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Cytosine chemoreceptor McpC in *Pseudomonas putida* F1 also detects nicotinic acid

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Soil bacteria are generally capable of growth on a wide range of organic chemicals, and pseudomonads are particularly adept at utilizing aromatic compounds. Pseudomonads are motile bacteria that are capable of sensing a wide range of chemicals, using both energy taxis and chemotaxis. Whilst the identification of specific chemicals detected by the $\geqslant$26 chemoreceptors encoded in *Pseudomonas* genomes is ongoing, the functions of only a limited number of *Pseudomonas* chemoreceptors have been revealed to date. We report here that McpC, a methyl-accepting chemotaxis protein in *Pseudomonas putida* F1 that was previously shown to function as a receptor for cytosine, was also responsible for the chemotactic response to the carboxylated pyridine nicotinic acid.

INTRODUCTION

N-heterocyclic aromatic compounds are abundant in nature, and provide good sources of carbon, nitrogen and energy for bacterial strains capable of their degradation (Fetzner, 1998; Kaiser *et al.*, 1996). Naturally occurring *N*-heteroaromatic compounds include key structural components of the nucleotides in DNA and RNA, the electron-carrying coenzymes NAD^+ and $NADP^+$, the energy currency molecules ATP and GTP, and vitamin B_6. In addition to natural *N*-heteroaromatic compounds, many structurally similar chemicals are produced industrially as solvents, pesticides and pharmaceuticals (Fetzner, 1998). Whilst there has been significant progress in elucidating the bacterial pathways by which many of these molecules are degraded (Fetzner, 1998; Kaiser *et al.*, 1996), little is known about whether these compounds are sensed as chemoattractants by motile bacteria.

One abundant *N*-heteroaromatic structure in nature is the pyridine ring, and the carboxylated pyridine nicotinic acid (also known as niacin and vitamin B_3) has served as a model for *N*-heteroaromatic compound degradation. Nicotinic acid is an intermediate in the biosynthesis and salvage pathways for the pyridine nucleotides NAD^+ and $NADP^+$, which are essential coenzymes in redox reactions in all domains of life. Additional roles for NAD in intracellular signalling, regulation of cell life span and circadian rhythms have been described in studies that highlight the importance and ubiquity of pyridine nucleotides in natural systems (Di Stefano & Conforti, 2013). The complete pathway for aerobic nicotinic acid degradation has been characterized in *Pseudomonas putida* KT2440 (Jiménez *et al.*, 2008, 2011). Members of the genus *Pseudomonas* are generally considered to be catabolically versatile and are particularly adept at degrading aromatic compounds; however, whilst the ability to utilize nicotinic acid seems to be characteristic of *P. putida*, it is rarely found in other *Pseudomonas* species (Jiménez *et al.*, 2010).

Consistent with their catabolic versatility, pseudomonads have complex chemotaxis systems that allow them to sense and respond to a wide range of chemicals in the environment (Kato *et al.*, 2008; Parales *et al.*, 2004; Sampedro *et al.*, 2014). The currently available sequenced *Pseudomonas* genomes have $\geqslant$26 genes predicted to encode methyl-accepting chemotaxis proteins (MCPs), which serve as chemoreceptors and signal transducers in the chemotaxis pathway (Parales *et al.*, 2004; Sampedro *et al.*, 2014). In contrast, *Escherichia coli* has four MCPs and Aer, an MCP-like energy taxis receptor (Hazelbauer *et al.*, 2008). Recent analyses of bacterial and archaeal genomes have shown that the number of MCPs encoded in a given genome is generally related to the metabolic diversity of the organism rather than the size of the genome (Lacal *et al.*, 2010). We have been using *P. putida* F1 as a model organism in which to study the range of attractants detected and the complexity of chemotactic signalling (Ditty *et al.*, 2013; Liu *et al.*, 2009; Luu *et al.*, 2013; Parales *et al.*, 2000, 2013). Previous work demonstrated that McpC, one of the 27 predicted MCPs/MCP-like proteins in *P. putida* F1, mediated the chemotactic response to the *N*-heteroaromatic pyrimidine base cytosine

Abbreviations: LBR, ligand-binding region; MCP, methyl-accepting chemotaxis protein.

(Liu *et al.*, 2009). In this study, we confirmed the function of the predicted nicotinic acid degradation pathway in *P. putida* F1, demonstrated that nicotinic acid serves as a chemoattractant for strain F1 and revealed that McpC mediates the chemotactic response to nicotinic acid.

METHODS

Bacterial strains and plasmids. The strains and plasmids used in this study are shown in Table 1. *E. coli* strains DH5α and DH5α(*λpir*) were used as hosts for cloned genes. *E. coli* HB101(pRK2013) was used as a helper strain for mobilizing plasmids in triparental matings, which were carried out on lysogeny broth (LB) plates (Davis *et al.*, 1980) at 30 °C for 24 h. *E. coli* strains were cultured in LB medium at 37 °C. *P. putida* strains were grown at 30 °C in minimal salts basal medium (MSB) (Stanier *et al.*, 1966) containing 10 mM succinate, 40 mM pyruvate or 5 mM nicotinic acid. MSB plates were solidified with 1.8 % Noble agar (BD Biosciences). Kanamycin, gentamicin and tetracycline were used at 100, 15 and 20 μg ml^{-1}, respectively, for *E. coli* strains, and at 50, 15 and 20 μg ml^{-1}, respectively, for *P. putida* strains.

