**Supplementary Information**

# Chemotaxis by *Pseudomonas putida* (ATCC 17453) Towards Camphor Involves Cytochrome $P450_{cam}$ (CYP101A1)

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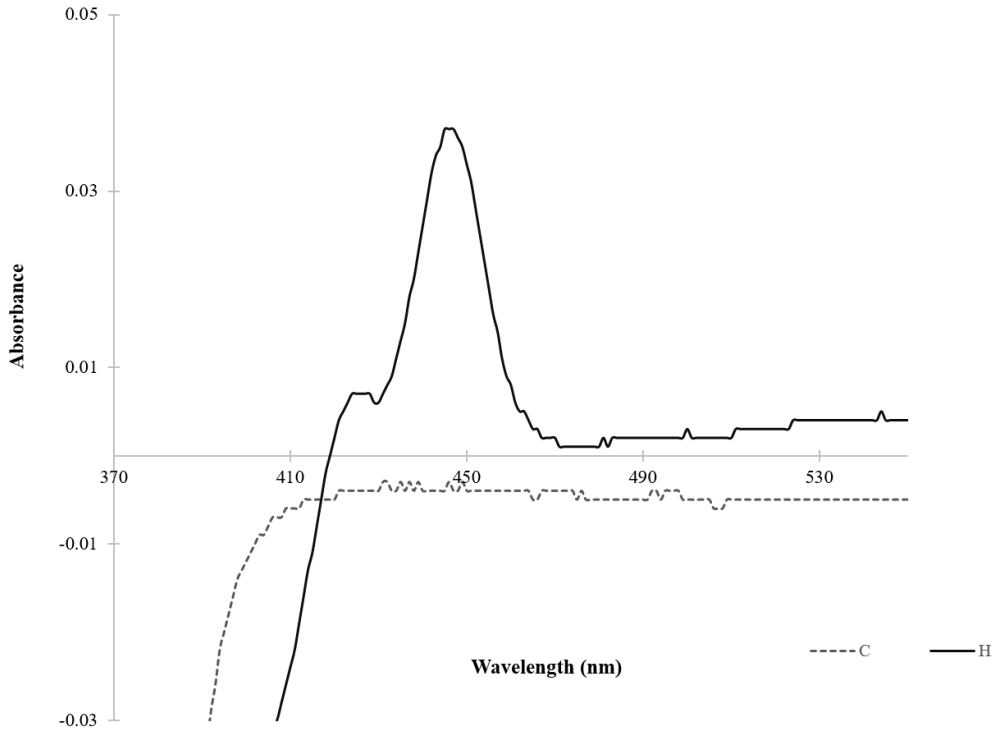



**Fig S1:** CO difference spectrum of crude ATCC 17453 lysate. The dotted line represents the spectrum of sodium dithionite reduced P450 and the solid line represents the spectrum of reduced P450, treated with CO.

