Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2015) **16**(7), 685–698 DOI: 10.1111/mpp.12227

Role of *Dickeya dadantii* 3937 chemoreceptors in the entry to Arabidopsis leaves through wounds

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SUMMARY

Chemotaxis enables bacteria to move towards an optimal environment in response to chemical signals. In the case of plant-pathogenic bacteria, chemotaxis allows pathogens to explore the plant surface for potential entry sites with the ultimate aim to prosper inside plant tissues and to cause disease. Chemoreceptors, which constitute the sensory core of the chemotaxis system, are usually transmembrane proteins which change their conformation when sensing chemicals in the periplasm and transduce the signal through a kinase pathway to the flagellar motor. In the particular case of the soft-rot pathogen *Dickeya dadantii* 3937, jasmonic acid released in a plant wound has been found to be a strong chemoattractant which drives pathogen entry into the plant apoplast. In order to identify candidate chemoreceptors sensing wound-derived plant compounds, we carried out a bioinformatics search of candidate chemoreceptors in the genome of *Dickeya dadantii* 3937. The study of the chemotactic response to several compounds and the analysis of the entry process to Arabidopsis leaves of 10 selected mutants in chemoreceptors allowed us to determine the implications of at least two of them (ABF-0020167 and ABF-0046680) in the chemotaxis-driven entry process through plant wounds. Our data suggest that ABF-0020167 and ABF-0046680 may be candidate receptors of jasmonic acid and xylose, respectively.

Keywords: chemoreceptors, *Dickeya dadantii* 3937, entry, jasmonic acid, xylose.

INTRODUCTION

Dickeya dadantii 3937 (*Dd*3937) is an enterobacterium included in the Top 10 List of phytopathogenic bacteria. This catalogue is based on the scientific/economic importance of the plant bacterial pathogens included (Mansfield *et al*., 2012). *Dickeya dadantii* causes soft rot in a wide range of plant species, including important crops, such as potato (Charkowski *et al*., 2012). Several mechanisms are needed for the adaptation and virulence of *D. dadantii* inside the plant. The most important virulence factor is the production of a large set of enzymes and isoenzymes (pectate lyases, polygalacturonases, pectin methylesterases, etc.) that disassemble the plant cell wall (Barras *et al*., 1994). Other processes involved in the adaptation of *D. dadantii* to the hostile plant conditions are as follows: (i) iron uptake, for which *D. dadantii* synthesizes two siderophores: chrysobactin and achromobactin (Munzinger *et al*., 2000; Persmark *et al*., 1989); (ii) mechanisms to counteract the presence of plant toxic substances, such as multidrug resistance (MDR) efflux pumps (Barabote *et al*., 2003; Maggiorani Valecillos *et al*., 2006); (iii) other mechanisms to overcome the presence of antimicrobial compounds (Costechareyre *et al*., 2013; Llama-Palacios *et al*., 2003, 2005; López-Solanilla *et al*., 1998; 2001; Rio-Alvarez *et al*., 2012). Moreover, the *hrp*-encoded type III secretion system (T3SS) is required for the full virulence of *D. dadantii* (Bauer *et al*., 1994; Yang *et al*., 2002; Yap *et al*., 2005).

All the above-cited mechanisms are needed for the adaptation and virulence of *D. dadantii* inside the plant. Nevertheless, bacteria spend the majority of their life outside the plant and bacterial entry into the plant is critical. The life cycle of *D. dadantii* during the infection of potatoes requires adhesion to the plant surface and penetration into the plant tissues, either via wound sites or through natural openings, such as stomata (Reverchon and Nasser, 2013). For this, an active bacterial movement towards entry sites is required. Chemotaxis, which enables bacterial cells to move towards certain stimuli and away from others (Porter *et al*., 2011), is crucial for the colonization of the host and for the establishment of a successful infection (Moens and Vanderleyden, 1996).

The bacterial chemotaxis system is the best-studied biological gradient sensor and has been analysed in detail in *Escherichia coli*

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(Sourjik and Wingreen, 2012). The system is composed of bacterial chemoreceptors or methyl-accepting chemotaxis proteins (MCPs). Bacterial chemoreceptors are hexagonally packed trimers of receptor dimers (Briegel *et al*., 2012) that recognize specific chemicals (even redox state) at the periplasm and regulate the flagellar function, assisted by a signal transduction pathway composed of the so-called Che proteins (Hazelbauer and Lai, 2010). The structure of a typical bacterial chemoreceptor is as follows: (i) a sensor domain or ligand-binding region (LBR), which can be either periplasmic or cytoplasmic, and which detects chemical signals and induces a conformational change; (ii) a HAMP-type adaptation domain (cytosolic), which transmits the molecular stimulus created by ligand binding at the LBR to the: (iii) MCP domain or signalling domain (cytosolic), which undergoes reversible methylation at multiple sites and transduces the signal to the downstream signalling cascade of Che proteins (Falke and Hazelbauer, 2001). Despite the fact that the chemotaxis system is conserved among bacteria, chemoreceptors differ largely in their protein topology. A large-scale comparative genomic analysis of the MCP signalling and adaptation domain family (from 312 prokaryotic genomes) allowed the identification of seven major chemoreceptor classes and three distinct structural regions within the cytoplasmic domain: signalling, methylation and flexible bundle subdomains (Alexander and Zhulin, 2007). Moreover, although most LBRs are periplasmic, others are cytoplasmic, and sometimes the LBR is lacking in some chemoreceptors (Lacal *et al*., 2010a). Much of the knowledge about the ligands for bacterial chemoreceptors comes from studies in *E. coli*, which has four chemoreceptors (Tar, Tsr, Trg and Tap) and an aeroreceptor (Aer). The ligand profile of each of the four chemoreceptors has been determined: the Tar receptor is primarily for aspartate and maltose, the Tsr receptor for serine and leucine, the Trg receptor for ribose and galactose, and the Tap receptor for dipeptides and pyrimidines (Lacal *et al*., 2010a). The Aer receptor, which lacks the periplasmic domain, mediates aerotaxis in response to oxygen-related cellular redox changes (Bibikov *et al*., 2004). Although there is little information on the ligands for other bacterial chemoreceptors, a considerable number of studies have focused on this issue, not only in animal-pathogenic bacteria (Nishiyama *et al*., 2012; Rahman *et al*., 2014; Sweeney *et al*., 2013), but also in environmental bacteria (Lacal *et al*., 2010b; Nichols *et al*., 2012; Oku *et al*., 2012).

In the case of plant-associated bacteria, active chemotaxis may represent an opportunity for the establishment of an interaction with the plant, either symbiotic or pathogenic. Nevertheless, fewer studies exist on chemotaxis and chemoreceptors in plant-pathogenic bacteria. Chemotaxis is needed for virulence, biofilm formation and competitive fitness in *Ralstonia solanacearum* (Yao and Allen, 2006, 2007) and for pathogenicity in *Agrobacterium tumefaciens* (Hawes and Smith, 1989). The chemotactic behaviour towards several compounds has been described in *Pseudomonas syringae* (Cuppels, 1988) and *Xanthomonas campestris* (Kamoun and Kado, 1990), although the role of chemotaxis in the interaction with the host has not been elucidated to date. However, a particular case is *Dd*3937. The contribution of motility and chemotaxis to the pathogenicity of *Dd*3937 has been studied (Antúnez-Lamas *et al*., 2009). Genes involved in the chemotaxis transduction system (*cheW*, *B*, *Y* and *Z*) and in the structure of the flagellar motor (*motA*) are required for swimming and entry into Arabidopsis leaves. Its capacity to mediate chemoattraction and chemorepellance in response to compounds such as sugars, amino acids and plant hormones, such as jasmonic acid (JA) (Antunez-Lamas *et al*., 2009), has been assessed. JA, which participates in wound signalling in plants (León *et al*., 2001), is a strong chemoattractant for *Dd*3937. Furthermore, this perception seems to drive the ingress of this bacterium inside plant tissues through wounds. These results suggest that *Dd*3937 may have at least one chemoreceptor responsible for the perception of JA. Moreover, jasmonate-dependent modifications of the pectin matrix of the plant cell wall during potato development function as a defence mechanism targeted by *Dd*3937 virulence factors (Taurino *et al*., 2014). This finding links the plant hormone, JA, with the plant cell wall modifications. In dicot secondary cell walls, the major hemicellulose is a polymer of β-(1,4)-linked xylose units, called xylan (Rennie and Scheller, 2014), and xylose is released when the plant cell wall is altered. Therefore, it can also be hypothesized that *Dd*3937 may possess chemoreceptors to detect plant cell wall-derived compounds.