Chemicals. Nicotinic acid (99.5 %), picolinic acid (99 %) and isonicotinic acid (99 %) were purchased from Acros Organics, and nicotinamide was from K & K Laboratories.

DNA methods. Genomic DNA from strain F1 was purified using a Puregene DNA Isolation kit (Gentra Systems). Plasmids were purified using a QIAprep Miniprep kit (Qiagen), and DNA fragments and PCR products were purified with a QIAquick Gel Extraction kit (Qiagen). Standard methods were used for the manipulation of plasmids (Ausubel *et al.*, 1993). Fluorescent automated DNA sequencing was carried out at the University of California, Davis sequencing facility using an Applied Biosystems 3730 automated sequencer.

Construction of a nicotinic acid catabolic mutant. The *nicB* gene in *P. putida* F1 (locus tag Pput_1889) was insertionally inactivated with the kanamycin resistance gene from pRK415Km (Luu *et al.*, 2013) using an Infusion-HD cloning kit (Clontech), and the suicide vector pEX18, which carried a gentamicin resistance gene and the *sacB* gene, conferring sucrose sensitivity (White & Metcalf, 2004). The primers used were pEX18_1889delfor (5′-CGACGGCCAGTGCCAAGCTTCACTCACAACAGGTGCCCAG-3′)/pEX18_1889delrev (5′-GCTATGACCATGATTACGAATTCCATCATTACGTCGATAGCTGGCA-3′), pput_1889_intfor (5′-TTGAATGGGCCCTACATGGTGTGGTCAGGTACGCAGAAC-3′)/pput_1889intrev (5′-GAGTTCGGTCCGATCAAGGTACCTGACCACACGCGGAT-3′) and pETKm_RsrIfor/pETKm_ApaI-rev (Luu *et al.*, 2013). To generate the mutant construct, 1 kb regions at the beginning and centre of the *nicB* gene were amplified by PCR, and the resulting PCR fragments were fused to the amplified kanamycin resistance gene and pEX18 using the complementary overhanging ends. The resulting plasmid (pVNF10) was introduced into *E. coli* DH5α(*λpir*), verified by restriction digestion and introduced into *P. putida* F1 by conjugation in the presence of *E. coli* HB101(pRK2013) (Simon *et al.*, 1983). Gentamicin- and kanamycin-resistant *P. putida* exoconjugants were subjected to counterselection in MSB containing 10 mM succinate and 20 % sucrose. Deletions in kanamycin-resistant, gentamicin-sensitive strains were verified by PCR.

Construction of a *P. putida* F1 Δ*aer2*Δ*mcpC* double mutant. The *aer2* gene was deleted from the Δ*mcpC* mutant XLF004 (Liu *et al.*, 2009) by homologous recombination using the *aer2* deletion construct pXLF019 (Luu *et al.*, 2013), generating strain XLF119.

Table 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Source or reference
Strains		
Escherichia coli		
DH5α	Cloning host	Life Technologies
DH5α(*λpir*)	Cloning host	William W. Metcalf University of Illinois
HB101	Host for mobilization plasmid pRK2013	Sambrook *et al.* (1989)
Pseudomonas putida		
F1	WT	Finette *et al.* (1984); Gibson *et al.* (1970)
VNF001	F1 *nicB*::Km	This study
XLF004	F1 Δ*mcpC*	Liu *et al.* (2009)
XLF019	F1 Δ*aer2*	Luu *et al.* (2013)
XLF119	F1 Δ*aer2*Δ*mcpC*	This study
Pseudomonas aeruginosa		
PAO1	WT	Stover *et al.* (2000)
Plasmids		
pEX18Gm	Cloning vector, *sacB*, Gm^r	Hoang *et al.* (1998)
pRK415	Broad-host-range cloning vector, Tc^r	Keen *et al.* (1988)
pRK2013	ColE1 *ori*, RP4 mobilization function, Km^r	Figurski & Helinski (1979)
pVNF10	*nicB* (locus tag Pput_1889) from strain F1 interrupted with a kanamycin resistance gene cloned in pEX18Gm, Gm^r	This study
pXLF019	*aer2* deletion construct	Luu *et al.* (2013)
pXLF204	*mcpC* (locus tag Pput_0623) from strain F1 cloned into *Xba*HI/*Eco*RI sites of pRK415, constitutively expressed from *lac* promoter of plasmid, Tc^r	Liu *et al.* (2009)

Chemotaxis assays. Qualitative capillary assays were carried out as described previously (Grimm & Harwood, 1997; Parales *et al.*, 2013). Briefly, bacterial cells in mid-exponential phase (OD_{660} 0.3–0.4) were harvested by centrifugation at 5000 x ***g*** for 5 min, washed once with chemotaxis buffer (CB; 50 mM potassium phosphate buffer, pH 7.0, 10 μM disodium EDTA and 0.05 % glycerol) (Parales *et al.*, 2000) and gently resuspended in CB to OD_{660}~0.10. Microcapillaries (1 μl) containing attractants in 2 % low-melting-temperature agarose (NuSieve GTG; Lonza) dissolved in CB were inserted into the suspension of bacterial cells. The response to nicotinic acid was tested at 50 mM. Negative (CB) and positive (2 % Difco Casamino acids; BD Biosciences) controls were included in all experiments. Responses were visualized at ×40 magnification on a Nikon Eclipse TE2000-S microscope, and photographed using an Evolution MicroPublisher 3.3 real-time viewing camera and Evolution MP/QImaging software (Media Cybernetics).