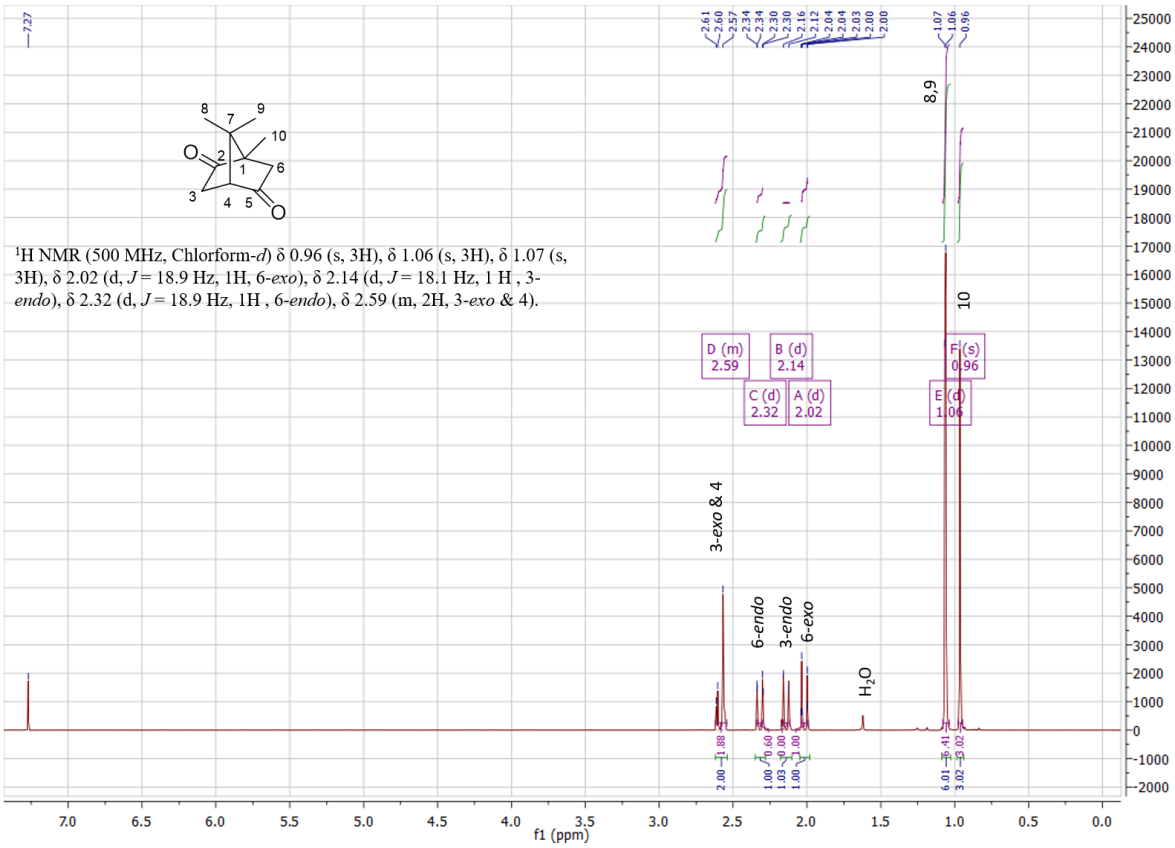
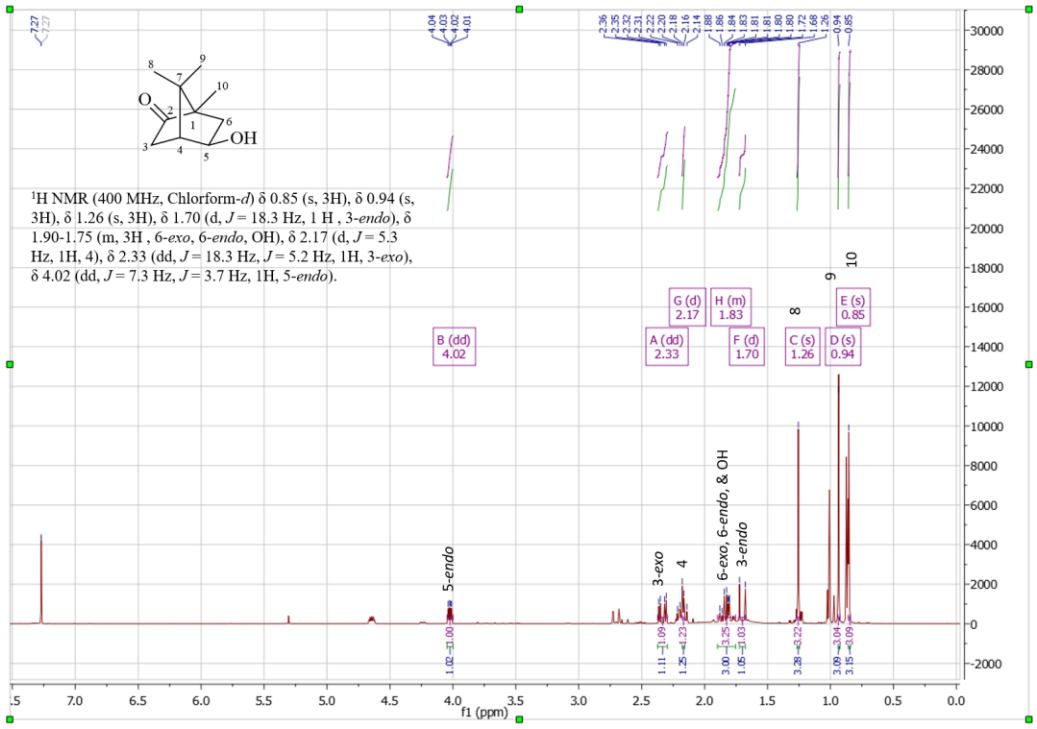



**Fig S2:** [1]H NMR spectrum of 5-ketocamphor in $CDCl_3$. The compound was isolated from wild-type *P. putida* culture supernatant by extraction (see section 2.1 of the text).