In this study, we carried out a bioinformatics analysis followed by an experimental approach to identify putative chemoreceptors involved in the perception of compounds released in plant wounds. Moreover, we analysed the role of these chemoreceptors in the entry to the plant apoplast and the colonization ability in Arabidopsis plants.

RESULTS

*Dd*3937 possesses an unusually high number of chemoreceptors among Enterobacteria

We screened the *Dd*3937 genome (http://asap.ahabs.wisc.edu/asap/home.php) for the presence of proteins containing the highly conserved MCP signalling domain. The *Dd*3937 genome encodes 47 proteins containing the MCP domain. Despite the fact that *Dd*3937 belongs to Enterobacteria, this result contrasts with the average number of MCP genes found in other Enterobacteria: 29 MCPs per genome (Lacal *et al*., 2010a). The amino acid sequences of these 47 chemoreceptors were searched for transmembrane regions (TMs) using the DAS server (Table 1). According to the presence of TMs, *Dd*3937 chemoreceptors were classified into six different topologies (Fig. 1A), as described previously (Lacal *et al*., 2010a). In the case of *Dd*3937, all topologies, including class Ia,

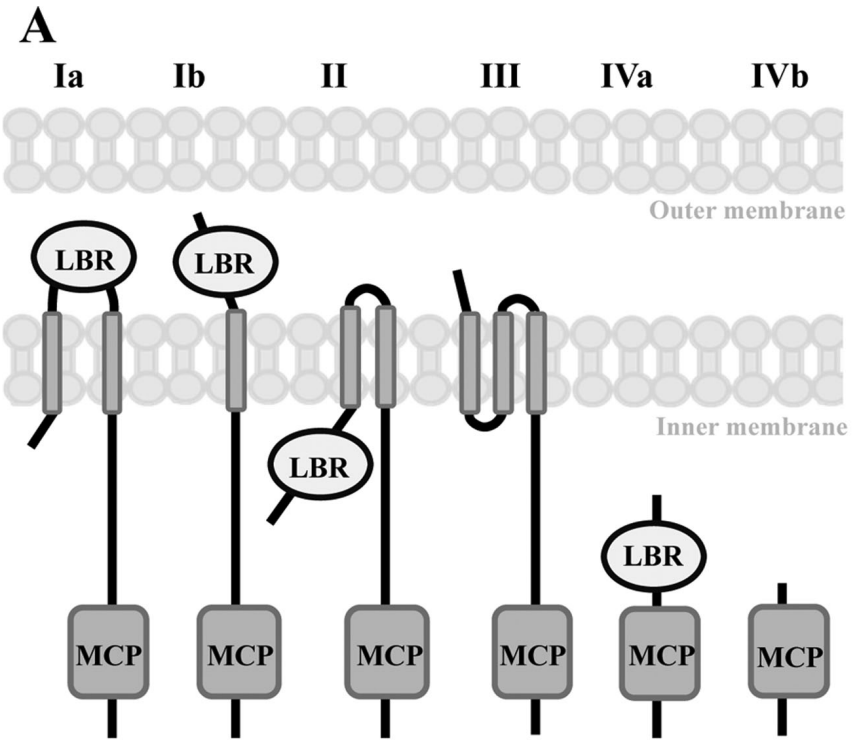
Table 1 Amino acid coordinates of methyl-accepting chemotaxis protein (MCP) domains and transmembrane regions (TMs) determine the topology of the *Dd*3937 chemoreceptor.

ID		TM						MCP		Topology class‡
ASAP*	UNIPROT†	i_1	j_1	i_2	j_2	i_3	j_3	i	j	
ABF-0014536	E0SHS4	10	32	181	203			334	501	Ia
ABF-0014618	E0SM30	36	58	214	232			367	531	Ia
ABF-0014722	E0SF47	7	29	190	209			344	511	Ia
ABF-0014824	E0SDP4	10	32	183	205			340	507	Ia
ABF-0015168	E0SH41	5	24	177	199			335	501	Ia
ABF-0015513	E0SBF1	20	42	200	222			360	523	Ia
ABF-0015600	E0SE76	12	34	278	295			431	598	Ia
ABF-0015603	E0SE77	38	60	300	317			451	618	Ia
ABF-0016115	E0SN86	13	35	269	285			421	588	Ia
ABF-0016585	E0SIA2	10	32	189	211			343	510	Ia
ABF-0016979	E0SEW8	10	32	183	205			343	510	Ia
ABF-0017097	E0SCJ7	17	39	272	290			427	593	Ia
ABF-0017662	E0SFJ0	5	27	187	204			342	508	Ia
ABF-0017665	E0SFJ2	36	58	213	235			372	538	Ia
ABF-0017668	E0SFJ3	10	32	184	206			344	511	Ia
ABF-0017672	E0SFJ4	10	32	184	206			347	514	Ia
ABF-0017674	E0SFJ5	20	39	201	223			359	526	Ia
ABF-0017863	E0SI11	10	32	189	211			346	510	Ia
ABF-0017896	E0SHY9	11	33	178	200			337	503	Ia
ABF-0018502	E0SEF0	10	32	187	209			346	512	Ia
ABF-0018541	E0SFF6	10	32	182	204			336	505	Ia
ABF-0018585	E0SJX6	20	42	199	221			357	524	Ia
ABF-0018754	E0SIM4	10	32	179	201			336	503	Ia
ABF-0018765	E0SIN3	20	42	204	226			356	518	Ia
ABF-0018892	E0SJ51	20	42	194	216			355	522	Ia
ABF-0019050	E0SAP6	15	37	270	292			427	591	Ia
ABF-0019306	E0SF78	13	35	282	299			435	601	Ia
ABF-0019718	E0SHT9	7	29	180	202			336	502	Ia
ABF-0019790	E0SF98	10	32	187	209			344	510	Ia
ABF-0019851	E0SM37	7	29	179	194			333	499	Ia
ABF-0019852	E0SM36	20	42	195	214			348	515	Ia
ABF-0019855	E0SM35	13	35	185	204			340	507	Ia
ABF-0019858	E0SM34	31	53	202	222			357	522	Ia
ABF-0020167	E0SDB0	10	29	178	200			340	507	Ia
ABF-0020252	E0SF13	25	47	288	314			439	604	Ia
ABF-0020431	E0SDX8	5	27	168	190			327	489	Ia
ABF-0046680	E0SMA1	58	77	348	370			504	670	Ia
ABF-0017537	E0SD21	10	32	142	147			421	586	Ia
ABF-0017419	E0SJN1	138	160					295	462	Ib
ABF-0014726	E0SF49	159	178	188	200			335	497	II
ABF-0019309	E0SF75	164	181	186	205			341	506	II
ABF-0014843	E0SDM9	147	151	165	180	185	196	337	499	III
ABF-0016380	E0SEN9	30	52	183	205	336	350	490	656	III
ABF-0018511	E0SEE2	13	30	196	203	315	330	468	633	III
ABF-0016436	E0SMM7							297	417	IVa
ABF-0017090	E0SCK2							474	615	IVa
ABF-0017824	E0SI40							17	131	IVa

*Nomenclature from the ASAP database (https://asap.ahabs.wisc.edu/asap/logon/php).
†Nomenclature from the UNIPROT database (http://www.uniprot.org/).
‡Topology class according to Lacal *et al*. (2010a).

show similar results to those reported by Lacal *et al*. (2010a). Furthermore, in the most abundant topology Ia, we grouped the LBRs according to their length (Fig. 1B). *Dd*3937 LBRs (Ia) can be clustered into two groups as described previously by Lacal *et al*. (2010a). Most LBRs (60%) are characterized by sizes corresponding to cluster I, the majority of the LBRs being those with sizes between 150 and 159 amino acids (Fig. 1B).