Quantitative capillary assays were carried out as described previously (Liu *et al.*, 2009). For these assays, cells were grown to OD_{660}~0.4 in MSB containing 5 mM nicotinic acid, harvested by centrifugation and resuspended in CB to a final OD_{660} 0.15. Responses to 5, 10, 50 and 100 mM nicotinic acid were tested. The response to 0.2 % Casamino acids was tested as a positive control and the response to 50 mM cytosine (the peak attractant concentration; Liu *et al.*, 2009) was tested for comparison. Competition assays were carried out with the competing attractant cytosine (50 mM) present in both the cell suspension and the capillary. Responses to 50 mM nicotinic acid, 0.2 % Casamino acids and buffer in the capillary were tested.

Minimal medium-soft agar swim plates (Harwood *et al.*, 1994) contained 2 mM nicotinic acid. For these assays, *P. putida* strains were grown overnight in 3 ml MSB medium containing 5 mM nicotinic acid at 30 °C with shaking. Cultures were harvested by centrifugation and the pellets were washed with 5 ml MSB, resuspended in MSB to OD_{660}~0.4, and 2 μl of each suspension was used to inoculate semi-solid (0.3 % Noble agar) minimal medium in 15 mm diameter Petri plates. For complementation experiments, 20 mg tetracycline ml^{-1} was included in the overnight growth medium as well as the soft agar plates. Cultures were incubated at 30 °C for 26–30 h. Photographs were taken using backlighting (Parkinson, 2007). For each experiment, the measured diameters of all strains were normalized to the mean diameter of WT *P. putida* F1 colonies (which was set to 1). All statistical analyses were conducted using JMP Pro version 10.0.

RESULTS

P. putida F1 is chemotactic to nicotinic acid and the response does not require nicotinic acid metabolism

The nicotinic acid degradation pathway has been characterized in *P. putida* KT2440 (Jiménez *et al.*, 2008, 2011) and a very similar gene cluster was identified in *P. putida* F1 (Fig. 1). Genomic analyses have suggested that key enzymes in the nicotinic acid pathway are conserved in several *P. putida* strains (Jiménez *et al.*, 2010; Tang *et al.*, 2012). As predicted by the genome sequence *P. putida* F1 was capable of growth on nicotinic acid as the sole carbon and energy source (doubling time 85±5 min; 5 mM nicotinic acid). The chemotactic response of *P. putida* F1 to 50 mM nicotinic acid was tested in qualitative capillary assays. Both pyruvate-grown (uninduced) and nicotinic acid-induced cells responded (Fig. 2). The response was specific to nicotinic acid as neither nicotinamide nor the other pyridine carboxylic acid isomers (isonicotinic acid and picolinic acid) elicited a response, and none of these *N*-heteroaromatic compounds served as growth substrates for strain F1 (data not shown).

In order to determine whether nicotinic acid was detected directly, we insertionally inactivated the *nicB* gene (Fig. 1), which encoded the catalytic component of nicotinic acid hydroxylase (Jiménez *et al.*, 2008). The resulting mutant (strain VNF001) was unable to grow on nicotinic acid (data not shown), but it had a WT response to nicotinic acid in qualitative capillary assays (Fig. 2), indicating that metabolism of nicotinic acid was not required for the chemotactic response and ruling out a role for energy taxis in the response.

