SUPPORTING INFORMATION

Role of the CrcB transporter of *Pseudomonas putida* in the multi-level stress response elicited by mineral fluoride

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Table S1. Oligonucleotides used in this study.

Name	Sequence (5'→3')	Purpose
rarA_up_U_fw	AGATCCUTCGCATGCTGTTGGTTTC	Upstream region of *rarA* amplification
rarA_up_U_rv	ATGGCAUGGCGAGCTCTGGTTACTC	
rarA_dw_U_fw	ATGCCAUGATTGCACTCATCGCCGC	Downstream region of *rarA* amplification
rarA_dw_U_rv	AGGTCGACUTTCGTTGGCCATGCGCTC	
prtR_up_U_fw	AGATCCUAAAGTTATTCCCTGATCGGGAAT	Upstream region of *prtR* amplification
prtR_up_U_rv	ATCGTCAUTTCAGTCTCCGCAAGGCC	
prtR_dw_U_fw	ATGACGAUCAGCTCAACTCCAGCCAGATCGG	Downstream region of *prtR* amplification
prtR_dw_U_rv	AGGTCGACUACCGCCGCTGAGCCTGCACAA	
lapA_up_U_fw	AGATCCUATTCATCTATAGAGTGCGGATTC	Upstream region of *lapA* amplification
lapA_up_U_rv	ATTGGACUCTCCGTGTGACCCGATGG	
lapA_dw_U_fw	AGTCCAAUGTGACAGACCACCGGGGCC	Downstream region of *lapA* amplification
lapA_dw_U_rv	AGGTCGACUTCGATTGGTCGACGGGTACG	
lapD_up_U_fw	AGATCCUCGGATTGGGATTTCTCCC	Upstream region of *lapD* amplification
lapD_up_U_rv	ATTCACATCUGTGCTCCTTCAGTAAACCG	
lapD_dw_U_fw	AGATGTGAAUGGCCTCTCGCGTGCCGCT	Downstream region of *lapD* amplification
lapD_dw_U_rv	AGGTCGACUACGCGGTATCGTCAAGCAACTGC	
flhA_up_U_fw	AGATCCUCTTACCGGCATACGACTCA	Upstream region of *flhA* amplification
flhA_up_U_rv	ACCGCGAGUCCTCTTGATGCAAAACTTTGA	
flhA_dw_U_fw	ACTCGCGGUGTGAGGTAGGGGATAATGCAAGTTA	Downstream region of *flhA* amplification
flhA_dw_U_rv	AGGTCGACUGCGCCAGGCCTGGCGCGG	
yijP_up_U_fw	AGATCCUTCCTGGCGCATGATGACGAT	Upstream region of *yijP* amplification
yijP_up_U_rv	ATTACACGUATGCCTCGTCGTACCTTGGC	
yijP_dw_U_fw	ACGTGTAAUCAGGCCAAGGTTCATGCAGGG	Downstream region of *yijP* amplification
yijP_dw_U_rv	AGGTCGACUCGCCACCCCGAGTCGCTG	
orn_up_U_fw	AGATCCUTACCAGATAGCGGTCGATCA	Upstream region of *orn* amplification
orn_up_U_rv	ACTCACAUGCAGGGGCTCCTCATATAG	
orn_dw_U_fw	ATGTGAGUCGAAACGGCGCAGATATTGTA	Downstream region of *orn* amplification
orn_dw_U_rv	AGGTCGACUATCGCCTGTTCTGCCGGC	
dusB_up_U_fw	AGATCCUAATGGCCCGGCAGGACCA	Upstream region of *dusB* amplification
dusB_up_U_rv	ATGCCAUAGGTGATCCCTGTTGTGGGGC	
dusB_dw_U_fw	ATGGCAUGACGATGATGACCGAGAA	Downstream region of *dusB* amplification
dusB_dw_U_rv	AGGTCGACUCATCCATCATTTCGGCGA	

vacB_up_U_fw	AGATCCUGGCACATAAATCGCAGGCAAGA	Upstream region of *vacB* amplification
vacB_up_U_rv	ATGACAUCAGAAGGGGTTCACCTTGGGGTAT	
vacB_dw_U_fw	ATGTCAUGAGTCAGCTGGAAAAAATCT	Downstream region of *vacB* amplification
vacB_dw_U_rv	AGGTCGACUGATCATCACCAGTGGCCC	

Table S2. Beneficial insertions in the tolerance of *P. putida* towards F^- stress.[a]