LBR sequences were analysed by CLUSTAL W2 (Larkin *et al*., 2007), searching for diversity among the sequences (Table S1, see Supporting Information). The results showed that the level of sequence conservation was generally low and no duplications were observed. Moreover, LBRs of each of the 47 MCP proteins in *Dd*3937 were searched for Pfam components (Finn *et al*., 2014), using a customized BioPerl (Stajich *et al*., 2002) script. Only hits



Relative abundance in *Dd*3937					
81 %	2 %	4 %	6 %	6 %	0 %

Overall relative abundance of MCPs in bacteria (Lacal *et al*., 2010a)					
70 %	4 %	3 %	10 %	5 %	8 %

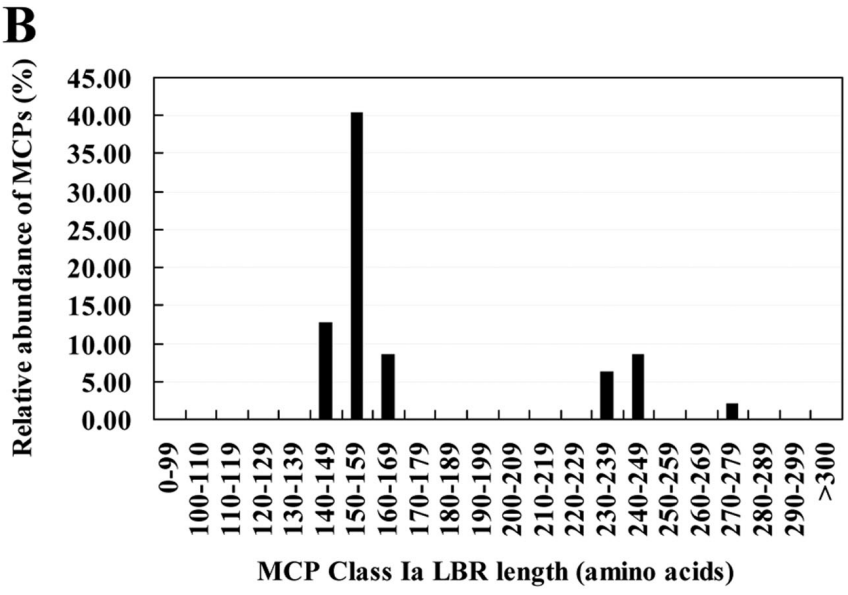


Fig. 1 (A) Classification of *Dd*3937 chemoreceptors into six different topologies (roman numerals); 47 MCP sequences from *Dd*3937 were analysed. Classification is based on clustering annotated by Lacal *et al*. (2010a). The prediction of transmembrane regions (TMs) was performed using the DAS server (Cserzo *et al*., 1997). Arabic numbers indicate the relative abundance of receptors with a given topology in *Dd*3937 and in bacteria according to Lacal *et al*. (2010a). MCP, methyl-accepting chemotaxis protein; LBR, ligand-binding region. (B) Relative abundance of *Dd*3937 MCP sequences (belonging to class Ia) according to the size of the LBR (amino acids).

with an E-value of less than or equal to 0.005 were retained (Table S2, see Supporting Information). The results showed that 23 of the LBRs fall into the annotated domain 4HB_MCP_1 (four helix bundle sensory module for signal transduction) (Ulrich and Zhulin, 2005), which is a ubiquitous sensory module in prokaryotic signal transduction. Five are homologues to the annotated domain TarH, a member of a family of transmembrane receptors that mediate the chemotactic response in certain enteric bacteria, such as *Salmonella typhimurium* and *E. coli* (Kim *et al*., 1996). Homology with other annotated domains involved in sensory processes can be found.

Global BLAST of LBRs from *Dd*3937 chemoreceptors suggests putative receptors for plant compounds

In order to identify *Dd*3937 chemoreceptors involved in the sensing of plant-specific compounds, LBRs were further analysed by bioinformatics methods. LBR sequences were extracted from the protein sequence as described in Experimental procedures. LBR sequences were subjected to local BLAST against a bacterial genome database (this database contains complete bacterial genomes available as of 14 February 2014 from GeneBank Ref Seq) using an *ad hoc* PERL script (for more details, see Experimental procedures). Table S3 (see Supporting Information) shows similar LBR sequences (E-value $\leq 10^{-30}$) to those found in other bacteria. Figure 2A shows the distribution of *Dd*3937 LBRs among the following groups of bacteria: (i) other *Dickeya* strains; (ii) plant-pathogenic bacteria; (iii) animal-pathogenic bacteria (considering the occurrence of a high number of similar LBR sequences in animal pathogens); and (iv) non-pathogenic bacteria (including bacteria present in plants, soil and water). Among the 47 LBRs of *Dd*3937, nine were widely distributed among bacteria regardless of their niche (animals, plants, soils and water). Another seven LBRs, apart from being present in plant-pathogenic bacteria, were also found in environmental (non-pathogenic) bacteria. Moreover, another four LBRs, apart from being present in *Dickeya* strains, were also found in environmental (non-pathogenic) bacteria. Only for one LBR were similar sequences found exclusively in animal and environmental bacteria. The largest group, 13 LBRs, was found in other plant-pathogenic bacteria besides the *Dickeya* genus, and another large group containing 12 members included LBRs exclusively present in the *Dickeya* genus. This means that 56% of *Dd*3937 LBRs show similarity only with other LBRs from phytopathogenic bacteria(Fig. 2B). Interestingly, one *Dd*3937 LBR

A

Topology class	ID[a]	In other *Dickeya* strains	In plant-pathogenic bacteria	In animal-pathogenic bacteria	In non-pathogenic bacteria
Ib	ABF-0014726	■	■	■	■
III	ABF-0014843	■	■	■	■
Ia	ABF-0016115	■	■	■	■
IVa	ABF-0016436	■	■	■	■
Ia	ABF-0017537	■	■	■	■
Ia	ABF-0018585	■	■	■	■
Ia	**ABF-0018754**	■	■	■	■
Ia	ABF-0018765	■	■	■	■
II	ABF-0019309	■	■	■	■
III	ABF-0016380	■	■		■
IVa	ABF-0017090	■	■		■
III	ABF-0018511	■	■		■
Ia	ABF-0018892	■	■		■
Ia	ABF-0019852	■	■		■
Ia	ABF-0019855	■	■		■
Ia	**ABF-0046680**	■	■		■
Ia	ABF-0014722	■		■	■
Ia	**ABF-0014536**	■	■		
Ia	ABF-0014824	■	■		
Ia	ABF-0015168	■	■		
Ia	ABF-0015513	■	■		
Ia	ABF-0016585	■	■		
Ia	ABF-0016979	■	■		
Ia	ABF-0017097	■	■		
Ia	**ABF-0017668**	■	■		
Ia	ABF-0017674	■	■		
Ia	ABF-0019050	■	■		
Ia	ABF-0019790	■	■		
Ia	ABF-0019851	■	■		
Ia	ABF-0018502	■	■		
Ia	**ABF-0018541**	■			■
IVa	**ABF-0017824**	■			■
Ia	ABF-0017896	■			■
Ia	ABF-0020431	■			■
Ia	ABF-0014618	■			
Ia	ABF-0015600	■			
Ia	ABF-0015603	■			
Ib	ABF-0017419	■			
Ia	**ABF-0017662**	■			
Ia	**ABF-0017665**	■			
Ia	**ABF-0017863**	■			
Ia	ABF-0017672	■			
Ia	ABF-0019306	■			
Ia	ABF-0019718	■			
Ia	ABF-0019858	■			
Ia	**ABF-0020252**	■			
Ia	**ABF-0020167**		■		

B

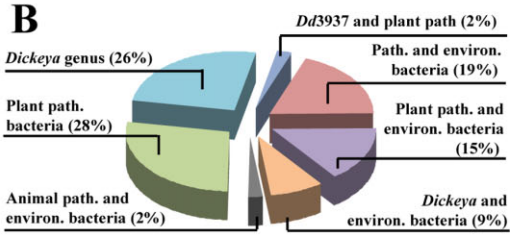


Fig. 2 (A) Distribution of *Dd*3937 ligand-binding regions (LBRs) among bacteria. [a]Nomenclature from the ASAP database (https://asap.ahabs.wisc.edu/asap/logon/php). In bold, genes selected for further analyses. Grey boxes indicate that similar sequences to a given *Dd*3937 LBR were found in the global BLAST analysis. (B) Schematic representation of the distribution of *Dd*3937 LBRs among bacteria according to their ecological niche. path., pathogenic; environ., environmental.

was not found in other *Dickeya* strains, but was found in other plant-pathogenic bacteria.