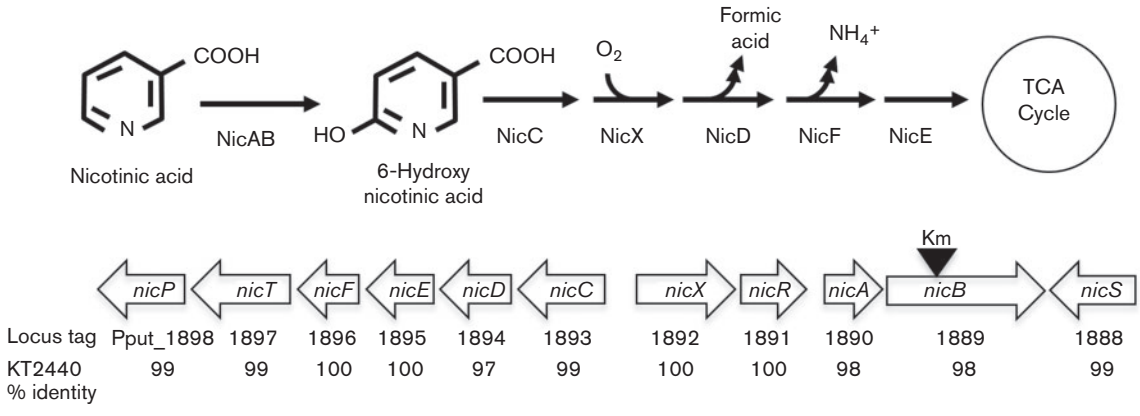

Fig. 1. Nicotinic acid degradation pathway and the *nic* gene cluster encoding pathway enzymes in *P. putida* F1. The *nic* genes are represented by open arrows. Locus tags for the *P. putida* F1 *nic* genes, together with the percent identities of the *P. putida* F1 and KT2440-deduced amino acid sequences, are indicated below the genes. The black triangle indicates the approximate location of the kanamycin resistance gene inserted into the *nicB* gene in strain VNF001.

McpC mediates the chemotactic response to nicotinic acid

The role of McpC in the detection of nicotinic acid was evaluated using qualitative capillary assays, which showed that the response to nicotinic acid by the Δ*mcpC* mutant strain XLF004 was severely reduced relative to that of WT *P. putida* F1 (Fig. 2). The chemotaxis defect was specific, as the response to Casamino acids was as strong as that of the WT (Fig. 2). The response was not completely eliminated in XLF004, but none of the other 26 single-deletion mutants (17 mutants each lacking one of the MCP-encoding genes and nine mutants each lacking one of the genes encoding MCP-like proteins; Liu, 2009; Parales *et al.*, 2013) had an obviously reduced response in qualitative capillary assays (data not shown). The swim-plate assay is more quantitative and can be used to detect subtle defects in chemotaxis; however, previous studies have shown that aerotaxis can mask defects in chemotaxis to specific chemicals in swim plates (Alvarez-Ortega & Harwood, 2007; Parales *et al.*, 2013). We therefore used quantitative swim-plate assays to examine 18 double mutants of *P. putida* F1 (each lacking the energy taxis receptor gene *aer2* and one of the 18 canonical MCP-encoding genes; Liu, 2009; Parales *et al.*, 2013) for defects in nicotinic acid chemotaxis. However, the only strain that demonstrated an obvious mutant phenotype in response to nicotinic acid lacked *aer2* and *mcpC* (Fig. 3a and data not shown). A slight but significant difference in colony size for WT strain F1 and the Δ*mcpC* single-mutant XLF004 was also detected (Fig. 3b). Growth studies demonstrated that all strains had similar growth rates in MSB medium containing 5 mM nicotinic acid (data not shown), indicating that the reduced colony size in the swim-plate assay was solely due to a chemotaxis defect. The response of the Δ*aer2* mutant was similar to that of the WT in qualitative capillary assays, and responses of the Δ*mcpC* mutant and Δ*mcpC*Δ*aer2* double mutant were comparable in this assay (Fig. 2), providing further evidence that energy taxis does not play a major role in the response to nicotinic acid. Introduction of a broad-host range plasmid carrying *mcpC* into strain XLF004 restored the response to nicotinic acid in both swim-plate assays (Fig. 3c, d) and qualitative capillary assays (Fig. 4).

