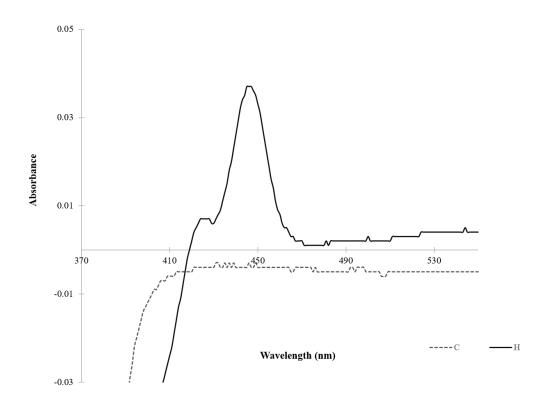
## **Supplementary Information**

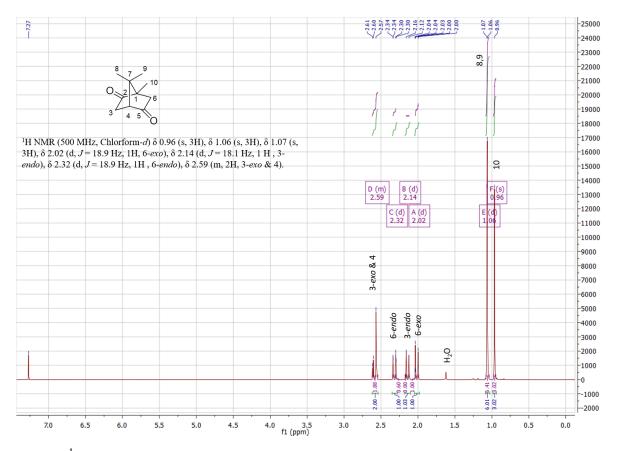
Chemotaxis by *Pseudomonas putida* (ATCC 17453) Towards Camphor Involves Cytochrome P450<sub>cam</sub> (CYP101A1)

PriyadarshiniBalaraman, ErikaPlettner<sup>\*</sup> Department of Chemistry SimonFraserUniversity 8888 University Drive, Burnaby, B.C.V5A 1S6 Canada

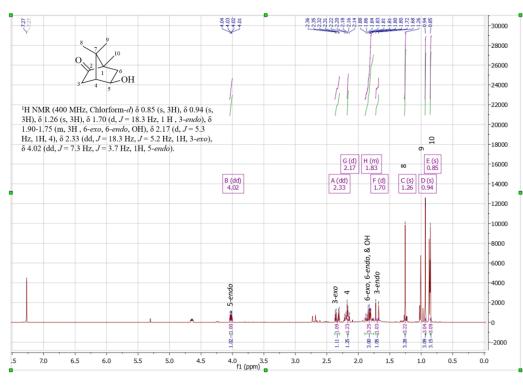
\* Author for correspondence e-mail: plettner@sfu.ca Tel.: 778-782-3586



**Fig S1:** CO difference spectrum of crude ATCC 17453 lysate. The dotted line represents the spectrum of sodium dithionite reduced P450 and the solid line represents the spectrum of reduced P450, treated with CO.

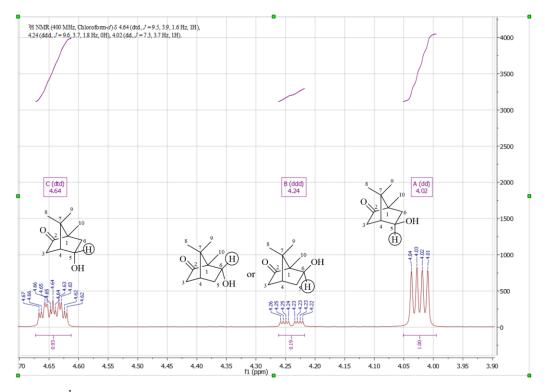


**Fig S2:** <sup>1</sup>H NMR spectrum of 5-ketocamphor in CDCl<sub>3</sub>. The compound was isolated from wild-type *P. putida* culture supernatant by extraction (see section 2.1 of the text).