Gene	Locus	Insertions	log(FC)	*P*-value	Description	References	Comments
orn	PP_4902	2	3.21	4.39×10^{-12}	Oligoribonuclease	(Yeom, et al., 2010, Escapa, et al., 2012)	Involved in PHA metabolism in *P. putida*. Exoribonuclease activity, produces 5'-phosphomonoesters. Changes in expression reported in the presence of ampicillin.
dusB	PP_4820	6	2.68	2.77×10^{-11}	NifR3 family TIM-barrel protein	(Steen, et al., 2013, Lahesaare, et al., 2016)	tRNA dihydrouridine synthase DusB. Differentially regulated in strain KT2440 after 30 min of O_2 depletion. Forms an operon with *PP_4821* (encoding Fis). Fis is involved in the regulation of LapF and cell surface hydrophobicity.
vacB/rnr	PP_4880	15	2.81	2.51×10^{-7}	Ribonuclease R	(Reva, et al., 2006, Fonseca, et al., 2011, Frank, et al., 2011)	Essential for growth of strain KT2440 at 4°C. Exoribonuclease activity, digests RNA with extensive secondary structure. Its absence leads to increased levels of many mRNAs (among them, *fleQ* and other flagella genes).
argD	PP_4481	1	2.52	2.47×10^{-6}	Bifunctional *N*-succinyldiaminopimelate-aminotransferase/acetylornithine transaminase	—	Involved in L-arginine biosynthesis.
kdsA-1	PP_1611	2	2.65	2.88×10^{-6}	2-Dehydro-3-deoxyphosphooctonate aldolase	(Walsh, et al., 1999)	Involved in lipopolysaccharide biosynthesis.

[a] *FC*, fold-change; *TIM-barrel*, triose phosphate isomerase barrel. The references in the table indicate reports of the corresponding gene or function in either *P. putida* KT2440 or related species.

SUPPLEMENTARY FIGURES

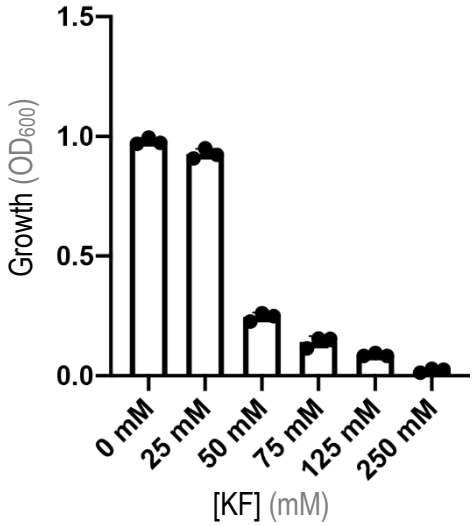

Figure S1 · **Effect of KF on bacterial growth.** Growth of *P. putida* KT2440 in M9 minimal medium containing 5 g L^{-1} glucose as the sole carbon source and supplemented with KF at the different concentrations indicated. Dots represent individual data per experiment with at least three independent cultures analyzed per condition. OD_{600}, optical density measured at 600 nm.

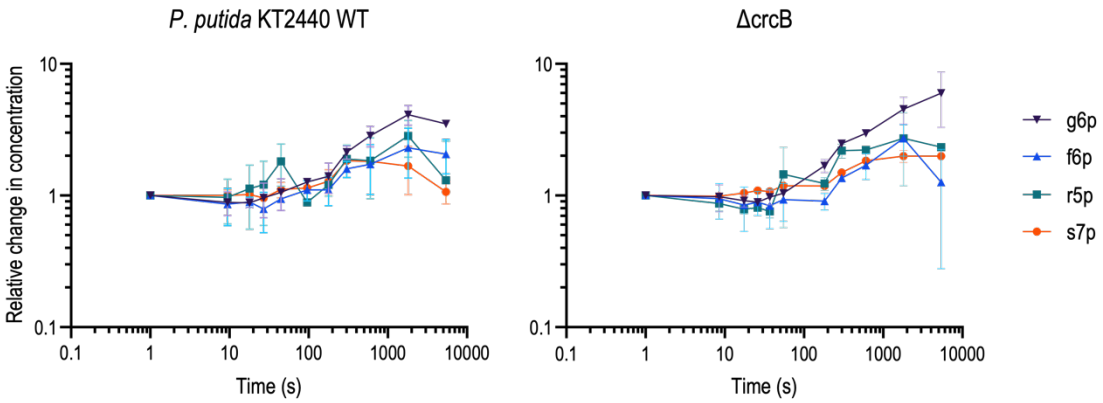

Figure S2 · Metabolomic analysis of sugar phosphates. Relative change in the metabolite concentration over time of metabolites of the upper glycolysis pathway in presence of NaF compared to control conditions in *P. putida* KT2440 and *P. putida* Δ*crcB.* Error bars correspond to standard deviations of two biological replicates. g6p, glucose 6-phosphate; f6p, fructose 6-phosphate; r5p, ribose 5-phosphate; and s7p, sedoheptulose 7-phosphate.