A)




B)

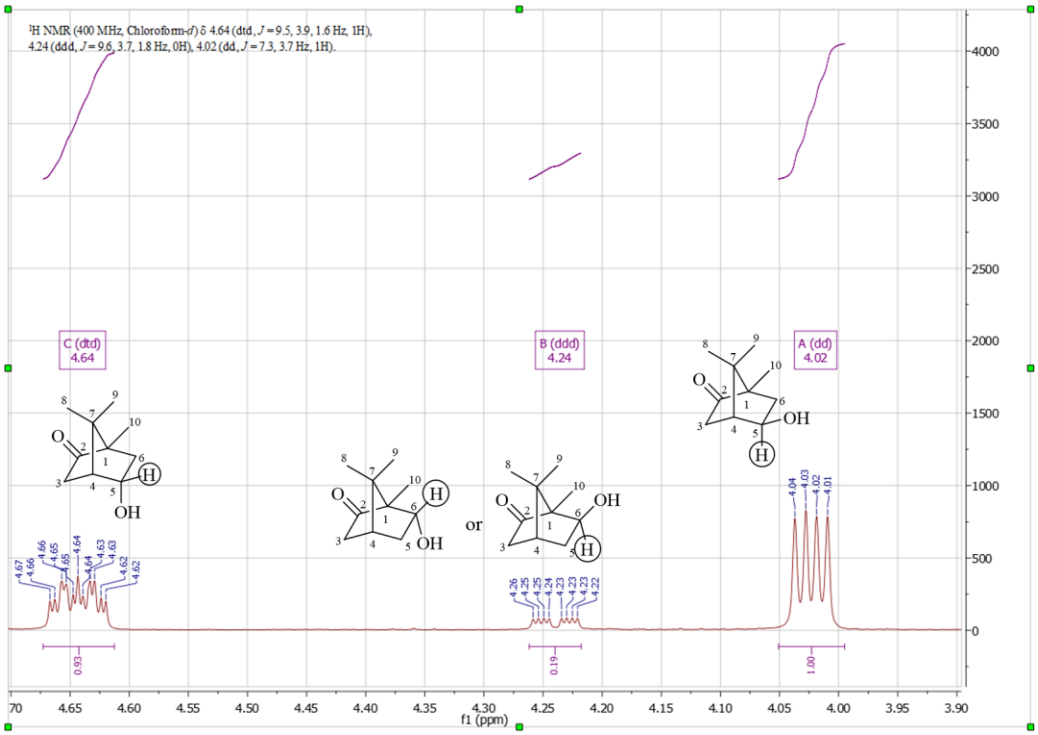



**Fig S3:** $^{1}$H NMR spectrum of the polar metabolite extract, with major component 5-*exo*-hydroxycamphor isolated from wild-type *P. putida* culture supernatant by extraction (see section

2.1 of the text). **A)** $^{1}$HNMR assignments for the 5-*exo*-hydroxycamphor.**B)** Other possible polar metabolites formed as per the literature.[1]