B)

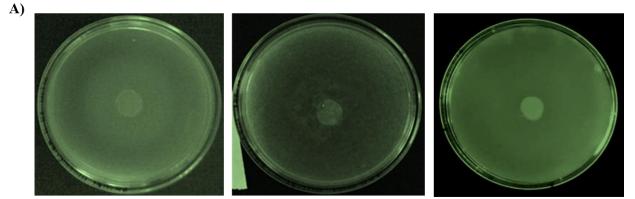
A)



**Fig S3:** <sup>1</sup>H NMR spectrum of the polar metabolite extract, with major component 5-*exo*-hydroxycamphor isolated from wild-type *P. putida* culture supernatant by extraction (see section

4

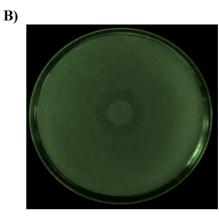
2.1 of the text). A) <sup>1</sup>HNMR assignments for the 5-*exo*-hydroxycamphor.**B**) Other possible polar metabolites formed as per the literature.[1]



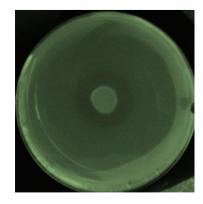
(D)-glucose

(1R)-(+)-camphor

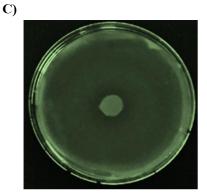
5-ketocamphor



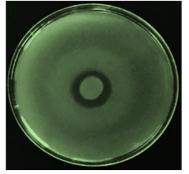
(+)-borneol



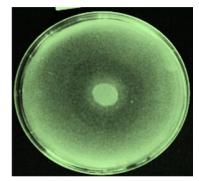
1-phenylimidazole



5-exo-hydroxycamphor

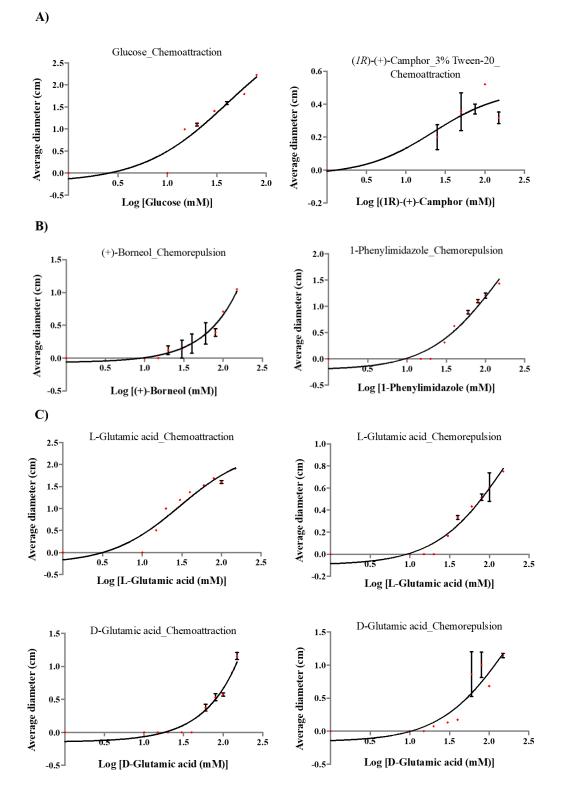


(L)-glutamic acid



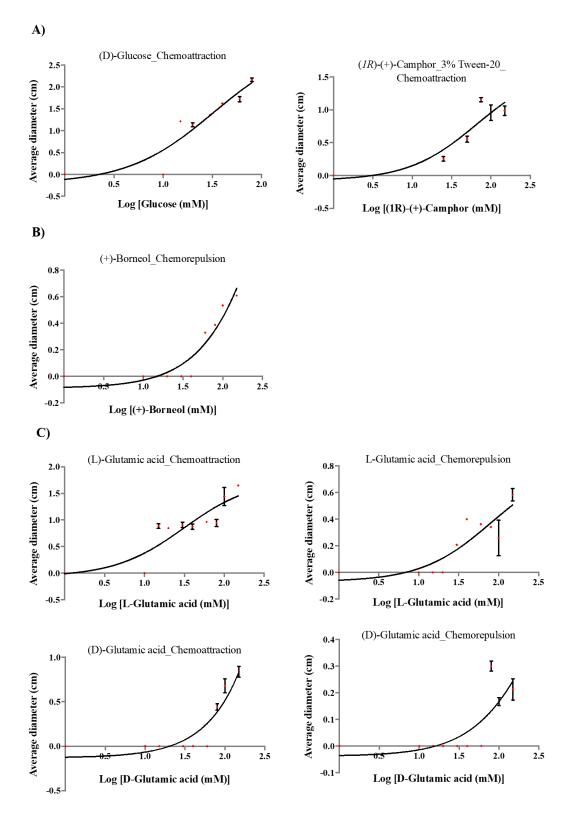
(D)-glutamic acid

**Fig S4:** Chemotactic response of *Pseudomonas putida* (ATCC 17453) in the chemical-in-plug assays towards various chemoeffectors. **A**) Chemoattractants; (D)-glucose, (*IR*)-(+)-camphor, and 5-ketocamphor. **B**) Chemorepellents; (+)-borneol and 1-phenylimidazole (150 