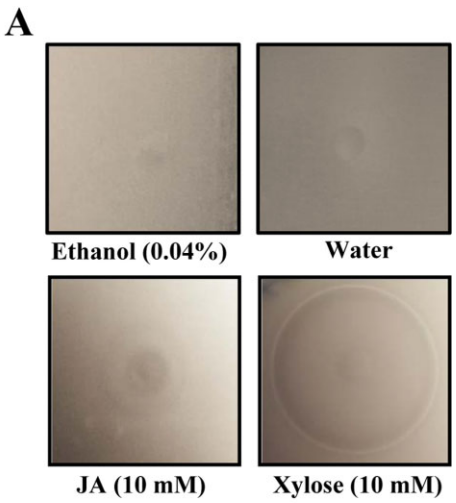
Mutants in individual chemoreceptors of *Dd*3937 are affected in the perception of JA and xylose

In order to unveil the role of the perception of plant molecules during the interaction of *Dd*3937 with plants, chemoreceptors from the different groups shown in Fig. 1 were selected. Thus, ABF-0018754 was selected as a control for chemotaxis assays as it is the candidate for serine perception in *Dd*3937. ABF-0046680 was chosen from the group of chemoreceptors whose LBRs showed similarity with those of plant-pathogenic bacteria, but also environmental bacteria. ABF-0014536 and ABF-0017668 were selected from the group of chemoreceptors whose LBRs showed similarity only with those of plant-pathogenic bacteria; ABF-0018541 and ABF-0017824 were selected from the group of chemoreceptors whose LBRs showed similarity with those of *Dickeya* strains (not with other plant-pathogenic bacteria) and environmental bacteria. ABF-0017662, ABF-0017665, ABF-0017863 and ABF-0020252 were selected from the group of chemoreceptors whose LBRs showed similarity only with those of *Dickeya* strains. Finally, ABF-0020167 was chosen because of its particularity of not showing similarity with other *Dickeya* strains, but with other plant-pathogenic bacteria. These chemoreceptors were mutagenized by different approaches (for more details, see Experimental procedures). ABF-0018754, chosen as a control for chemotaxis assays, restored serine perception when heterologously expressed in the non-chemotactic *E. coli* UU1250 strain (Bibikov *et al*., 2004) (Fig. S1, see Supporting Information).

In order to elucidate how the mutations in the chemoreceptors cited above affected the movement of *Dd*3937 towards putative entry sites, such as wounds, the chemotactic response of wild-type (WT) and mutant strains towards JA and xylose was analysed. As mentioned previously, JA secreted in plant wounds is a strong chemoattractant for *Dd*3937 (Antunez-Lamas *et al*., 2009) and xylose is released when the plant cell wall is altered.

The analysis of the chemotaxis towards these plant compounds was performed using the classical 'in-plug' assay (Tso and Adler, 1974), which is based on the ability of bacteria to swim in soft agar and move towards/away from a given chemical. The results were recorded from soft agar–bacteria plates based on either the observation of attraction haloes or of the absence of a chemotactic response (Fig. 3). Independent of the intensity of the haloes, these were considered as a positive chemoattraction when an apparent ring of accumulated cells was found at a certain distance from the plug.

From the results of the 'in-plug' assays, we selected mutants *14536*, *17824*, *18541*, *20167* and *46680* for quantitative chemotaxis assays towards JA, and mutants *18541*, *20167*, *20252* and *46680* for quantitative chemotaxis assays towards xylose. For this purpose, a Palleroni-like assay was performed using 5-μL tips instead of glass capillaries, as handling allows a high-throughput screening in comparison with the classic Palleroni assay (Palleroni, 1976). This assay allows the calculation of the chemotaxis index



B

	JA	Xylose
WT	+	+
14536	Ø	+
17662	+	+
17665	+	+
17668	+	+
17824	Ø	+
17863	+	+
18541	Ø	Ø
18754	+	+
20167	Ø	Ø
20252	+	Ø
46680	Ø	Ø

Fig. 3 (A) Typical attraction haloes of *Dd*3937 wild-type (WT) towards jasmonic acid (JA) and xylose in the 'in-plug' assay. A drop containing a given chemical, either JA (10 mM) or xylose (10 mM), or their corresponding controls, ethanol (0.04% v/v) and water, was deposited on the (0.25%) soft-agar bacteria, and haloes were recorded after 20 min. (B) Chemotactic response of *Dd*3937 strains towards JA and xylose. Chemotactic response of *Dd*3937 WT and mutant strains was evaluated in the presence of a concentration gradient of JA (10 mM) and xylose (10 mM). The chemotactic response was considered as attraction (+) or non-chemotactic response (Ø) in comparison with that of the WT strain for each chemical. Similar results were obtained in three independent experiments.

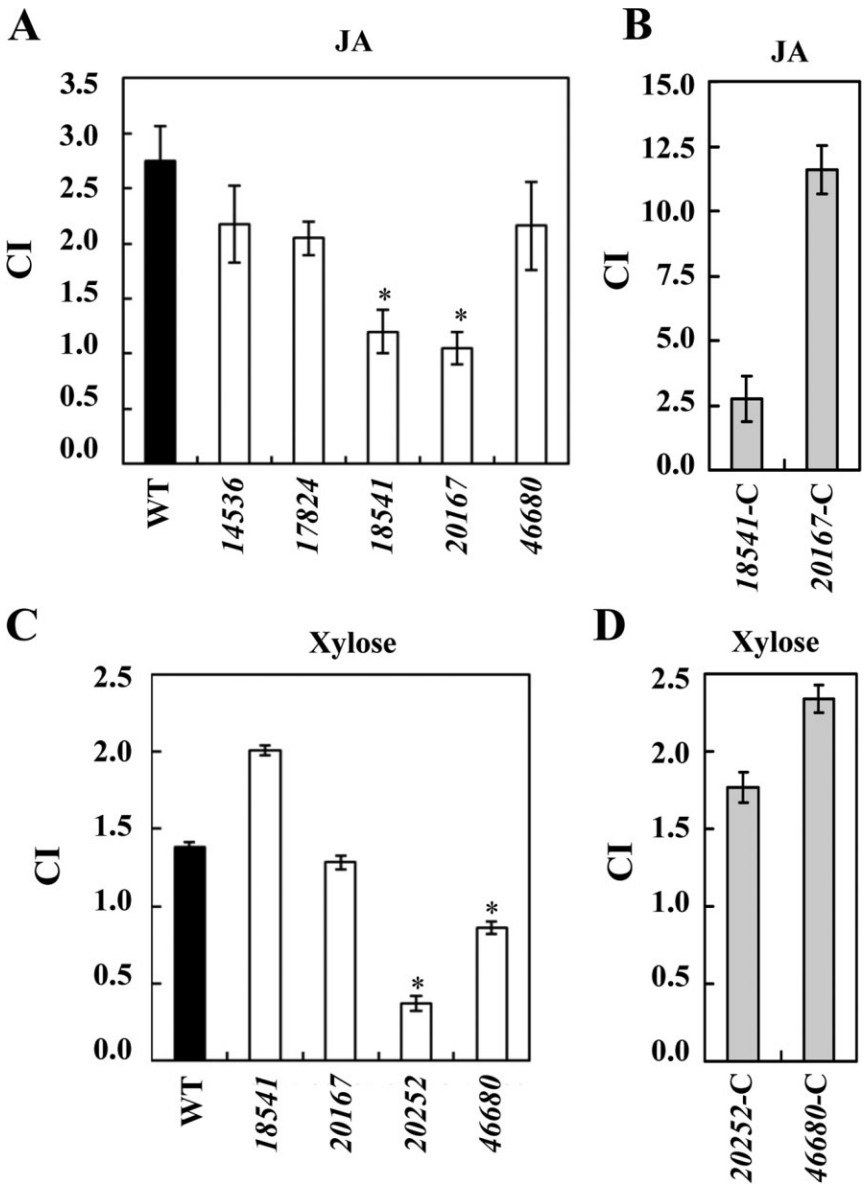


Fig. 4 Quantitative chemotaxis assays of *Dd*3937 strains towards jasmonic acid (JA) and xylose. Bars represent the chemotaxis index (CI) for wild-type (WT) and mutant strains towards 1 mM JA (A) and 1 mM xylose (C). Those mutants showing a different chemotaxis towards JA or xylose were complemented and the chemotaxis towards 1 mM JA (B) or xylose (D) was tested using the same procedure. CI is defined as the number of cells that accumulate after 20 min within a tip containing a given compound divided by the number of cells accumulated within a tip containing the solvent. The means and standard errors are shown. Error bars represent the standard error of the mean (SEM). Asterisk indicates significant differences between WT and mutant CI determined according to Student's *t*-test ($P < 0.05$). Similar results were obtained in three independent experiments.