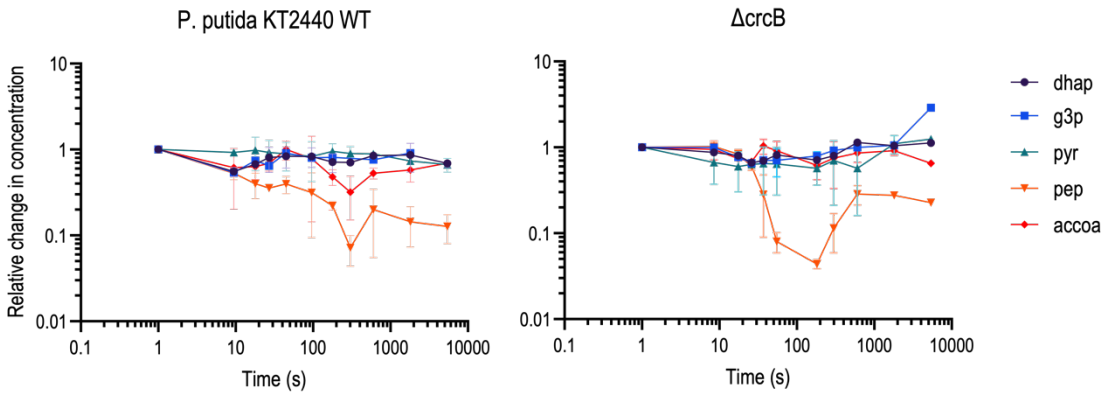

Figure S3 · Metabolomic analysis of intermediates of the lower glycolysis. Relative change in the concentration over time of metabolites of the lower glycolysis pathway in presence of NaF compared to control conditions in *P. putida* KT2440 and *P. putida* Δ*crcB.* Error bars correspond to standard deviations of two biological replicates. dhap, dihydroxyacetone phosphate; g3p, glyceraldehyde 3-phosphate; pyr, pyruvate; pep, phosphoenol pyruvate; and accoa, acetyl-coenzyme A.

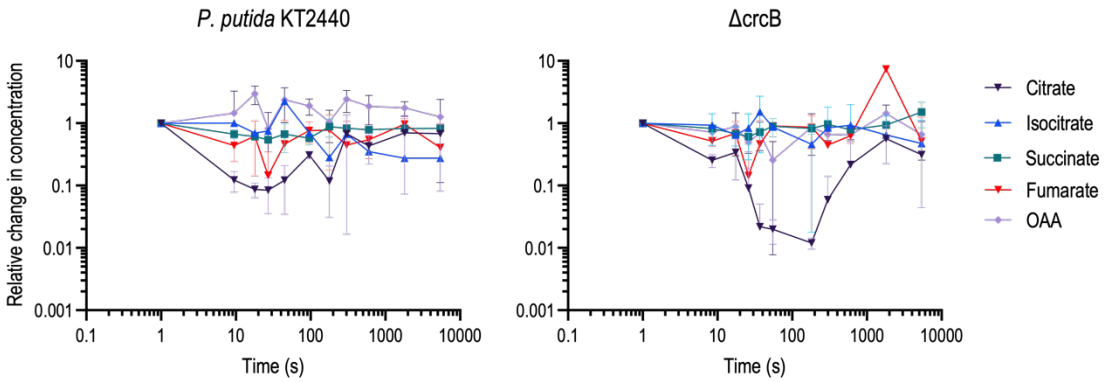

Figure S4 · **Metabolomic analysis of intermediates of the tricarboxylic acid cycle.** Relative change in the concentration over time of metabolites of the tricarboxylic acid cycle in presence of NaF compared to control conditions in *P. putida* KT2440 and *P. putida* Δ*crcB.* Error bars correspond to standard deviations of two biological replicates. OAA, oxaloacetate.