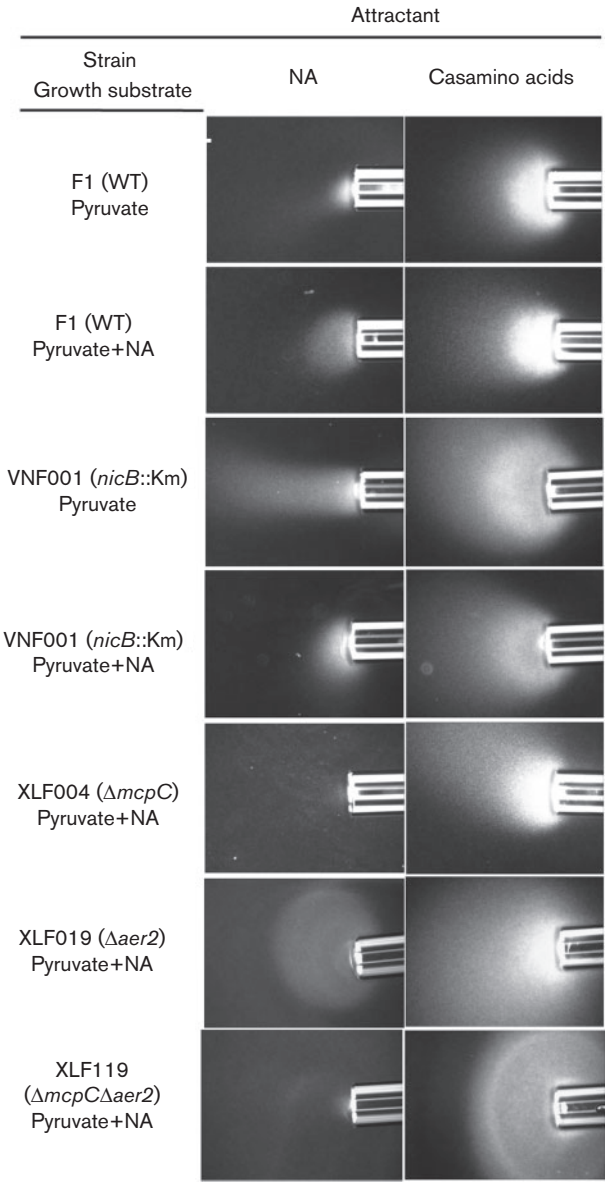

Fig. 2. Chemotactic response of WT and mutant *P. putida* strains to nicotinic acid in qualitative capillary assays. *P. putida* F1 and the *nicB* (VNF001), Δ*mcpC* (XLF004), Δ*aer2* (XLF019) and Δ*aer2*Δ*mcpC* (XLF119) mutants were grown either with 40 mM pyruvate (uninduced) or 40 mM pyruvate plus 5 mM nicotinic acid (NA; induced) as indicated. Nicotinic acid was provided as the attractant at 50 mM. Also shown are positive control responses of each strain to 2 % Casamino acids. No response was detected when only chemotaxis buffer was present in the capillary (not shown). Assays were repeated at least three times and representative photographs are shown. Photographs were taken after 7 min.

Sensitivity of McpC for nicotinic acid and cytosine

We used quantitative capillary assays to determine the sensitivity of McpC for nicotinic acid by testing the response of WT *P. putida* F1 to a range of nicotinic acid concentrations (5–100 mM). The strongest response to nicotinic acid was at the highest tested concentration and this was much weaker than the response to 50 mM cytosine (Fig. 5a). The response to cytosine in this experiment in which cells were grown with 5 mM nicotinic acid was comparable to the previously reported responses of succinate- and succinate plus cytosine-grown *P. putida* F1 (13 900 ± 1600 and 14 200 ± 1700 cells, respectively; Liu *et al.*, 2009). The presence of 50 mM cytosine as a competing attractant was able to eliminate the response to nicotinic acid (Fig. 5b). These results showed that cytosine was a stronger attractant than nicotinic acid and provided