(CI). As bacterial cells can move actively towards/away from a given chemical contained in the capillary, values of CI greater than 1.0 are considered as chemoattraction and values less than 1.0 as chemorepulsion (Fig. 4). The results in Fig. 4 indicate that, in the case of JA, only *18541* and *20167* mutants showed a reduced chemoattraction towards this plant hormone. In the case of xylose, *20252* and *46680* mutants were affected in the perception of this sugar. As a control for the quantitative (capillary) assays, chemotaxis towards serine of the WT and mutant *18754* was assayed in each experiment (data not shown). Strains overexpressing the WT chemoreceptors restored the ability to develop a chemoattraction response in the presence of the respective compound (Fig. 4B, D).

To check that these results were not the product of an altered chemotactic ability, *Dd*3937 mutants were tested for chemotaxis towards serine. All mutants, except *18754* (candidate serine receptor of *Dd*3937), showed the same chemotaxis towards serine as did the WT strain (Fig. S2, see Supporting Information). Moreover, to demonstrate that these mutants do not have a general defect in motility, we carried out a swimming assay in soft agar-rich medium. This assay, which reflects the sum of taxis towards compounds present in the medium, showed that the spreading of these strains was not altered with respect to that of the WT strain (Fig. S3, see Supporting Information).

*Dd*3937 chemoreceptors contribute in a different manner to entry into Arabidopsis leaves

As stated above, how pathogens enter plant tissues is critical for pathogenesis. To ascertain the particular relevance of the selected

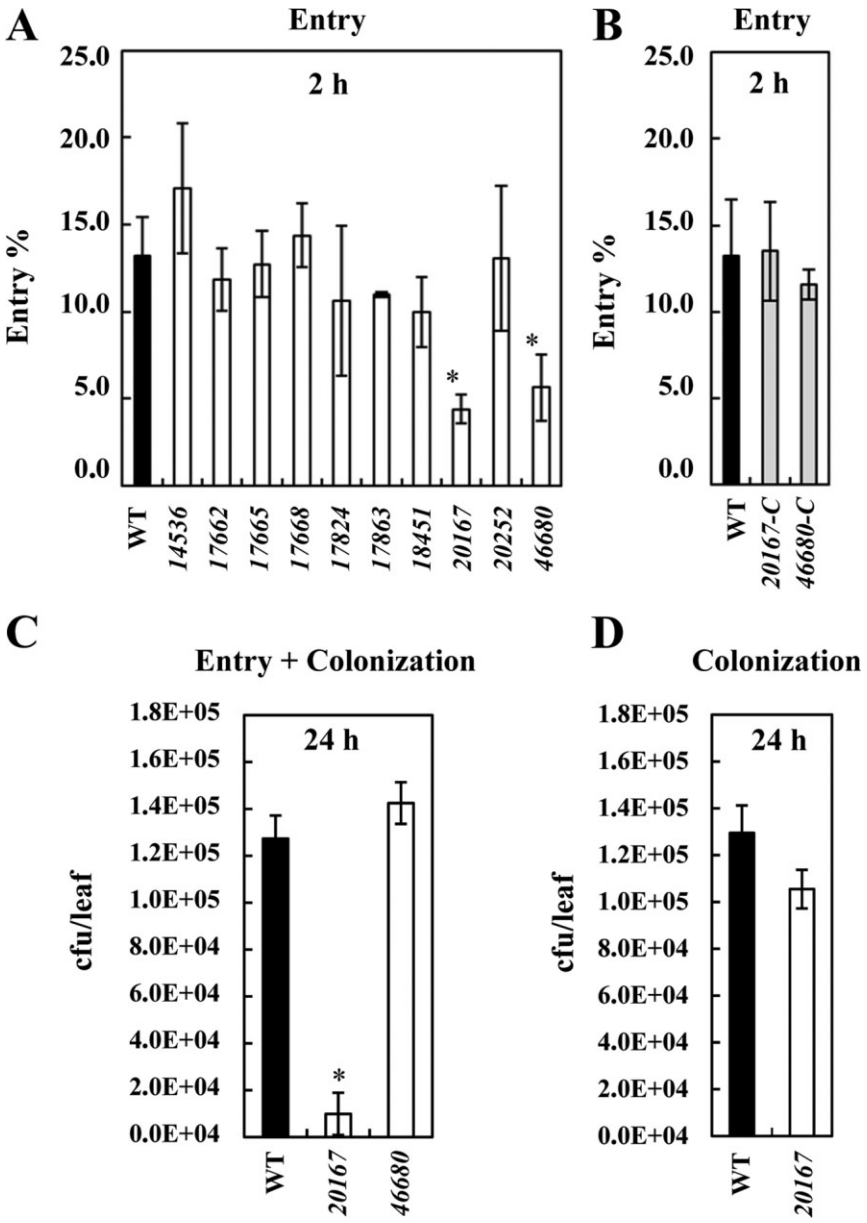


Fig. 5 (A) Entry of *Dd*3937 wild-type (WT) and mutant strains in *Arabidopsis thaliana* leaves. For each strain, 10^5 cells were placed on a wound. This process was repeated in 15 leaves from at least five different plants. Bacterial populations inside the leaf were estimated after 2 h. (B) Entry of *Dd*3937 WT and complemented strains in *A. thaliana* leaves. For each strain, 10^5 cells were placed on a wound. This process was repeated in 15 leaves from at least five different plants. Bacterial populations inside the leaf were estimated after 2 h. (C) Entry-dependent colonization assay in *A. thaliana* leaves. 10^5 cells of WT or mutant (*20167* and *46680*) strains were placed on a wound. This process was repeated in 15 leaves from at least five different plants. Bacterial populations inside the leaf were estimated after 24 h. (D) Non-entry-dependent pathogenicity assay in *A. thaliana* leaves. 2×10^6 cells of WT or *20167* strains were infiltrated into the abaxial side of leaves. This process was repeated in 15 leaves from at least five different plants. The bacterial population inside the leaf was estimated after 24 h. cfu, colony-forming unit. For (A–D), means and standard errors are shown. Error bars represent standard error of the mean (SEM). Asterisk indicates significant differences between WT and mutant population (estimated either as percentage entry or total population inside the leaves) determined according to Student's *t*-test ($P < 0.05$). Similar results were obtained in three independent experiments.

*Dd*3937 chemoreceptors in this process, and how this could affect the subsequent development of symptoms, WT and mutant strains were challenged to entry and colonization assays in Arabidopsis leaves. *Arabidopsis thaliana* has been described and characterized previously as a susceptible host of *Dd*3937 (formerly *Erwinia chrysanthemi* 3937) (Dellagi *et al*., 2005; Fagard *et al*., 2007).

To compare the ability to detect plant compounds released in a wound, we first performed entry assays in *A. thaliana* Col-0 leaves, as described previously (Antunez-Lamas *et al*., 2009). For each particular strain, the cells which entered the plant apoplast were measured relative to the initial inoculum of cells (cells recovered from the leaf/cells put on the wound; expressed as a percentage), and these ratios were used for comparison with the WT strain ratio. Figure 5A shows the differences in entry ability of the 10 MCP mutants tested in comparison with the WT strain. Two mutants, *20167* and *46680*, were significantly affected in their ability to enter the plant apoplast in contrast with the WT strain. Entry assays in *A. thaliana* leaves were performed as described previously. *20167*-C and *46680*-C complemented strains showed entry percentages close to those of the WT levels (Fig. 5B).

