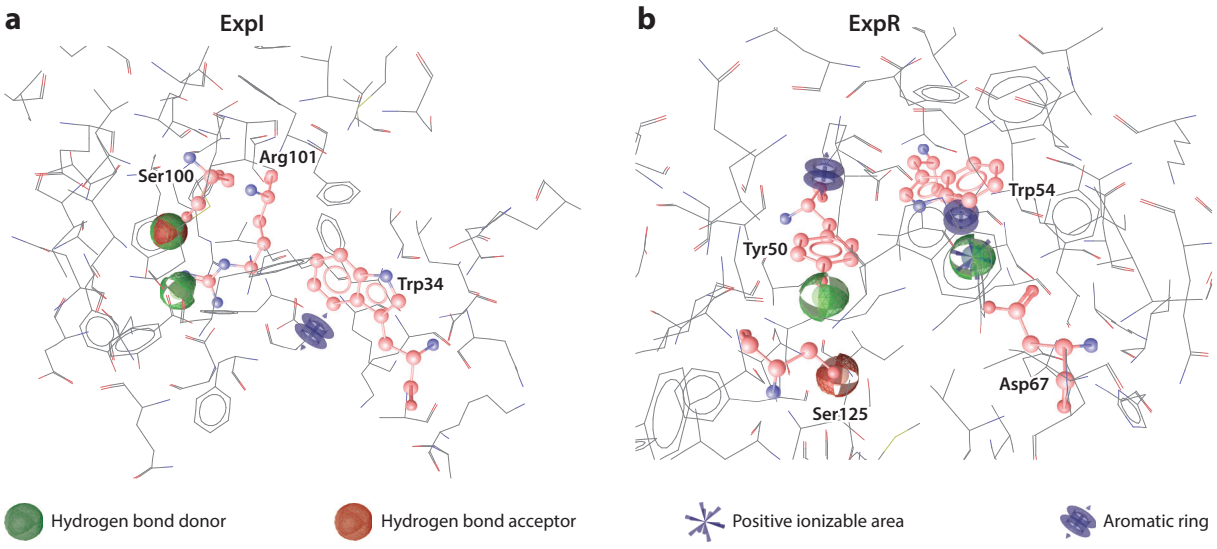


Figure 4

Structure-based pharmacophore models based on the homology models' structure of (*a*) ExpI and (*b*) ExpR.

share a similar overall shape composed of a linear linker capped by two phenyl rings. Tannic acid is also predicted to be the best binder to LasI and its homolog TofI; in the latter case, it is accompanied by the structurally distinct ellagic acid and bisdesmethoxycurcumin. The structural diversity of ligands with the best docking scores may hint at the promiscuity of the TofI binding site. Finally, tannic acid, rosmarinic acid, bisdesmethoxycurcumin, curcumin, phenylethylcinnamamide, phloretin, quercetin, and piperine are predicted to be the best binders to LasR. It is interesting to note that from among the 35 ligands considered in this work, only 10 are predicted to be among the strongest binders to any QS protein and only seven to more than a single QS protein.

Looking at **Figure 3**, we observe that certain protein residues interact (i.e., are found within a distance of 4 Å) with more than 60% of the ligands. In ExpI, these residues are Ser100, Arg101, Phe102, Ile142, Val143, Ser144, and Met147. In LasI, they are Met42, Ser99, and Arg100. In TofI, they are Trp33, Ser103, Arg144, Thr145, and Phe146. In LasR, they are Leu36, Gly38, Tyr47, Ile52, Tyr56, Trp60, Arg61, Tyr64, Asp73, Thr75, Val76, Trp88, and Tyr93. In ExpR, they are Lys30, Ser32, Met34, Pro41, Met44, Pro41, Met44, Ile46, Tyr50, Trp54, Tyr58, Gln64, Asp67, Val69, Val70, Ile106, Thr111, Met123, and Ser125. Using multiple sequence alignment, we identified a subset of these residues common to all family members. For the LasI/TofI/ExpI family, these residues are S100 (ExpI)/S103 (TofI)/S99 (LasI), R101 (ExpI)/R104 (TofI)/R100 (LasI), and W34 (ExpI)/W33 (TofI). For the LasR/ExpR family, they are Y50 (ExpR)/Y56 (LasR), W54 (ExpR)/W60 (LasR), D67 (ExpR)/D73 (LasR), and S125 (ExpR)/S129 (LasR). These residues are also conserved using ConSurf analysis (**Figure 2**).

Based on the list of binding site residues that interact most frequently with the different ligands, we generated two site-based pharmacophore models for the LasI/TofI/ExpI and LasR/ExpR protein families (see **Figure 4**) using LigandScout 4.4 (133). The LasI/TofI/ExpI pharmacophore consists of four pharmacophoric features, including one aromatic ring, one hydrogen bond acceptor, and two hydrogen bond donor features. The LasR/ExpR model differs and consists of

six pharmacophoric features, including two aromatic rings, two hydrogen bond donors, one hydrogen bond acceptor, and one positive ionizable feature. We take these models to represent the general requirements for an efficient binding of small molecules with these two protein families. This information may prove useful for the future identification of novel QS inhibitors.