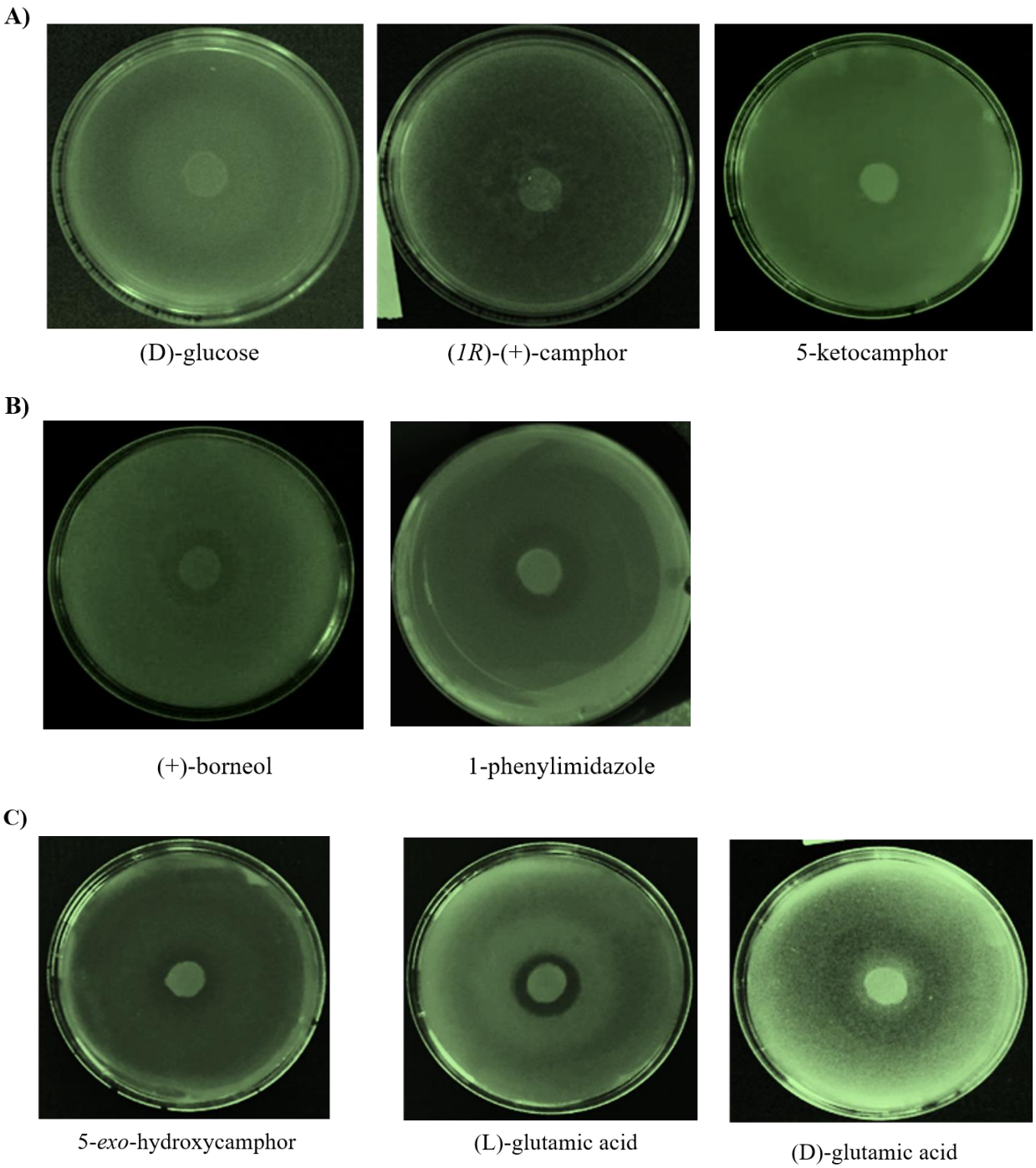



**Fig S4:** Chemotactic response of *Pseudomonas putida* (ATCC 17453) in the chemical-in-plug assays towards various chemoeffectors. **A)** Chemoattractants; (D)-glucose, (*1R*)-(+)-camphor, and 5-ketocamphor. **B)** Chemorepellents; (+)-borneol and 1-phenylimidazole (150 mM). **C)** Compounds that showed 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.

