Supplementary material

Table S1) Bacterial mutants of *Pseudomonas putida* KT2440 used for chemotaxis assays. Shown are the coordinates of the insertion of the mini-Tn5 cassette into the genome as determined by sequencing as well as an analysis of the gene products of the ORFs mutated in the 12 mutants. Given is their size, the domains predicted by SMART (http://smart.embl-heidelberg.de/), the number of transmembrane regions as determined by DAS (http://www.sbc.su.se/~miklos/DAS/).
Mutants were obtained from the *Pseudomonas* Reference Culture Collection. They were generated by mini-Tn*5* mutagenesis, as described before using pUT-Km as source for the mini-Tn*5* transposon (Duque *et al*., 2007). The collection consists of more than 3000 independent mutants.
Duque E, Molina-Henares AJ, de la Torre J, Molina-Henares MA, del Castillo T et al (2007) Towards a genome-wide mutant library of *Pseudomonas putida* strains KT2440. In *Pseudomonas, volume V*, Ramos JL, Filloux A (eds) pp 227-251. Springer, Dordrecht, The Netherlands

ORF MCP	Coordinates for Mini-Tn5 insertion	Size (amino acids)	domains predicted in SMART	No. of TM[1]
pp0562	653530	653	HAMP, MA	2
pp1228	1403877	688	HAMP, MA	2
pp1371	1561561	624	HAMP, MA	2
pp1819	2045068	646	HAMP, MA	2
pp1940	2193684	716	HAMP, MA	2
pp2120	2419352	555	HAMP, MA	2
pp2249	2566591	643	HAMP, MA	2
pp2310	2639862	492	HAMP, MA	2
pp2643	3029741	550	HAMP, MA	2
pp3950	4456080	725	HAMP, MA	1
pp4658	5284039	639	HAMP, MA	2
pp4989	5687728	686	HAMP, MA	2

[1] Prediction of transmembrane helices according to the DAS programme (Cserzo et al. (1997) Prot. Eng. 10, 673.

Figure legends for supplementary figures

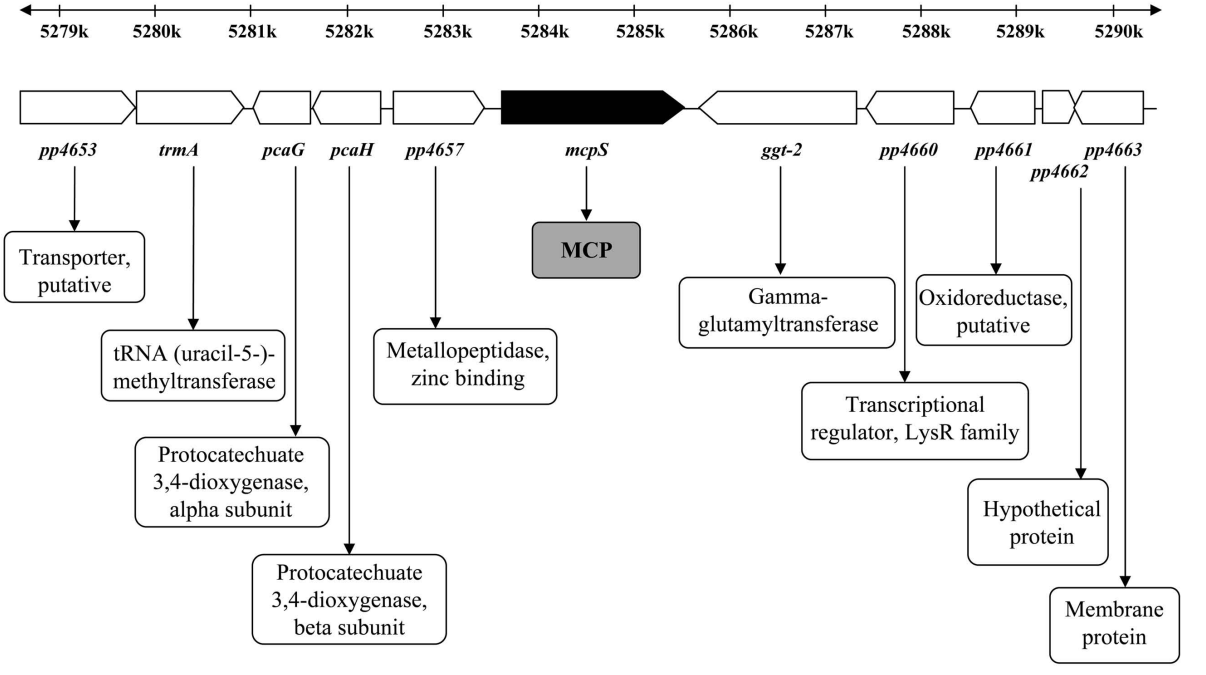
Fig. S1: Genomic environment of the *mcpS* gene. Gene annotation according to the *Pseudomonas* genome database (http://www.pseudomonas.com/).