mM). **C**) Compounds that showed mixed responses; (L)-glutamic acid, (D)-glutamic acid, and 5-*exo*-hydroxycamphor ("polar metabolite mix"). The chemotactic responses were recorded after 8 h of incubation.



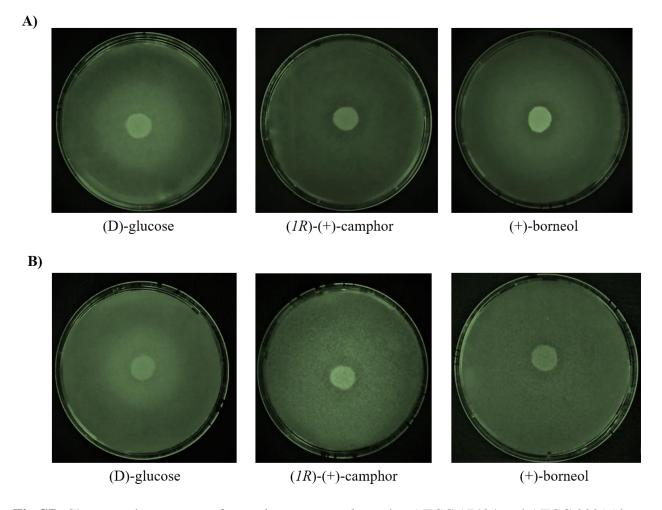
**Fig S5:** Chemotaxis of P450<sub>cam</sub>-induced cultures of *Pseudomonas putida* (ATCC 17453) towards various concentrations of the selected chemoeffectors, as determined by in-plug assays. These

curves were used to calculate the  $EC_{50}$  values in **Table 1**. A)Chemoattractants; (D)-glucose, and (1R)-(+)-camphor. B) Chemorepellent, (+)-borneol. C) Compounds that showed mixed responses; (L)-glutamic acid, and (D)-glutamic acid. Each point represents the average of response diameter average  $\pm$  S.E. of 3 replicates.

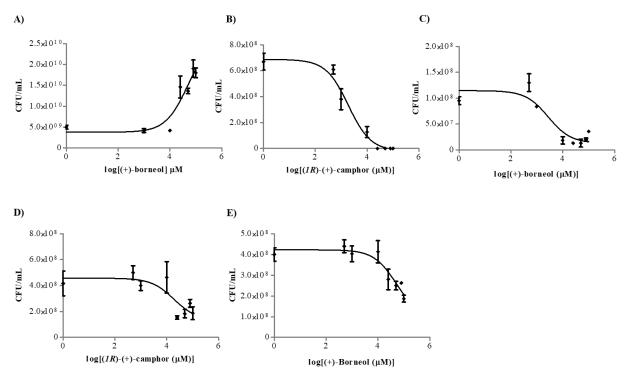


**Fig S6:** Chemotaxis of  $P450_{cam}$  non-induced cultures of *Pseudomonas putida* (ATCC 17453) towards various concentrations of the selected chemoeffectors, as determined by in-plug assays. These curves were used to calculate the EC<sub>50</sub> values in**Table 1**. A)Chemoattractants; (D)-