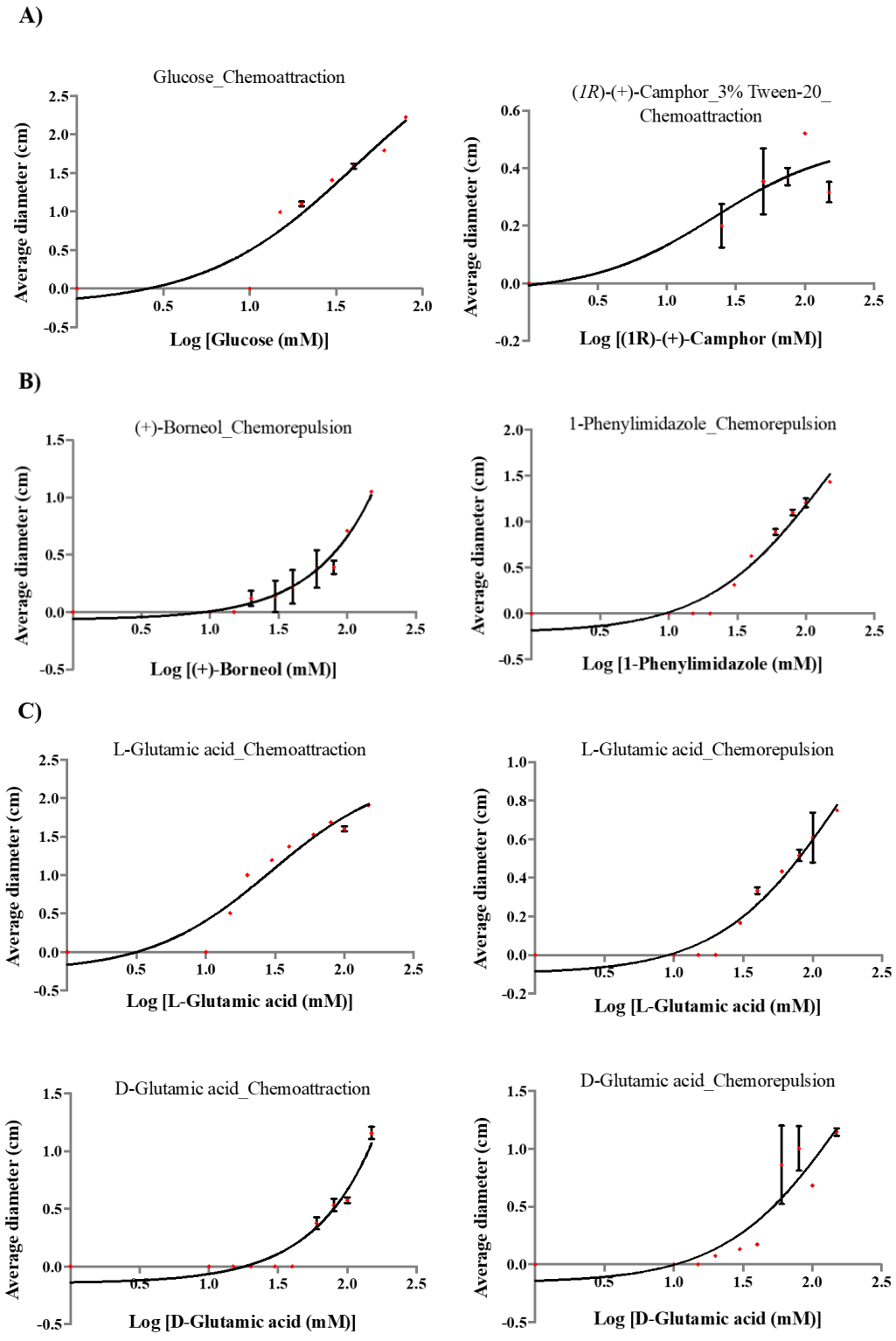



**Fig S5:** Chemotaxis of $P450_{cam}$-induced cultures of *Pseudomonas putida* (ATCC 17453) towards various concentrations of the selected chemoeffectors, as determined by in-plug assays. These

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 ± S.E. of 3 replicates.