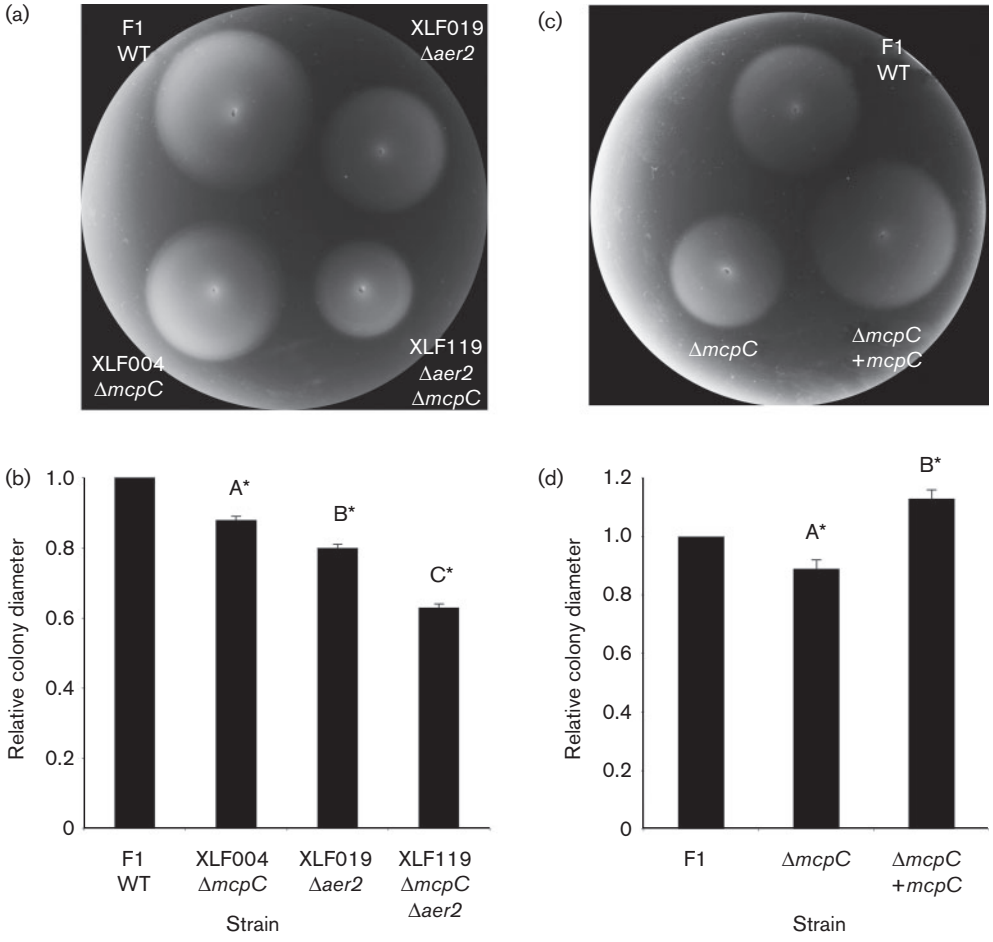

Fig. 3. Chemotactic response of WT, mutant and complemented *P. putida* strains to nicotinic acid in soft agar swim plates. (a) Representative swim plate showing responses of *P. putida* strains F1 (WT), XLF004 (Δ*mcpC*), XLF019 (Δ*aer2*) and XLF119 (Δ*aer2*Δ*mcpC*) to 2 mM nicotinic acid. (b) Quantitative analysis of swim-plate assay results in (a) (n=3). Mean colony diameters were normalized to the diameter of WT F1. (c) Representative swim plate showing complementation of the *mcpC* deletion. Shown are responses of *P. putida* strains F1 (pRK415) (WT), XLF004 (pRK415) (Δ*mcpC*) and XLF004 (pXLF204) (Δ*mcpC* containing *mcpC*) to 2 mM nicotinic acid. (d) Quantitative analysis of swim-plate assay results in (c) (n=3). Mean colony diameters were normalized to the diameter of F1 (pRK415). Bars, SD. Means with different letters are significantly different. *P<0.05, one-way ANOVA interaction, Tukey's multiple comparison test. Ninety-five per cent confidence intervals (indicated by asterisks) are used to describe significant differences from the normalized WT controls.

evidence that both attractants were detected by the same binding site on McpC.

Heterologous expression of *mcpC* confers the ability to sense nicotinic acid in *Pseudomonas aeruginosa*

Previous studies demonstrated that *P. aeruginosa* PAO1 does not encode a McpC orthologue and is not chemotactic to cytosine (Liu *et al.*, 2009). A search of the *P. aeruginosa* PAO1 genome sequence (Jiménez *et al.*, 2010; Stover *et al.*, 2000) did not identify genes encoding a nicotinic acid degradation pathway. We confirmed that *P. aeruginosa* PAO1 was unable to grow in minimal medium containing 5 mM nicotinic acid (data not shown) and did not respond to nicotinic acid when tested using the qualitative capillary assay (Fig. 6). However, when *mcpC* from *P. putida* F1 was expressed in *P. aeruginosa* PAO1, the strain acquired the ability to sense nicotinic acid (Fig. 6).

DISCUSSION

The mechanism of aromatic compound sensing in *Pseudomonas* strains seems to depend on the particular aromatic compound under study. Responses to (methyl)phenols and phenylacetic acid are mediated through energy taxis via Aer2 (Luu *et al.*, 2013; Sarand *et al.*, 2008), whilst responses to naphthalene, 2-nitrobenzoate, toluene, 4-chloroaniline, catechol and aromatic amino acids are sensed by chemotaxis

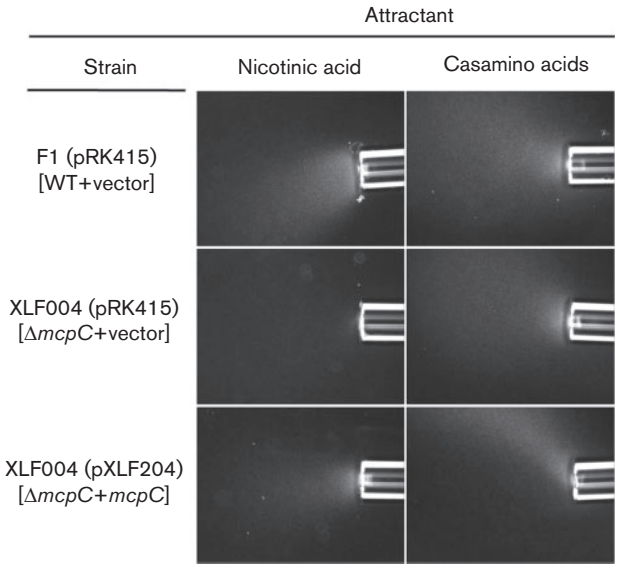

Fig. 4. Qualitative capillary assays demonstrating complementation of the *mcpC* deletion. Responses of *P. putida* F1 (pRK415) (WT), XLF004 (pRK415) (Δ*mcpC*) and XLF004(pXLF204) (Δ*mcpC* containing *mcpC*) are shown. Nicotinic acid was provided at 50 mM. Also shown are positive control responses of each strain to 2 % Casamino acids. No response was detected when only chemotaxis buffer was present in the capillary (not shown). Assays were repeated at least three times and representative photographs are shown. Photographs were taken after 7 min.

via specific MCPs (Grimm & Harwood, 1999; Iwaki *et al.*, 2007; Lacal *et al.*, 2011; Oku *et al.*, 2012; Parales *et al.*, 2000; Taguchi *et al.*, 1997; Vangnai *et al.*, 2013).