To ascertain whether the delay in entry into the plant apoplast exhibited by *20167* and *46680* mutants could affect the subsequent establishment of infection, the same assay described above was performed, changing the incubation time from 2 to 24 h (Fig. 5C). Figure 5C shows that the delay in entry of *20167* observed at 2 h affected the population inside the leaves at 24 h,

whereas *46680* reached WT population levels at 24 h. To check that the decrease in the *20167* population inside the leaves at 24 h was not a result of impairment in colonization ability, but a direct consequence of reduced entry, a non-entry-dependent colonization assay was performed. For this, WT and *20167* cell suspensions were infiltrated into the abaxial side of *A. thaliana* leaves and bacterial populations were estimated at 24 h. Figure 5D shows that, when infiltrated, the population of the *20167* mutant is at the same level as that of the WT strain.

DISCUSSION

The *E. coli* chemotactic pathway has provided a paradigm for sensory systems in general. However, the increasing number of sequenced bacterial genomes shows that, although the central sensory mechanism seems to be common to all bacteria, there is added complexity in a wide range of species (Krell *et al*., 2011; Wadhams and Armitage, 2004). Indeed, the analysis of bacterial genomes reveals that motile bacteria differ enormously in the number of chemoreceptors. Chemoreceptor abundance in a bacterial genome ranges from 64 in *Magnetospirillum magnetotacticum* (Alexandre *et al*., 2004) to a single chemoreceptor in other bacteria (Lacal *et al*., 2010a). Forty-seven chemoreceptors have been identified in *Dd*3937 through bioinformatics analyses. In comparison with its close Enterobacteria relatives (*Shigella*, *Salmonella*, *Yersinia*, *Escherichia* spp.), which show a small number of chemoreceptors, for instance five in *E. coli* (Lacal *et al*., 2010a; Wadhams and Armitage, 2004), *Dd*3937 possesses a large number of chemoreceptors. This is congruent with the hypothesis that microorganisms living in complex and variable environments must be able to detect a greater variability of changing conditions. Indeed, genome sequencing has revealed that many microorganisms from aquatic and soil environments possess large numbers of chemoreceptors (Alexandre *et al*., 2004). Moreover, to assess the relationship between the abundance of chemoreceptors and lifestyle, regardless of taxonomic classification, Lacal *et al*. (2010a) analysed 264 bacterial genomes for the presence of chemoreceptors, and similar results were obtained, showing that the number of chemoreceptors per genome depends mostly on bacterial lifestyle and metabolic diversity, but weakly on genome size. Among the 264 bacterial genomes analysed, only three phytopathogenic bacteria, *Xylella fastidiosa*, *Xanthomonas oryzae* pv. *oryzae* and *Agrobacterium tumefaciens* C58, were included. These authors also analysed the topology of the chemoreceptors defining several classes. In the case of *Dd*3937, the most abundant topology is that of class Ia, as it mainly occurs in bacteria (Lacal *et al*., 2010a). For the rest of the classes, similar results to those shown for bacteria were obtained in *Dd*3937. Moreover, the analysis of the LBR length in the most abundant topology class (Ia) also showed the same clustering into two defined groups described by Lacal *et al*. (2010a).

The detailed analysis of the distribution of *Dd*3937 LBRs among known bacterial genomes allowed us to determine that more than 50% of the LBRs show high similarity only with sequences from other plant-pathogenic bacteria. These *in silico*-based data suggest that these chemoreceptors would be implicated in the perception of plant-derived compounds. The large number of putative chemoreceptors involved in this process demonstrates the relevance of the perception of plant compounds during the interaction. Indeed, the identification of host–microbe interaction factors in the genome of *Dd*3937 with supervised machine learning shows that methyl-accepting chemotaxis genes are highly enriched among the predicted host–microbe interaction factors in this bacterium (Ma *et al*., 2014).

The results from the 'in-plug' chemotaxis assays indicated that more than one mutant strain showed an altered chemotactic response towards JA or xylose with respect to the WT strain. A possible explanation of these findings would be that a given compound can be detected by more than one chemoreceptor. Indeed, there are several examples in the literature showing this (Kato *et al*., 2008). The best studied example is the recognition of amino acids by the paralogous receptors PctA, PctB and PctC of *Pseudomonas aeruginosa* (Rico-Jiménez *et al*., 2013). However, the opposite phenomenon has also been described: one chemoreceptor is able to sense more than one compound. For example, McpS, a *Pseudomonas putida* KT2440 chemoreceptor for tricarboxylic acid (TCA) cycle intermediates, is involved in the chemotactic response towards different ligands, such as malate, succinate and fumarate (Lacal *et al*., 2010b). Other examples of multi-ligand chemoreceptors are as follows: Tcp receptor in *S. typhimurium*, which mediates positive chemotaxis to citrate and negative chemotaxis to phenol (Yamamoto and Imae, 1993), PA2652 of *P. aeruginosa*, which mediates chemotaxis to malate, but not to any of the remaining TCA cycle intermediates (Alvarez-Ortega and Harwood, 2007), and McpX of *Vibrio cholera*, which functions as a chemoreceptor for multiple amino acids (Nishiyama *et al*., 2012). Another hypothesis which cannot be ruled out is that a mutation in a given chemoreceptor alters the chemotactic response of the remaining receptors, through gene expression reprogramming. There is much information missing about the gene regulation of chemoreceptors. For example, it is unknown whether all receptors are constitutively expressed, whether they are inducible under certain stimuli and whether there is fine regulation depending on the metabolic needs. It has been shown that the responses of *E. coli* to two opposing chemoattractant gradients are dependent on the chemoreceptor ratio (Kalinin *et al*., 2010). Therefore, if *Dd*3937 chemoreceptor ratios are being altered through individual mutations, one can expect several changing chemotactic behaviours, as found in this study.

The analysis of the chemotactic response to xylose and JA using a quantitative methodology based on capillary assays showed

that, of the five mutants found to be altered in chemotaxis towards JA in the in-plug assay, only two were affected in the capillary assays. In the case of xylose, of the four mutants altered, only two consistently showed a reduced chemotaxis in the capillary assays. This discrepancy could be a result of the nature of the medium employed, i.e. semi-solid agar in the 'in-plug' assay and liquid medium in the capillary assay. Both the availability of the chemoattractant to be perceived and the effects of the conditions during the assay on the motility ability could be responsible for the different results obtained. An important element to take into account is that chemotaxis is a flagellum-dependent phenomenon and the number of flagella is intimately linked to the characteristics of the medium (Jarrell and McBride, 2008).

Although other chemoreceptors could be involved in JA perception, we focused our studies on the role of ABF-0018541 and ABF-0020167 in the active entry process through wounds as the corresponding mutant strains consistently showed a decreased chemotaxis towards this hormone in the two assays performed. Our experiments showed that only the *20167* mutant displayed impaired entry ability to the plant apoplast, which suggested a pivotal role for this receptor during this process. In the case of xylose, although *20252* and *46680* mutants showed reduced chemoattraction towards this sugar in both 'in-plug' and quantitative assays, only *46680* was impaired in the entry process, which also suggested a key role for this receptor during the entry to the plant apoplast. However, when analysing the colonization ability, although *20167* and *46680* mutants displayed impaired entry ability, only the *20167* strain was altered in the establishment of a sufficiently large population to prosper in the hostile plant conditions. When *20167* was infiltrated, the bacterial population reached the same level as that of the WT strain. Therefore, the altered perception of compounds produced in wounds, such as JA, is the main contributor to the impaired colonization ability of this strain, which does not seem to be related to the colonization ability or fitness inside the plant tissue. In the case of *46680*, although under laboratory conditions the mutant is not affected in its colonization ability, a role in this process under natural conditions cannot be ruled out.

It is noteworthy that, in our *in silico* analysis, similar sequences to the LBR from ABF-0020167 were only found in plant-pathogenic bacterial genomes. It is also noteworthy that no matches with other *Dickeya* strains were found. Moreover global BLAST analyses did not show any match of this LBR with *Pectobacterium carotovorum*, a phylogenetically related bacterium which does not sense JA (Antunez-Lamas *et al*., 2009). Therefore, ABF-0020167 could be considered as a potential receptor for JA, although further analyses are required to demonstrate the interaction of its LBR with this plant hormone. It is noteworthy that ABF-0018541 has also been identified as a putative JA chemoreceptor in our capillary assays. The sequence similarity between the LBR of these two chemoreceptors is very low, but it cannot be ruled out that a similar tertiary structure exists, allowing the binding of the same type of molecules. Indeed, both LBR sequences show homology with the annotated Pfam domain 4HB_MCP_1 (four helix bundle sensory module for signal transduction) (Ulrich and Zhulin, 2005), which is a ubiquitous sensory module in prokaryotic signal transduction. A detailed structural analysis of these regions will shed light on their implications in JA binding.