Fig. S2: Compounds used for ITC experiments with McpS-LBD. Compounds which bind are highlighted in blue and the corresponding thermodynamic parameters are given in Fig. 3 of the article.

Fig. S3) Agarose plug assays of *P. putida* KT2440 and its *mcpS* deficient mutant towards succinate, fumarate, malate and oxaloacetate. Compounds were present at a concentration of 5 mM. The wild-type strain showed a strong chemotactic response towards succinate, malate and fumarate. This phenotype was characterised by the formation of well-defined rings, which started to appear after 4 min. A chemotactic response to oxaloacetate was also observed, but the intensity of ring formation was weaker and delayed in time. Using this assay no chemotaxis towards butyrate, citrate and isocitrate was detected. The more sensitive capillary assays, however, revealed a weak response towards these latter 3 compounds.

Fig. S4) Agarose plug assays of *P. putida* KT2440 towards mixtures of toluene with butyrate and isocitrate. No chemotaxis is observed towards butyrate and isocitrate using this technique (a weak response is only seen using the capillary method which has a higher sensitivity) but a significant response is seen towards toluene. Chemotaxis towards toluene is mediated by a different MCP. However, the presence of 5 mM butyrate or isocitrate did not alter chemotaxis towards toluene. This is consistent with the notion that isocitrate and butyrate have no nonspecific effect on the chemotactic apparatus which would result in cell paralysis.

Fig S1


5279k
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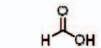
pp4653
trmA
pcaG
pcaH
pp4657
mcpS
ggt-2
pp4660
pp4661
pp4662
pp4663
Transporter, putative
tRNA (uracil-5-)-methyltransferase
Protocatechuate 3,4-dioxygenase, alpha subunit
Protocatechuate 3,4-dioxygenase, beta subunit
Metallopeptidase, zinc binding
MCP
Gamma-glutamyltransferase
Transcriptional regulator, LysR family
Oxidoreductase, putative
Hypothetical protein
Membrane protein

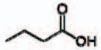
Fig. S2

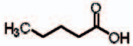
Monocarboxylic acids

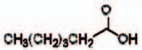

Formic acid

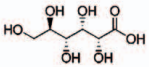

Pyruvic acid


Lactic acid

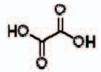

Butyric acid

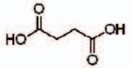

Valeric acid

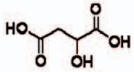

Hexanoic acid

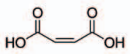

Gluconic acid

Dicarboxylic acids


Oxalic acid


Succinic acid


Malic acid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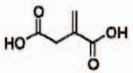
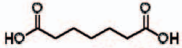
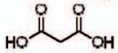
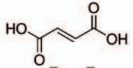
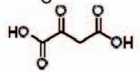
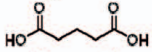