Based on previous results (Liu *et al.*, 2009) and results reported here, we now have evidence that McpC is responsible for chemotaxis to the *N*-heteroaromatic compounds cytosine and nicotinic acid. The response to these two chemicals is quite specific, as the nicotinic acid isomers and nicotinamide, and the pyrimidine bases thymine and uracil (Liu *et al.*, 2009), are not detected. Whilst a nicotinic acid chemotaxis defect was seen in the Δ*aer2* mutant in swim-plate assays, we interpret this as an aerotaxis defect. This conclusion is consistent with previous results in *P. putida* and *P. aeruginosa*, which have shown that responses in swim-plate assays can result from a combination of both aerotaxis and chemotaxis to specific chemicals; in some cases, aerotaxis masks chemotaxis phenotypes (Alvarez-Ortega & Harwood, 2007; Parales *et al.*, 2013). In addition, the WT response of the *nicB* mutant demonstrates that metabolism of nicotinic acid by *P. putida* F1 is not required for the chemotactic response, as would be expected if the signal were processed primarily via energy taxis (Alexandre, 2010). In *E. coli*, MCPs serve as the primary chemoreceptors for some attractants, whilst other attractants are detected by a specific periplasmic binding protein; the complex then interacts with a specific MCP to transmit the signal (Wadhams & Armitage, 2004). It seems likely that McpC binds both cytosine and nicotinic acid directly without the participation of a periplasmic binding protein,

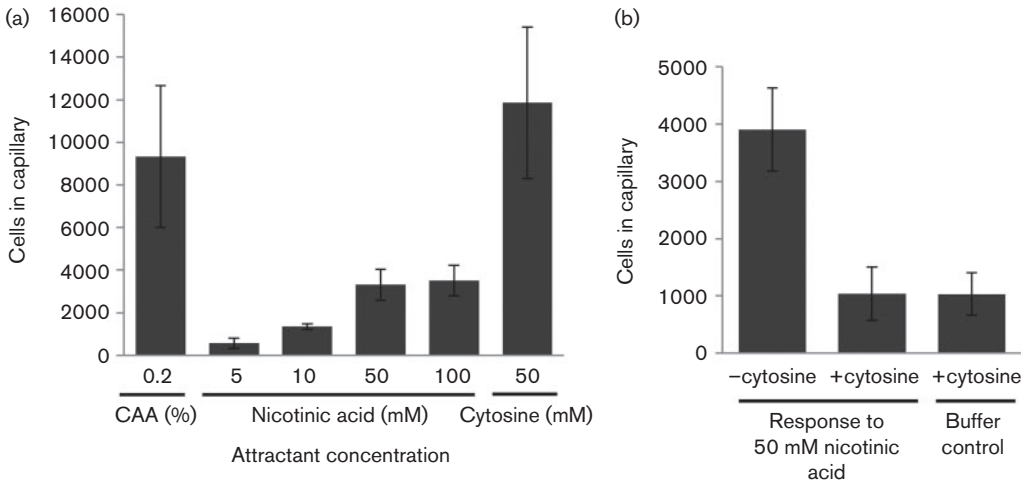

Fig. 5. Sensitivity of *P. putida* F1 to nicotinic acid and cytosine measured by quantitative capillary assays. (a) Chemotactic responses to various concentrations of nicotinic acid compared to the response to 50 mM cytosine (the previously determined peak attractant concentration; Liu *et al.*, 2009). Response to the positive control attractant 0.2 % Casamino acids (CAA) is also shown. The mean number of cells that accumulated in capillaries containing buffer only was subtracted from each dataset (590 ± 140 cells). (b) Response to 50 mM nicotinic acid (attractant in the capillary) in competition capillary assays in which 50 mM cytosine (competitor) was present or absent in both the capillary and cell suspension as indicated. Cultures were grown in MSB containing 5 mM nicotinic acid and results represent the mean ± SEM of at least three independent experiments (nine or more capillaries in total), except the cytosine data in (a) (two independent experiments, six capillaries in total).

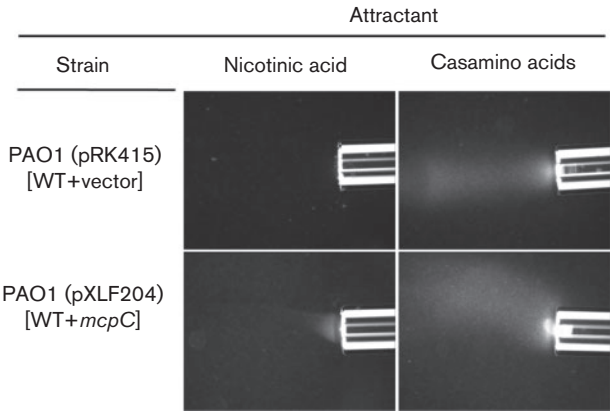

Fig. 6. Chemotactic responses of *P. aeruginosa* PAO1 (pRK415) and PAO1 (pXLF204) (carrying *mcpC*) to 50 mM nicotinic acid in qualitative capillary assays. Cells were grown overnight in MSB containing 27.5 mM glucose and 60 μg tetracycline ml^{-1}. As cultures grown in the presence of tetracycline were poorly motile, cultures were reinoculated in the same medium lacking tetracycline and were grown for two to three doublings prior to assays. Also shown are positive control responses of each strain to 2 % Casamino acids. No response was detected when only chemotaxis buffer was present in the capillary (not shown). Assays were repeated at least three times and representative photographs are shown. Photographs were taken after 7 min.

as heterologous expression of *mcpC* alone was sufficient to confer the ability to respond to both compounds in *P. aeruginosa* PAO1 (Fig. 6) (Liu *et al.*, 2009).