With regard to the ABF-0046680 chemoreceptor, the *46680* mutant was impaired in the ability to sense xylose, but further analyses are required to demonstrate whether: (i) the LBR of ABF-0046680 binds directly to xylose; and (ii) xylose is being released at wound sites.

Moreover, it is expected that the contribution of these chemoreceptors to the efficiency of infection by *Dd*3937 will depend on the host. As demonstrated previously, the chemotaxis process depends on the presence of a concentration gradient of the compound being sensed (Kalinin *et al*., 2010), and the gradient created in a wound for a given compound may be different for different hosts.

In summary, the study of *Dd*3937 chemoreceptors by bioinformatics analyses and experimental approaches has allowed us to identify the ABF-0020167 and ABF-0046680 chemoreceptors as putative receptors for biomolecules of great importance in plant defence: JA and xylose, respectively. Moreover, entry assays through wounds performed in *A. thaliana* leaves suggest that the perception of plant compounds released from a wound by the chemoreceptors ABF-0020167 and ABF-0046680 is pivotal to the entry into the plant apoplast and to the achievement of a population density sufficient to prosper in the plant apoplast. Further research is needed to demonstrate the interaction between the LBRs and these molecules.

EXPERIMENTAL PROCEDURES

Identification of MCP domains

For sequence analyses, the *Dd*3937 genome sequence was downloaded from the ASAP database (http://asap.ahabs.wisc.edu/asap/home.php). The *Dd*3937 genome was searched for genes encoding chemoreceptors. The search for the MCP domain was performed using an internal pipeline. The MCP domain was downloaded from the Pfam website of the Sanger Centre (http://pfam.sanger.ac.uk/) using the Pfam identifier PF00015. This alignment was used to build hidden Markov models (HMMs) by means of HMMER v2.3.2 (Eddy, 1998). Finally, a customized BioPerl (Stajich *et al*., 2002) script combined with HMMER searched for HMM hits using an E-value of 0.005 to search for MCP-containing proteins.

Identification of TMs and determination of LBR sequence

TM domains were identified one by one using the DAS algorithm at the DAS server (Cserzo *et al*., 1997, 2002). The complete sequences of

Table 2 Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant characteristics	Source or reference
Strains		
E. coli		
DH5α	*supE44 ΔlacU169 (Φ80 lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Hanahan (1983)
CC118 λ*pir*	*Δ (ara, leu) araD ΔlacX74 galE galK phoA20 thi-1 rps rpoB argE*(Am) *recA1* lysogenized with λ*pir* phage; Sp^r	Herrero *et al*. (1990)
S17-1λ*pir*	λ*pir* lysogen of S17-1	de Lorenzo, CIB, Madrid, Spain
UU1250	*Δaer-1 Δ(tar-tap)5201 Δtsr-7028 Δtrg-100 ygjG*::Gm *zbd*::Tn*5*	Bibikov *et al*. (2004)
K12	Wild-type strain	CBGP, Madrid Spain
D. dadantii 3937		
3937	Wild-type strain	Kotoujansky *et al*. (1985)
14536	*14536*::Tn*5* Sp^r *derivative of 3937*	This work
17662	*17662*::Tn*5* Sp^r *derivative of 3937*	This work
17665	*17665*::Tn*7* Km^r *derivative of 3937*	This work
17668	*17668*::Tn*7* Km^r *derivative of 3937*	This work
17824	*17824*::miniTn*5* Sp^r *derivative of 3937*	This work
17863	*17863*::miniTn*5* Sp^r *derivative of 3937*	This work
18541	*18541*::miniTn*5* Sp^r *derivative of 3937*	This work
18754	*18754*::miniTn*5* Sp^r *derivative of 3937*	This work
20167	*20167*::pKNG101 Sm^r *derivative of 3937*	This work
20252	*20252*::Tn*5* Sp^r *derivative of 3937*	This work
46680	*46680*::pKNG101 Sm^r *derivative of 3937*	This work
Plasmids and BACs		
pKNG101	R6K origin *sacB* marker exchange vector; mob^+, Sm^r	Kaniga *et al*. (1991)
pGEM-T easy®	Ap^r	Invitrogen
pGEM-T easy-18754	Ap^r	This work
pGEM-T easy-4387	Ap^r	This work
TrueBlue-BAC2®	Cm^r	Genomics One
TrueBlue-BAC2-18541	Cm^r	This work
TrueBlue-BAC2-20167	Cm^r	This work
TrueBlue-BAC2-20252	Cm^r	This work
TrueBlue-BAC2-46680	Cm^r	This work

chemoreceptors were searched for TMs considering a cut-off value of –2.5. Then, the LBR was extracted from the protein sequence as: (i) the sequence in between two TM domains when present; (ii) the sequence in between the amino terminus and the TM when only one TM was present; (iii) the sequence in between the amino terminus and the MCP domain when no TM was present; or (iv) the sequence in between the carboxyl terminus and the MCP domain when no TM was present. When three TMs were found, only the periplasmic LBR was considered. LBRs were identified in all MCPs and aligned with CLUSTAL W2, which renders the percentage identity matrix. The sizes of LBRs for the chemoreceptors belonging to topology Ia were clustered into groups of 10-amino-acid intervals.

Identification of Pfam domains in the LBR sequence

HMMs based on protein families were downloaded from the Pfam (Finn *et al*., 2014) website (ftp://ftp.ebi.ac.uk/pub/databases/Pfam/current_release/). We focused on the Pfam component, so-called Pfam-A, corresponding to a number of high-quality, manually curated families. LBRs of each of the 47 MCP-associated proteins in Dd3937 were searched for the above Pfam domains using a customized BioPerl (Stajich *et al*., 2002) script. Only hits with an E-value of less than or equal to 0.005 were retained.

Global BLAST analysis of LBR sequences

Amino acid sequences of LBRs were searched against a database of bacterial genome sequences. For this, the complete bacterial sequences of 2997 chromosomes and 2164 plasmids, available as of 14 February 2014, were downloaded from GenBank Refseq. Local BLAST (E-value $\leq 10^{-30}$) was performed using an *ad hoc* PERL script. The output file was processed manually.

Microbiological methods

The bacterial strains and plasmids used in this study are listed in Table 2. Unless indicated, strains of *E. coli* were grown at 37 °C in Luria–Bertani (LB) medium (Sambrook *et al*., 1989). Unless indicated, strains of *Dd*3937 were cultivated at 28 °C in nutrient broth (NB) (per litre: yeast extract, 1 g; beef extract, 2 g; NaCl, 5 g; bactopeptone, 5 g) or King's B (KB) medium (King *et al*., 1954). When required, antibiotics were used at the following final concentrations (μg/mL): ampicillin, 100; chloramphenicol, 30; gentamycin, 20; kanamycin, 20; spectinomycin, 50; streptomycin, 20. Bacterial growth was monitored at an optical density at 600 nm (OD_{600}) using a Jenway 6300 spectrophotometer Jenway (Stone/Staffordshire/United Kingdom). For *Dd*3937 strains used in this study, $OD_{600} = 1.0$ corresponds approximately to 5×10^8 colony-forming units (cfu).

DNA manipulation

The plasmids used in this study are described in Table 2. To analyse the functions of the genes of interest in *Dd*3937, mutants in these genes were tracked by genomic polymerase chain reaction (PCR) from a mutant grid

available in our laboratory using two gene-specific primers and a transposon-specific primer. Those mutants not found in the grid were made by two different approaches: In short, the *17662* and *17668* mutants were constructed by Tn*7 in vitro* mutagenesis using a Genome Priming System (GPS-1) kit (New England Biolabs, Ipswich/Massachusetts/USA), and marker exchange was performed as described previously by Roeder and Collmer (1985). Construction of the *20167* and *46680* mutants was carried out by amplifying with *Pfu* DNA polymerase (Biotools, Madrid/Spain) an internal sequence between 700 and 800 bp from the *Dd*3937 genome and cloning into pKNG101 (Kaniga *et al*., 1991). The resulting plasmid was transferred into *Dd*3937 via triparental mating (de Lorenzo and Timmis, 1994). As pKNG101 cannot replicate in *Dd*3937, single crossover integrants were selected by resistance to streptomycin. In all cases, the mutations were verified by DNA sequencing.