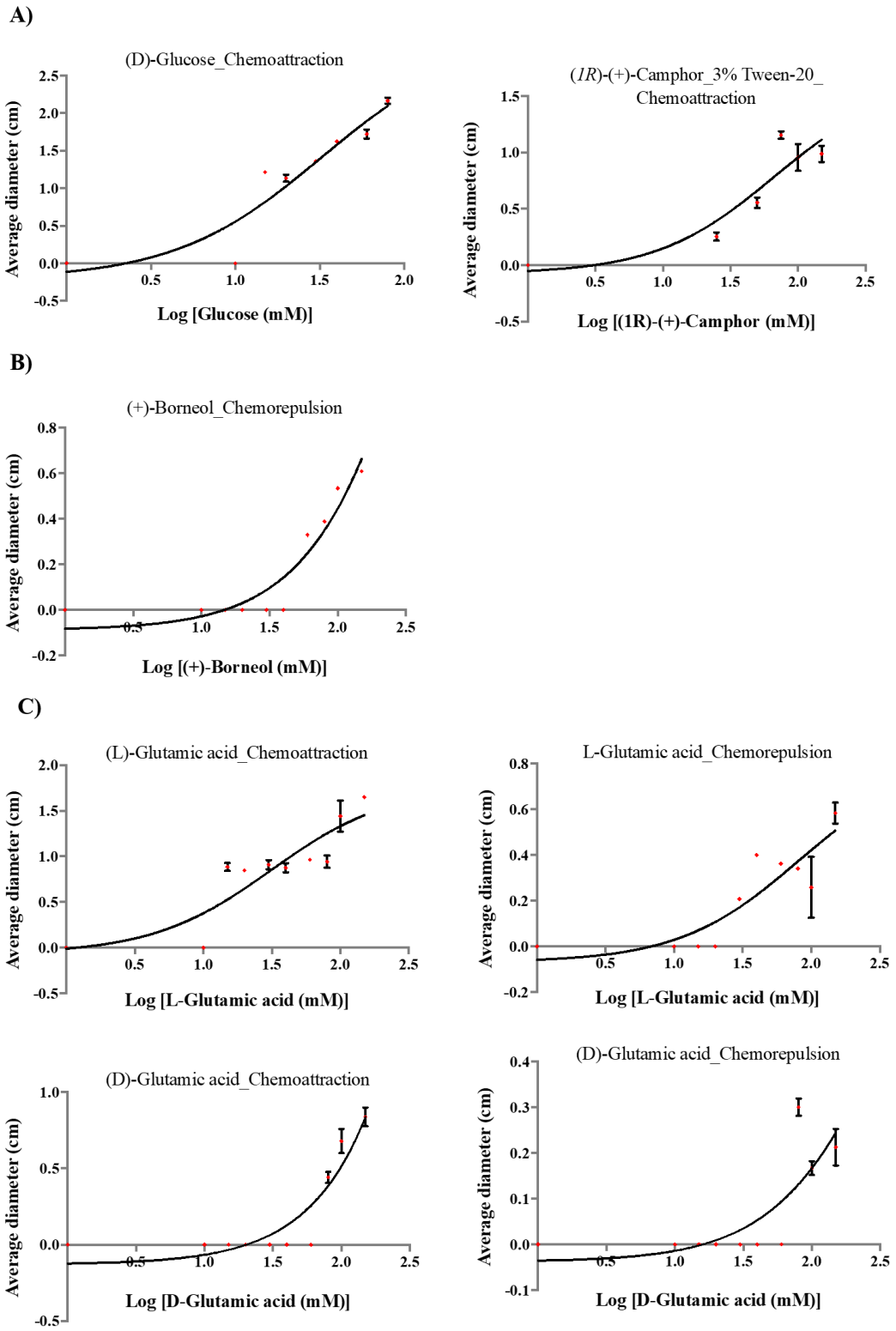



**Fig S6:** Chemotaxis of $P450_{cam}$ non-induced cultures of *Pseudomonas putida* (ATCC 17453) towards various concentrations of the selected chemoeffectors, as determined by in-plug assays. These curves were used to calculate the $EC_{50}$ values in**Table 1**. **A)**Chemoattractants; (D)-

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 ± S.E. of 3 replicates.