As reported previously, McpC has a length of 647 aa and exhibits the canonical MCP structure, with a periplasmic ligand-binding region (LBR) flanked by two hydrophobic transmembrane helices, a HAMP (histidine kinase, adenylyl cyclase, methyl-accepting chemotaxis protein, and phosphatase) domain, and a cytoplasmic signal transduction region (Liu *et al.*, 2009). The cytoplasmic region is in class 40H (comprised of 40 heptads; Alexander & Zhulin, 2007) and has two potential methylation sites, but does not contain any C-terminal pentapeptide tether regions, which are known to be binding sites for CheB and CheR in *E. coli* (Li & Hazelbauer, 2006). The LBR falls into cluster II (Lacal *et al.*, 2010), spanning 262 aa, and automated annotation identified the presence of a conserved Cache (Ca^{2+} channel and chemotaxis receptor) domain, which is predicted to sense small molecules (Anantharaman & Aravind, 2000; Finn *et al.*, 2014). A closer examination revealed that the predicted Cache domain is one of two inserted PAS (Per, ARNT, Sim)-like PDC (PhoQ, DcuS, CitA) domains, in an architecture seen in the sensor domains of many bacterial histidine kinases, including chemoreceptors (Cheung & Hendrickson, 2010; Zhang & Hendrickson, 2010).

A homology search comparing the McpC periplasmic LBR to known structures in the Protein Data Bank returned an uncharacterized MCP from *Vibrio cholerae* (ID: 3C8C; Y. Patskovsky and others, unpublished) as the top hit. The LBRs of McpC and the *Vibrio* MCP are 30 % identical in amino acid sequence. Webb *et al.* (2014) recently reported a homologue of the *Vibrio* MCP (which binds alanine) in *Sinorhizobium meliloti* called McpU, which senses proline. The LBRs of the *Vibrio* MCP and McpU are 26 % identical, and have conserved aspartate residues at positions 172 and 201 (*Vibrio* MCP) and 155 and 182 (McpU) that coordinate their amino acid ligands via hydrogen bonds with the amino group (Webb *et al.*, 2014). The aspartate at one of the corresponding residues in McpC, position 191, is conserved. However, position 163 contains a tryptophan, which may reflect the hydrophobic nature of the ligands nicotinic acid and cytosine.

The response to nicotinic acid was not completely abolished in the Δ*mcpC* mutant; however, two different screens to identify additional receptors that sense nicotinic acid did not identify any mutants with obvious phenotypes. It is possible that multiple additional MCPs participate in the response to nicotinic acid and phenotypes of mutant strains lacking any single MCP were too subtle to detect in these assays. These results suggest that McpC may not be the only chemoreceptor that detects nicotinic acid in *P. putida* F1.

Expression of the *nic* genes in *P. putida* KT2440 is tightly controlled by a regulatory circuit involving two repressors that respond to different effectors (Jiménez *et al.*, 2011). Although regulation of the *nic* genes has not been investigated in *P. putida* F1, based on the conserved gene order and deduced amino acid sequences of the nine structural and two regulatory genes in *P. putida* KT2440, it is expected that *nic* genes in both strains are regulated in a similar fashion. The chemotactic response of *P. putida* F1 to nicotinic acid appeared to be constitutive, as was the response of *P. putida* F1 to cytosine (Liu *et al.*, 2009). It seems unlikely that *mcpC* would be specifically regulated with genes for nicotinic acid degradation as the receptor also mediates the response to cytosine, which serves as a nitrogen source for *P. putida* F1 that is metabolized by a completely different pathway. The *mcpC* gene (locus tag Pput_0623) is not co-localized with the *nic* genes for nicotinic acid degradation (locus tags Pput_1888–Pput_1898), nor does it appear to be co-regulated based on the constitutive chemotaxis phenotype.

Many human, animal and plant pathogens lack pathways for *de novo* biosynthesis of NAD, and are nicotinic acid or nicotinamide auxotrophs; a few examples include *Shigella* species, enteroinvasive *E. coli* strains, *Salmonella enterica* (serovar Dublin), group A streptococci and *Erwinia amylovora* (Bergthorsson & Roth, 2005; Paternoster *et al.*, 2010; Prunier *et al.*, 2007; Sorci *et al.*, 2013). The use of nicotinic acid-degrading strains of *Pseudomonas rhizosphaerae* and *Pseudomonas fluorescens* as biocontrol agents to protect plants from the plant pathogen *Erwinia amylovora*, a nicotinic acid auxotroph, was reported recently (Paternoster *et al.*, 2010). It is unknown whether the tested *Pseudomonas* strains are chemotactic to nicotinic acid, but it is possible, as the

genome of *P. fluorescens* Pf0-1 has a predicted orthologue of McpC that is >79 % identical in amino acid sequence. *P. fluorescens* Pf0-1, however, lacks genes for nicotinic acid degradation. It is expected that a strain with the ability not only to degrade but also to sense and respond to nicotinic acid as a specific chemoattractant could be a more efficient biocontrol strain. It would therefore be of interest to investigate the chemotactic abilities of bacterial strains that are proposed for use as biocontrol agents.

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