For gain-of-function assays, ABF-0018754 and AIH-0004387 genes were amplified using a *Pfu* DNA polymerase from *Dd*3937 and *E. coli* K12 genomic DNA, respectively. The primers (5'→3') used were as follows: 18754F, GCCCATGTCATTTGATAC; 18754R, AGCGATTAAAAGGTTTCCC; 04387F, ATTCACTCGCGCTAATCTCC; 04387R, CTTATCGGGCATTTTCAT GGC. PCR products were cloned into pGEM-Teasy® vector and the resulting plasmids were transferred into *E. coli* UU1250 via electroporation (Sambrook *et al*., 1989).

For complementation of the mutants, ABF-0020167 and ABF-0046680 genes were amplified using a *Pfu* DNA polymerase from *Dd*3937 genomic DNA employing the following primers (5'→3'): 18541F_PstI, *CTGCA G*TAGCAATTGTCTCCAATTG; 18541R_XmaI, *CCCGG*GTTGTAACGCCATCA TC; 20167F_PstI, *CTGCAG*TTTGCGTTCAAGAGCCTC; 20167R_XmaI, *CCCG GG*TATCAAGGTGCAGTGC; 20252F_PstI, *CTGCAG*TACATGGAATGACGT TCACA; 20252R_XmaI, *CCCGGG*CCAAACAGGTGTGATGGTTA; 46680F_ BamHI, *GGATCC*ATCATTAATTCTCATGTTG; 46680R_PstI, *CTGCAG*TGTA TCAGCGTTGACCGC; the restriction sites used for cloning into TrueBlueBAC2© (Genomics One) are shown in italic type. The cloned genes were sequenced to confirm that no PCR errors had occurred, and TrueBlueBAC2-20167 or TrueBlueBAC2-46680 was introduced into the corresponding mutant by electroporation (Sambrook *et al*., 1989).

Gain-of-function assays

Starter cultures of *E. coli* cells were grown overnight in liquid LB medium to the late exponential phase and then 20-fold diluted into liquid LB medium and incubated at 37 °C to an OD_{600} of 0.6. Cultures were centrifuged at 6500 ***g*** and washed twice with 10 mM $MgCl_2$. Sediments were used for inoculation. Tryptone broth (TB) (Parkinson, 1976) 0.35% agar plates, supplemented or not with 1 mM serine (final concentration), were inoculated with bacterial sediments using a toothpick. Plates were incubated for 48 h at 37 °C. Five plates were inoculated for each experiment.

Swimming assays

*Dd*3937 cells were scraped from NB plates incubated overnight and suspended in 10 mM $MgCl_2$ to an OD_{600} of 1.0. A 5-μL drop from each bacterial suspension was inoculated at the centre of each of five KB 0.25% agar plates. Plates were incubated for 24 h at 28 °C with high humidity. After that, bacterial haloes were recorded. Significant differences between the CIs of WT and mutant strains were determined and statistically compared according to Student's *t*-test ($P < 0.05$).

The chemical 'in-plug' bacterial chemotaxis assay

*Dd*3937 cells were scraped from NB plates incubated overnight and suspended in 10 mM $MgCl_2$ at the desired final concentration. Then the bacterial suspension was mixed with Minimal Medium A (MMA) agar medium (Antúnez-Lamas *et al*., 2009) to a final concentration of 0.25% agar and 2.5×10^8 cfu/mL. Once polymerized, a 5-μL drop containing a given chemical was placed on the agar surface (Tso and Adler, 1974). Xylose, serine and JA were used at 10 mM (final concentration). Plates were incubated for 20 min at room temperature. After that, bacterial haloes were recorded. Five replicates were analysed for each experiment. Water was used as a solvent for xylose and serine, and JA was dissolved in ethanol.

Capillary assays

The chemotaxis of *Dd*3937 towards several compounds was measured using a modified Palleroni assay (Palleroni, 1976). CI was defined as the number of cells that accumulated after 20 min within a 5-μL tip containing 2 μL of a given compound divided by the number of cells accumulated within a 5-μL tip containing 2 μL of the solvent in which the compound was dissolved. Values of CI significantly greater than 1.0 were identified as a positive response of the cells to the chemical compound. Values of CI significantly less than 1.0 were identified as a negative response of the cells to the chemical compound. Three replicates (capillaries) were analysed in each experiment. Xylose, serine and JA were tested at 1 mM. Significant differences between the CIs of WT and mutant strains were determined and statistically compared according to Student's *t*-test ($P < 0.05$).

Plant bioassays

Entry and entry-dependent colonization assays of *Dd*3937 strains in *A. thaliana* leaves were carried out as described previously by Antunez-Lamas *et al*. (2009) using 4-week-old Col-0 ecotype plants. A 10-μL drop containing 10^5 cfu was placed on a hole made with a needle on the adaxial side of leaves. Ten leaves were inoculated with each strain. Three leaves were mock infiltrated with 10 mM $MgCl_2$. After 2 or 24 h at 28 °C, leaves were cut and washed with sterile water, and viable cell counts in leaves were determined by serial dilution and plating.

For non-entry-dependent colonization assays, 10 leaves were infiltrated into the abaxial side with a suspension containing 2×10^6 cfu/mL by syringe infiltration. Three leaves were mock infiltrated with 10 mM $MgCl_2$. After 24 h at 28 °C, leaves were processed as described above.

In both kinds of experiment, significant differences between WT and mutant cells inside the leaves were determined and statistically compared according to Student's *t*-test ($P < 0.05$).

ACKNOWLEDGEMENTS

This research was supported by the Spanish Plan Nacional I+D+I grants AGL-2009-12757 and AGL2012-32516. I. Río-Álvarez was supported by the FPI program (MICINN-Spain). C. Muñoz-Gómez was supported by the FPI program (MINECO-Spain). P. M. Martínez-García was supported by the Campus de Excelencia Internacional Andalucía Tech and AGL2011-30343-C02-01. We thank Dr Tino Krell for critical reading of the manuscript and helpful comments, and for providing the *E. coli* UU1250 strain. We also thank Dr Miguel Angel Torres for helpful review of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1 Complementation of non-chemotactic strain *Escherichia coli* UU1250 with putative serine chemoreceptors. Tryptone broth (TB) 0.35% agar plates (A) and TB 0.35% agar plates supplemented with 1 mM serine (B) were inoculated with wild-type (WT), UU1250 or transformed *E. coli* UU1250 strains expressing the *E. coli* serine chemoreceptor (AIH-0004387) or the putative serine chemoreceptor of *Dd*3937 (ABF-0018754). Results were recorded at 48 h. Similar results were obtained in three independent experiments.

Fig. S2 'In-plug' chemotactic response of *Dd*3937 strains towards serine. A drop containing water or serine (10 mM) was deposited on the (0.25%) soft-agar bacteria. All the *Dd*3937 strains tested [wild-type (WT) and mutants] showed no chemotactic response towards water; a representative image is shown. Haloes were recorded after 20 min. Similar results were obtained in three independent experiments.

Fig. S3 Swimming ability of *Dd*3937 strains in rich medium. King's B (KB) 0.25% agar plates were inoculated with *Dd*3937 wild-type (WT) and mutant strains at the exponential phase. Diameters of haloes were recorded at 24 h. The means and standard errors are shown. Error bars represent standard error of the mean (SEM). Similar results were obtained in three independent experiments.

Table S1 Percentage identity matrix of the Dd3937 ligand-binding region (LBR).

Table S2 Pfam matches for Dd3937 ligand-binding regions (LBRs). (a) Nomenclature from the ASAP database (https://asap.ahabs.wisc.edu/asap/logon/php). In grey, methyl-accepting chemotaxis proteins (MCPs) under study in this work.

Table S3 Global BLAST of Dd3937 ligand-binding regions (LBRs). Amino acid sequences of LBRs were searched against a database of bacterial genome sequences using BLAST (E-value $\leq 10^{-30}$). * indicates that the periplasmic LBR was selected when two LBRs were present. (a) Nomenclature from the ASAP database (https://asap.ahabs.wisc.edu/asap/logon/php).