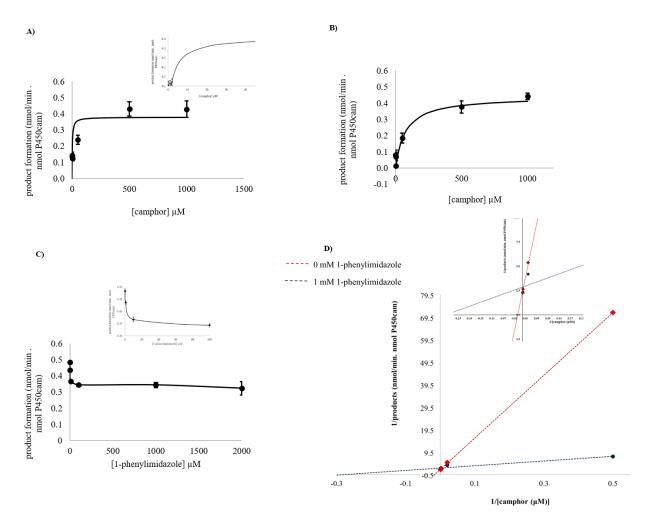
glucose, and (1R)-(+)-camphor. **B**) Chemorepellent, (+)-borneol. **C**) Compounds that showed mixed responses; (L)-glutamic acid, and (D)-glutamic acid. Each point represents the average of response diameter average  $\pm$  S.E. of 3 replicates.



**Fig S7:** Chemotactic response of *Pseudomonas putida* strains ATCC 17484 and ATCC 33015 in the chemical-in-plug assays towards various chemoeffectors. **A**) Chemotactic response of ATCC 17484 towards (D)-glucose, (1R)-(+)-camphor, and (+)-borneol. **B**) Chemotactic response of ATCC 33015 towards (D)-glucose, (1R)-(+)-camphor, and (+)-borneol. The chemotactic responses were recorded after 8 h of incubation.



**Fig S8:** Survival assay of three *Pseudomonas putida* strains, *Strains1*, 2, &3, towards (1R)-(+)-camphor and/or (+)-borneol. **A)** Survival dose response of ATCC 17453 (*Strain 1*) in the presence of (+)-borneol. **B)** Survival dose response of ATCC 33015 (*Strain 2*) in the presence of (1R)-(+)-camphor. **C)** Dose response of *Strain 2* in presence of (+)-borneol. **D)** Dose response of ATCC 17484 (*Strain 3*) in presence of (1R)-(+)-camphor. **E)** Dose response of *Strain 3* in presence of (+)-borneol.Each point represents the average CFU/mL ± S.E. of 3 replicates.



**Fig S9:** Enzyme kinetics assay under well aerated condition to establish the enzyme activity of  $P450_{cam}$  and the inhibition by 1-phenylimidazole (a P450 inhibitor). **A**) Enzyme activity assay of  $P450_{cam}$  with varying concentrations of (+)-camphor (0 to 1 mM) in the absence of 1-phenylimidazole. **B**) Enzyme activity assay of  $P450_{cam}$  with varying concentrations of (+)-camphor (0 to 1 mM) in the presence of 1 mM 1- phenylimidazole. **C**) Enzyme inhibition assay of  $P450_{cam}$  with varying concentrations of 1- phenylimidazole (0 to 1 mM) in the presence of 1 mM(1R)-(+)-camphor. **D**) Lineweaver-Burk plot to estimate the  $K_i$  and nature of inhibition by 1-phenylimidazole. The inset shows that the lines intersect on the y axis, as expected for a competitive inhibitor.

Each point in **A** & **B** represents the amount of products formed (nmol/min. nmol P450<sub>cam</sub>)  $\pm$  S.E. of 5 replicates. Each point in **C** represents the amount of products formed (nmol/min. nmol P450<sub>cam</sub>)  $\pm$  S.E. of 3 replicates.