Itaconic acid

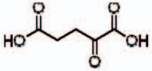

Pimelic acid


Malonic acid

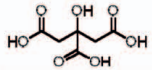

Fumaric acid

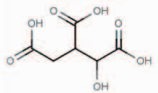

Oxaloacetic acid

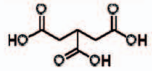

Glutaric acid


α-Ketoglutaric acid

Tricarboxylic acids

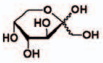

Citric acid


Isocitric acid

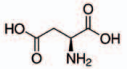

Tricarballylic acid

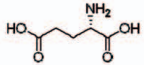
Carbohydrates


D-Glucose


D-Fructose

Aminoacids


L-Aspartic acid


L-Glutamic acid

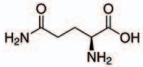
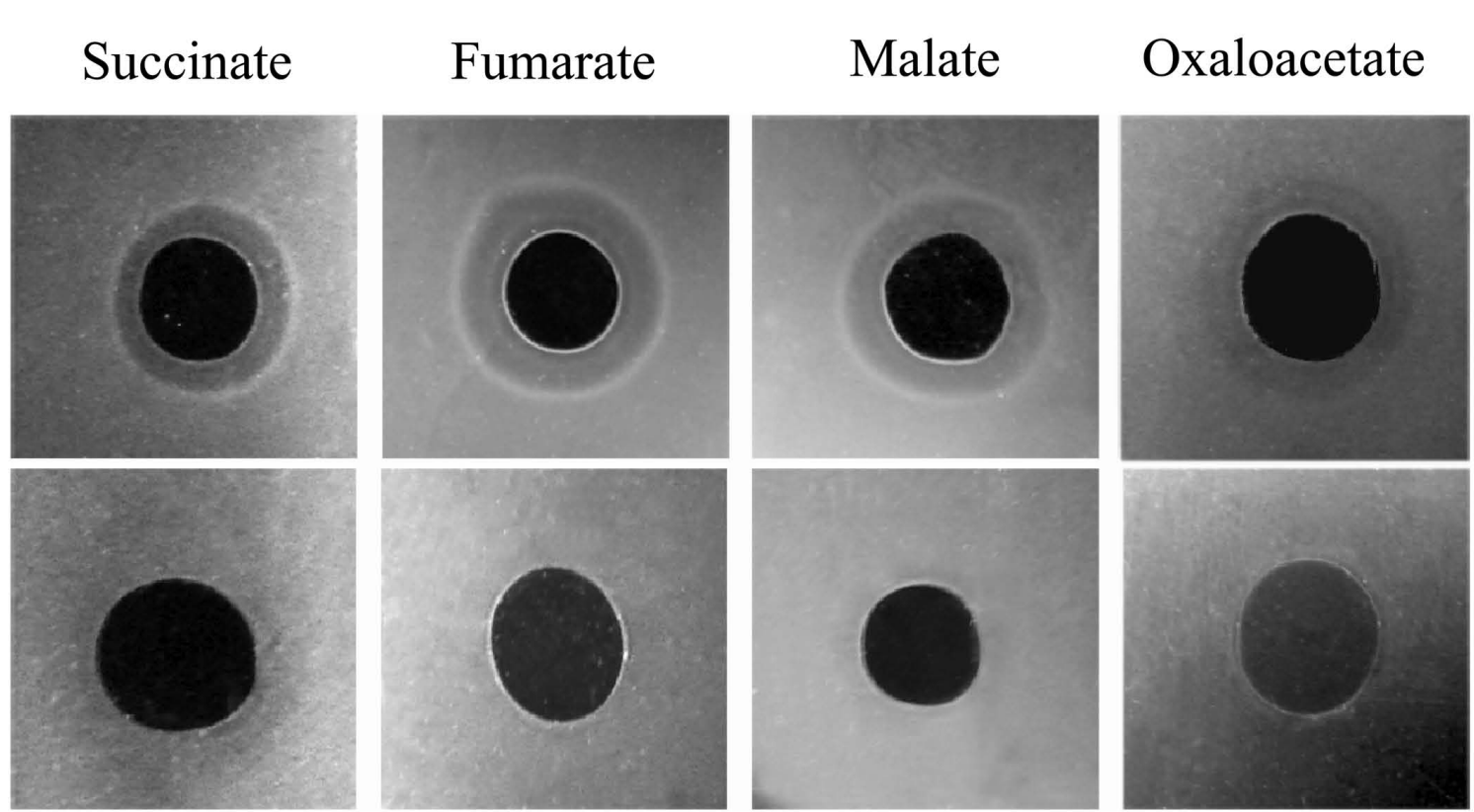