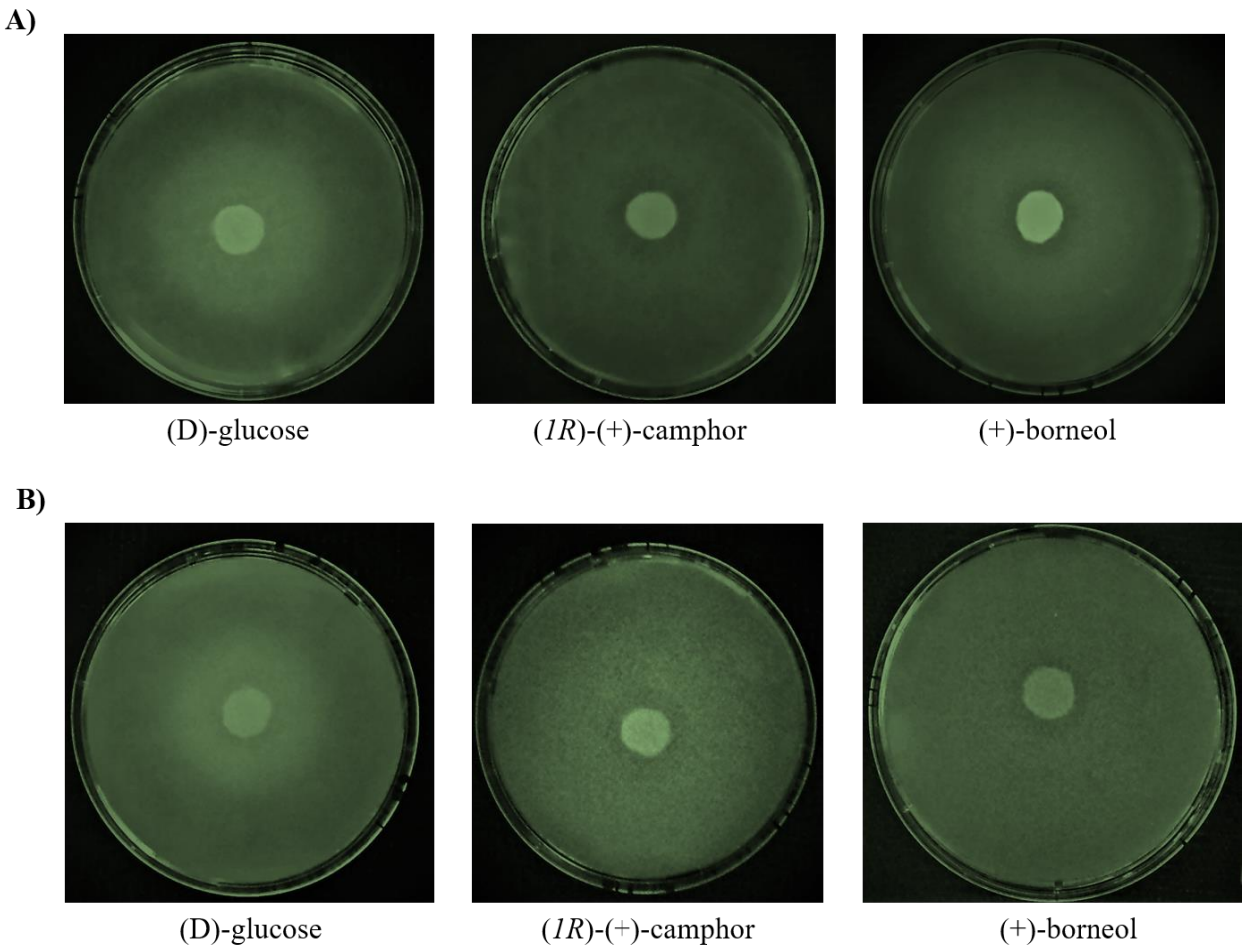



**Fig S7:** Chemotactic response of *Pseudomonas putida* strains ATCC 17484 and ATCC 33015 in the chemical-in-plug assays towards various chemoeffectors. **A)** Chemotactic response of ATCC 17484 towards (D)-glucose, (*1R*)-(+)-camphor, and (+)-borneol. **B)** Chemotactic response of ATCC 33015 towards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

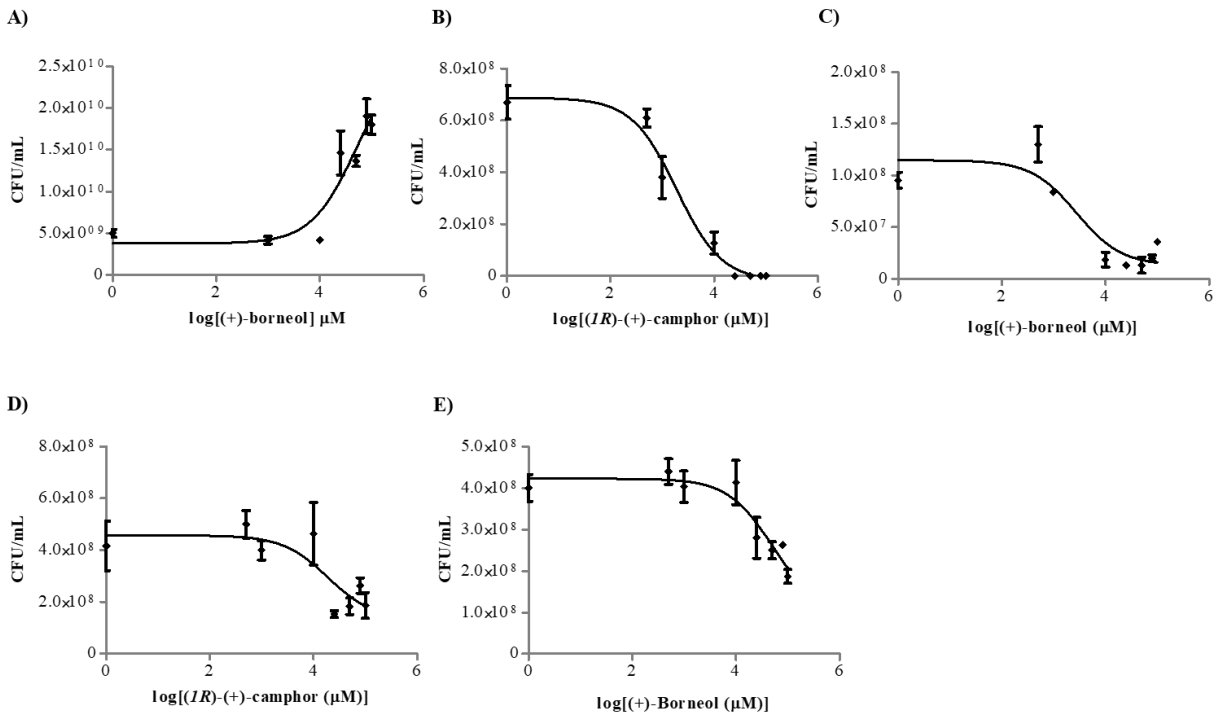



**Fig S8:** Survival assay of three *Pseudomonas putida* strains, *Strains1*, *2*, *&3*, towards (*1R*)-(+)-camphor and/or (+)-borneol. **A)** Survival dose response of ATCC 17453 (*Strain 1*) in the presence of (+)-borneol. **B)** Survival dose response of ATCC 33015 (*Strain 2*) in the presence of (*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.