GenBank : AB771747.1

Protein ID = BAN13299.1

"MEQASARPRQKTTAPDLATAITSARQAWQSHERAHQVLEADKQHLTGKAERLGAQLEEA VQQSRQLSERIELLSHATSEGVWEIRSGAGNLDDQSVTAWFSPQFRALLGFQDEQDFANQ LDSWLSRAEPASRGTLLRDMVVALKAGHHAYRAELRLATKGGDLRWFEISAHMATATSDS PMRLHGRLRDIHDHRQHERLITRFELSRELMNDGLWDMEVIAGDPLNPSNPLWWSDQFLH MLGFDNPEDFPNVLSSWTSRIHEEDQEQMFKLFQAHLEDKSGIPGFDMTYRIRLKSGEYH WFRARCHTQRSANGLPVRLIGSLVDVQAAHQEELVRQEQAQQRESLELTLQKLAEIVSAI RAIASQTNLLALNAAIEAARAGDAGRGFAVVADEVRKLATRTSEATQQATEMLT"

**Fig S10:** Amino acid sequence of the protein sequence of the methyl accepting chemotaxis protein (MCP) on the CAM plasmid.

A)	B)	
4_MCP_Thermotoga 3_MCP_chemotaxis 2_Biofilm disper 1_Futative MCP_A 5_Chemotaxis pro 6_MCP_Beudmona 7_MCP_Beudmona 3_MCP_chemotaxis 2_Biofilm disper 1_Futative MCP_A 5_Chemotaxis pro 6_MCP_Beudmona 7_MCP_Beudmona	<ul> <li>a) a) a</li></ul>	s putida]

## C) Multi-way Trasnlation alignment.

Sequences: 7. Scoring matrix: BLOSUM 62										
Sequence name	NCBI #	Organism	Start	End	# Match	Non match	% Match			
1_Putative methyl-accepting chemotaxis protein	BAN13299.1	Pseudomonas Putida ATCC 17453 (aerobic, gram-negative bacterium)	1	413						
2_Biofilm dispersion protein	NP_250114.1	Pseudomonas aeruginosa PAO1 (aerobic, gram-negative bacterium).PMID: 24645272	1	417	122	382	24			
3_Chemotaxis signal transduction system methyl accepting sensory transducer with 4HB_MCP sensory domain	NP_716136.1	Shewanella oneidensis MR-1 (anaerobic, gram-negative bacterium) Chemotaxis towards anaerobic electron acceptors. 10.1186/1471-2164-11-319	1	543	102	469	17			
4_Methyl-accepting chemotaxis protein	NP_228952.1	Thermotoga maritima MSB8	1	539	107	460	18			
5_Multispecies: chemotaxis protein	WP_010871656.1	Synechocystis	1	869	140	739	15			
6_Methyl-accepting chemotaxis protein	NP_251342.1	Pseudomonas aeruginosa PAO1Positive responses to oxygen (Aer and Aer-2), inorganic phosphate (CtpH and CtpL), malate (PA2652), amino acids and gamma aminobutyrate (GABA) (PctABC), ethylene (TlpQ), and chloroethylenes (McpA). https://doi.org/10.1111/1574- 6976.12081	1	561	114	470	19			
7_Chemotaxis signal transduction system methyl accepting sensory transducer with 4HB_MCP sensory domain	NP_717054.1	Shewanella oneidensis MR-1 (anaerobic, gram-negative bacterium)	1	543	91	489	15			

**Fig S11:** Multi-way alignment of the sequences obtained by Smart Blast NCBI search (using MCP sequence, **Fig S7**, on the CAM plasmid as the query sequence) for a detailed pairwise global alignments in the Clone 9 Manager software (Sci-Ed Software, Denver, CO). **A**)Alignment of the Blast search sequences to the putative MCP sequence on the CAM plasmid. Highlighted sequences represent the regions of similarity between the aligned sequences. **B**)Dendrogram representation for the phylogenetic relationship between the aligned sequences. **C**)The table provides a summary of the sequence alignment, including the sequences used to align, NCBI numbers of the sequences, number of matched and non-matched sequences, and the calculated percent match.

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## Reference

 de Jesus HCR, Jeller AH, Debonsi HM, Alves PB, Porto ALM: Multiple Monohydroxylation Products from rac-Camphor by Marine Fungus Botryosphaeria sp Isolated from Marine Alga Bostrychia radicans. Journal of the Brazilian Chemical Society 2017, 28(3):498-504.