Application of Plant-Derived Phenolic Compounds to Control Plant Disease

Plant phenolics were reported to improve disease resistance by increasing the plant's defense responses to pathogens or directly by modifying pathogen growth and/or virulence. Pepper plants treated with phenolic compounds displayed decreased symptoms of bacterial spot disease (1). Similarly, tissue culture potato grown in the presence of SA displayed reduced symptoms of *Dickeya solani* infection (26). Phenolics also affect bacterial multiplication (27), membrane properties (113), secretion systems (57, 134), chemical cross-talking (49, 51, 52), and virulence (27, 49, 51, 52). Using molecular biology and biotechnological approaches, overproduction of phenolic compounds in transgenic tobacco and *Arabidopsis* resulted in increased disease resistance (31, 32). Increased concentrations of phenolics among other secondary metabolites in hybrid *Ornithogalum* spp. and in wild potato increased resistance and inhibited virulence factors of a bacterial soft-rot pathogen (53, 54). However, in some cases increased phenolic production may also have negative consequences, mainly on the rate of photosynthesis, which is not a desirable trait for cultivated plants (46). Overexpression of phenolic compounds through manipulation of specific metabolic pathways has agricultural potential for the development of plant resistance to bacterial pathogens.

CONCLUSIONS

Many plant-associated bacteria interact with their hosts through QS. The ability to manipulate, inhibit, or block QS systems offers a strategy by which plants may attenuate bacterial diseases. Phylogenetic analysis of LuxI and LuxR homologs suggests that pressure from the host or environment is providing strong selection pressure on these genes because insertions, deletions, and mutations in active sites are common in both LuxI and LuxR. Host QS suppressors could provide a high level of selection pressure on these genes. Bacterial plant pathogens that rely on AHL-based QS to regulate and synchronize their interactions with plants are an example of how a spectrum of signaling molecules (autoinducers) are utilized by microbes to orchestrate infection of their plant host. Targeting LuxI-type AHL synthases or LuxR-type receptors by plant-produced molecules that inhibit or promote the synthesis/perception of signaling molecules that control bacterial group behavior represents a sophisticated chemical strategy by which plants disarm pathogenic bacteria. Experimental evidence of such interactions between QS proteins and their plant-derived ligands is now starting to accumulate.

In the past decades, the incorporation of computational tools to explore such potential interactions has helped identify specific amino acids that are involved in plant–microbe interactions and ranking different plant molecules according to their potential antivirulence activity, often in accord with experimental evidence. The agreement between experiments and computations suggests that computational tools traditionally used for drug discovery may serve as a powerful platform to explore molecular interkingdom signaling interference between bacterial pathogens and their host plants.

The summary of the findings presented in this review reveals a dynamic field of research that aims to uncover how sessile organisms like plants have developed a repertoire of secondary metabolites through evolution, which they exploit to manipulate the cellular targets of their enemies.

SUMMARY POINTS

1. QS presents an attractive target for the prevention of biofilm formation, production of PCWDEs, and pathogenicity by manipulating the bacterial capability to synchronize pathogenic attack.
2. Plant-derived compounds that act as AHL mimics or AHL inhibitors are involved in QS interference and seem to play a major role in determining the outcome of plant–microbe interactions.
3. Insertions, deletions, and mutations in the ligand binding sites of LuxR and LuxI appear to be common.
4. Interkingdom signaling involves not only LuxR solos as a subfamily of proteins implicated in communication between bacteria and plants but also LuxI-type proteins and direct interference with autoinducer synthesis.
5. Synthesis of small molecules by plants through common metabolic pathways may be manipulated with breeding and biotechnological tools (such as CRISPR-Cas9) to increase plant defense against bacterial pathogens.
6. Such approaches, and their combinations with common antibiotics, may improve the control of bacterial pathogens, prevent the selection pressure on the development of resistance, and reduce the use of antibiotics.
7. The specificity of some compounds to certain bacterial pathogens in selected hosts may enable the retention of beneficial microbes in the plant environment.
8. The time has come for researchers to use computational tools developed for pharmaceutical purposes to develop new strategies for management of bacterial diseases of plants.

FUTURE ISSUES

1. Multiple small molecules from plants possess significant anti-QS activities. Of these, only a few, such as SA, are also recognized as plant-signaling molecules that act as interkingdom signals. An important task would be to learn how such signaling molecules affect the expression of a wide spectrum of bacterial genes in the laboratory and natural environments.
2. The new class of genome-editing technology, CRISPR-Cas9, is now being applied to dozens of crop plants. It allows the manipulation of plant metabolic pathways to produce specific low-molecular-weight compounds. An important task will therefore be to manipulate plants to produce interkingdom signals that alter bacterial communities to support agricultural systems.
3. It would be interesting to learn the specificity of interkingdom signals and whether they have special receptors in other organisms.
4. The field of bacterial virulence poses new challenges to computational paradigms that may be deployed for the study and design of specific small molecules that restrict plant-pathogenic bacteria. To meet these challenges, existing drug discovery tools may need to be modified and new tools may need to be developed. These tools should be incorporated into robust workflows and skillfully applied to the design and optimization of new crop protectors.

DISCLOSURE STATEMENT

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