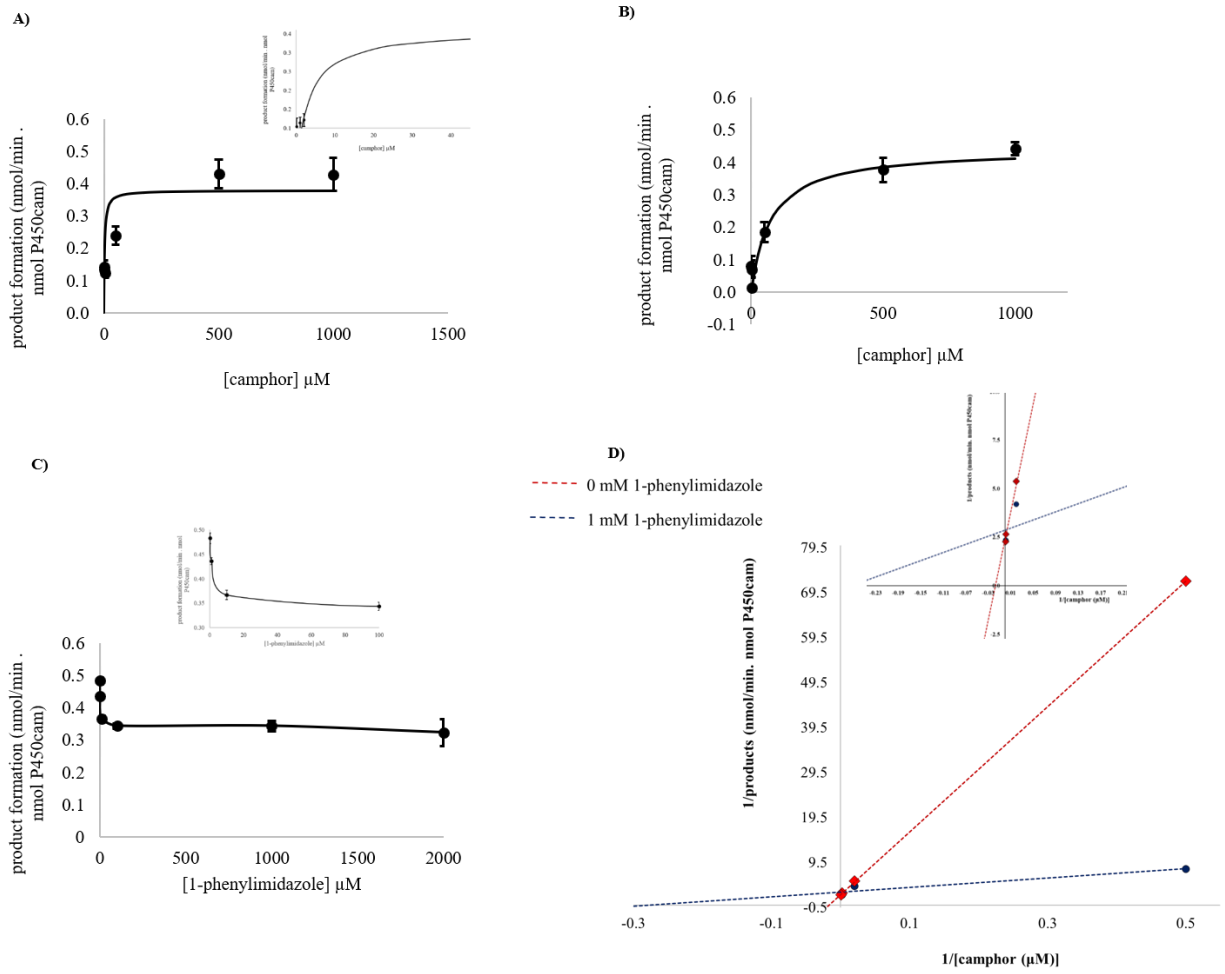



**Fig S9:** Enzyme kinetics assay under well aerated condition to establish the enzyme activity of $P450_{cam}$ and the inhibition by 1-phenylimidazole (a P450 inhibitor). **A)** Enzyme activity assay of $P450_{cam}$ with varying concentrations of (+)-camphor (0 to 1 mM) in the absence of 1-phenylimidazole. **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 $P450_{cam}$) ± S.E. of 5 replicates. Each point in **C** represents the amount of products formed (nmol/min. nmol $P450_{cam}$) ± S.E. of 3 replicates.

GenBank : AB771747.1

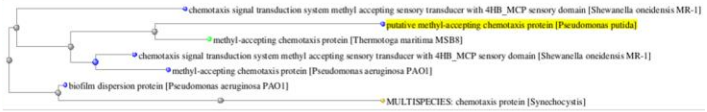
Protein ID = BAN13299.1

"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)




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.

## Reference

1. 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.