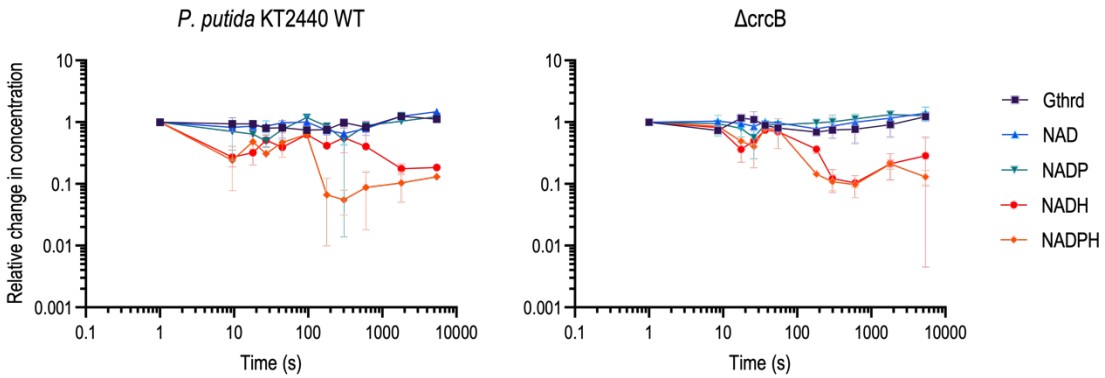

Figure S5 · Metabolomic analysis of redox cofactors and related metabolites. Relative change in the concentration over time of metabolites involved in redox balance in presence of NaF compared to control conditions in *P. putida* KT2440 and *P. putida* Δ*crcB.* Error bars correspond to standard deviations of two biological replicates. Gthrd, reduced glutathione.

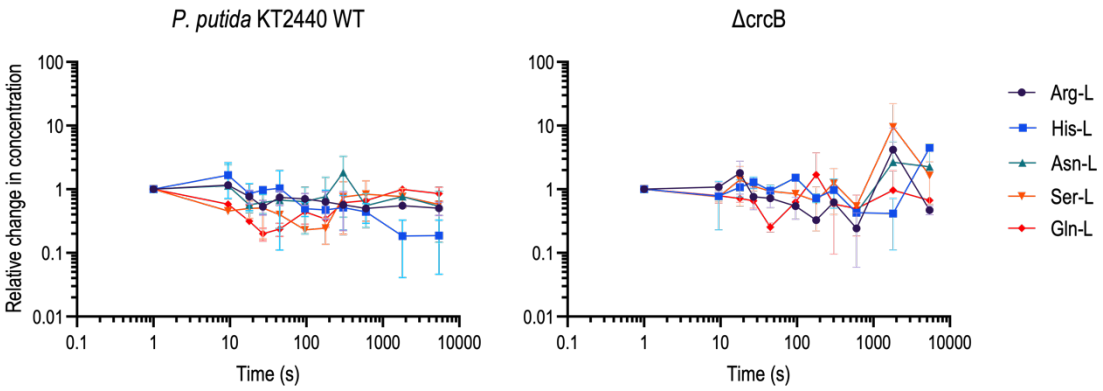

Figure S6 · **Metabolomic analysis of intracellular amino acids.** Relative change in the concentration over time of amino acids in presence of NaF compared to control conditions in *P. putida* KT2440 and *P. putida* Δ*crcB.* Error bars correspond to standard deviations of two biological replicates.

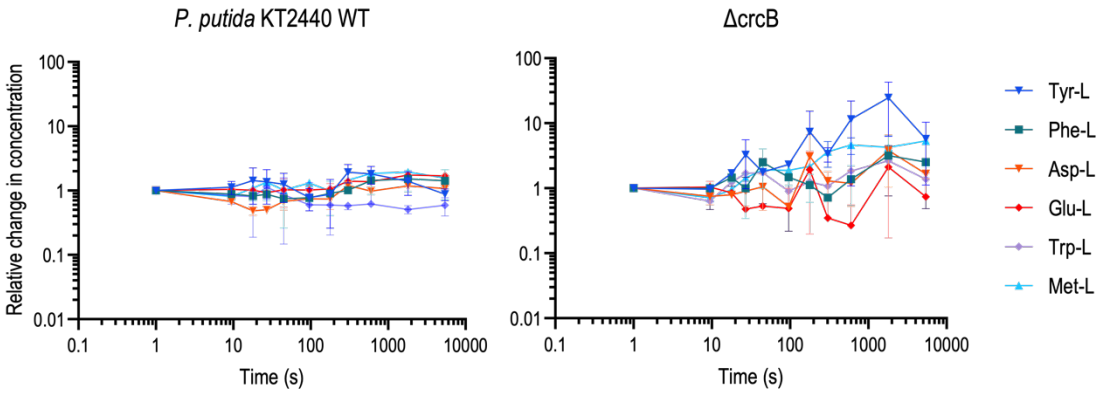

Figure S7 · Metabolomic analysis of intracellular (aromatic and sulphur-containing) amino acids. Relative change in the concentration over time of amino acids in presence of NaF compared to control conditions in *P. putida* KT2440 and *P. putida* Δ*crcB.* Error bars correspond to standard deviations of two biological replicates.