L-Glutamine

Fig. S3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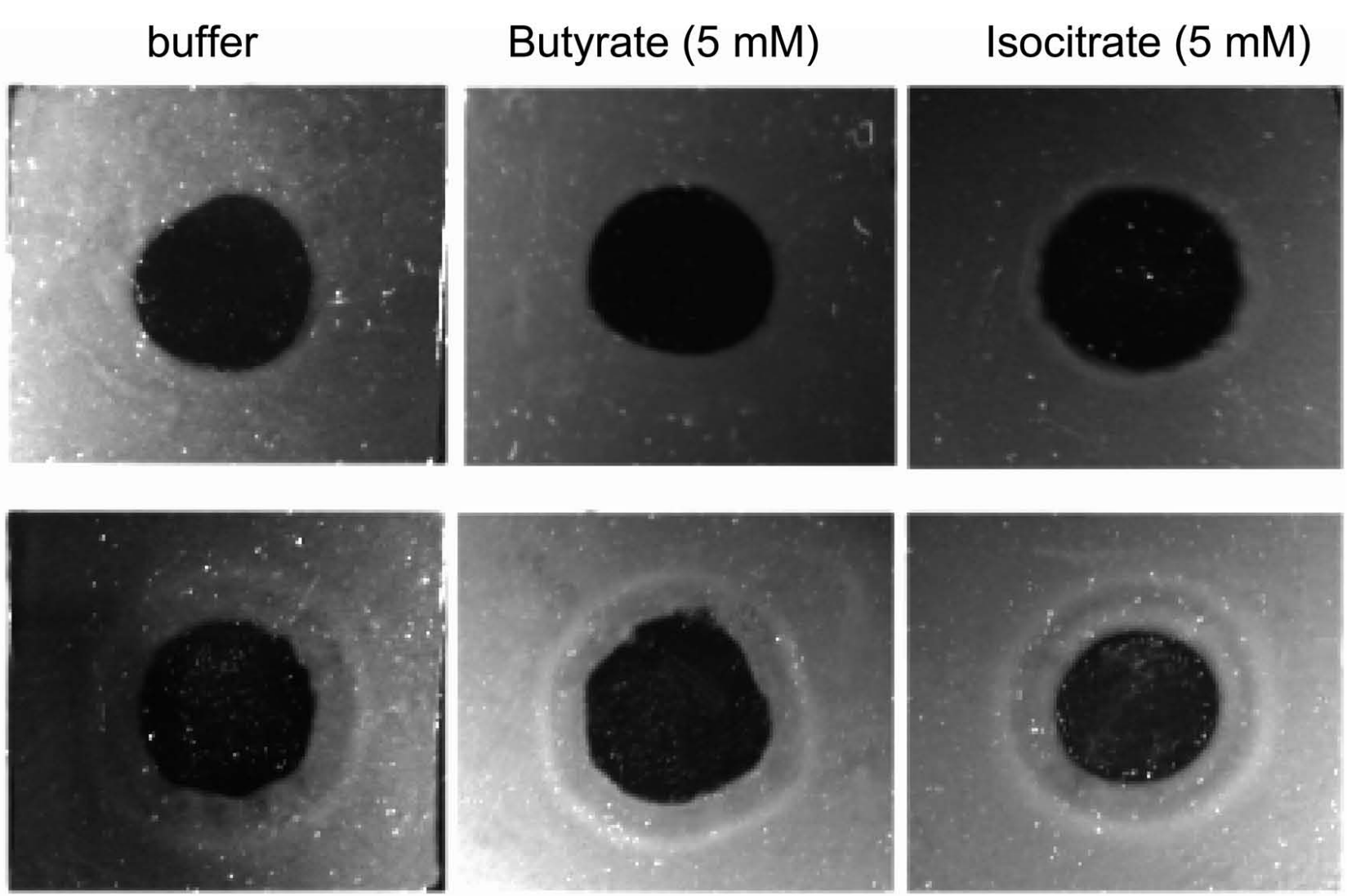
Fumarate
Malate
Oxaloacetate
P. putida KT2440
P. putida KT2440 mcpS::Tn5

Fig.S4


buffer
Butyrate (5 mM)
Isocitrate (5 mM)
Toluene (5 mM)
Toluene (5 mM)
+
Butyrate (5 mM)
Toluene (5 mM)
+
Isocitrate (5 mM)