

## Supplemental material

### The heterotrophic bacterium *Cupriavidus pinatubonensis* JMP134 oxidizes sulfide to sulfate with thiosulfate as a key intermediate

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#### **This supplemental document contains:**

**Figure S1:** Sulfide oxidation by complementary strains of *C. pinatubonensis* JMP134 mutants.

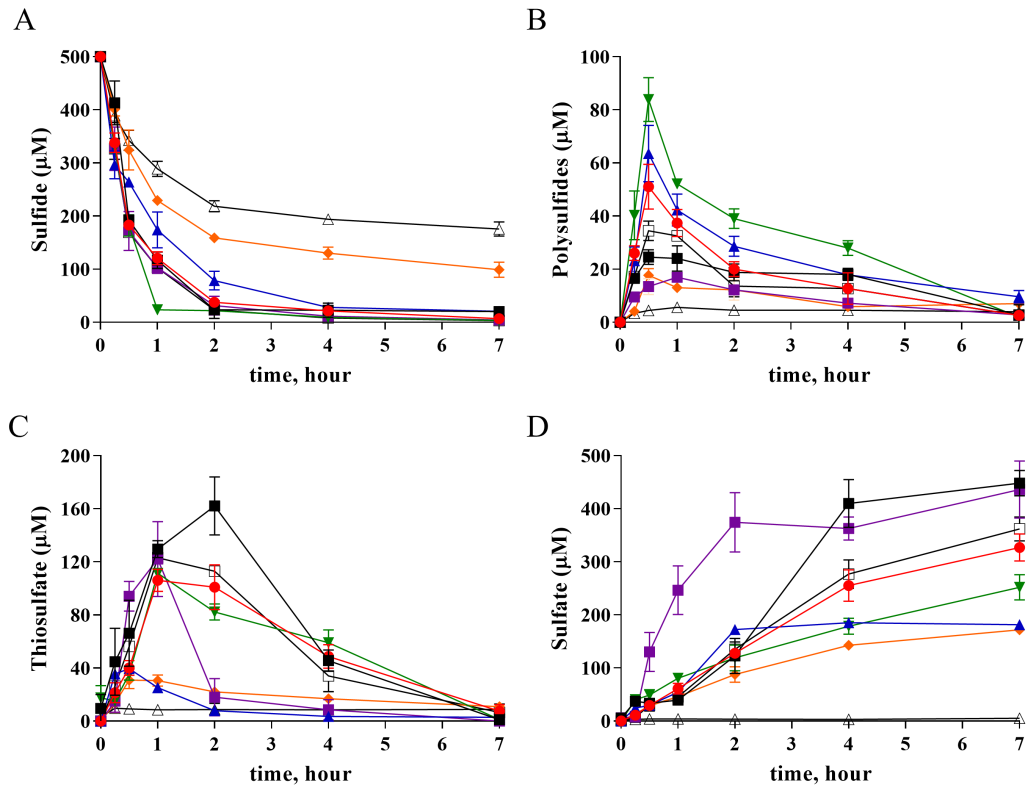
**Figure S2:** The deletion of *sorA* does not affect sulfide oxidation to sulfate.

**Figure S3:** SorA is responsible for the oxidation of added sulfite.

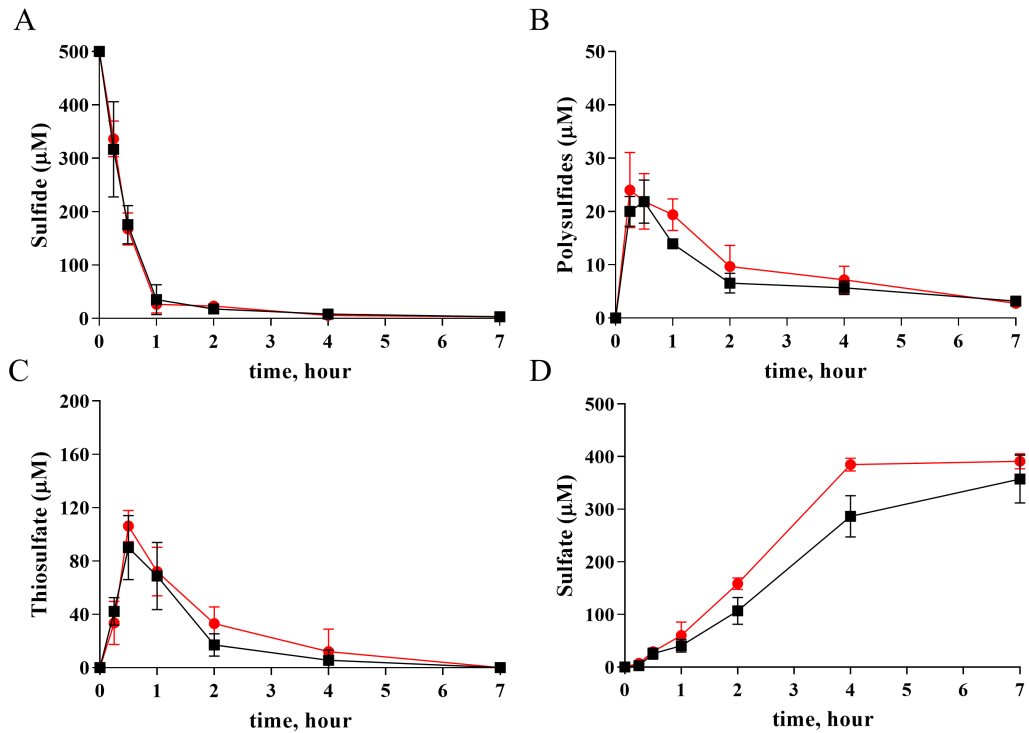
**Figure S4:** Thiosulfate-induced  $\Delta$ *sorA* cells also oxidized sulfite to sulfate.

**Table S1:** Sulfur-oxidizing enzymes in *C. pinatubonensis* JMP134.

**Table S2:** Sulfur-oxidizing enzymes in GB17, OCh 114, and DSS-3.



**Fig. S1. Sulfide oxidation by complemented strains of *C. pinatubonensis* JMP134 mutants.** Cells were harvested, washed, and re-suspended at  $OD_{600}$  of 2.0 in 100 mM HEPES buffer, pH 7.4. Sulfide was added to 500  $\mu$ M to initiate the reaction. Sulfide, polysulfides, thiosulfate, and sulfate were determined at different times. *C. pinatubonensis* JMP134 ( $\square$ ),  $\Delta$ *soxYZ*::*soxYZ* ( $\blacksquare$ ),  $\Delta$ *pdo1pdo2*::*pdo2* ( $\bullet$ ),  $\Delta$ *pdo1pdo2soxYZ*::*soxYZ* ( $\blacktriangle$ ),  $\Delta$ *fccB*::*fccB* ( $\blacktriangledown$ ),  $\Delta$ *sqrpdo1pdo2soxYZ*::*soxYZ* ( $\blacklozenge$ ),  $\Delta$ *sqrpdo1pdo2*::*sqrpdo2* ( $\blacksquare$ ), and HEPES buffer ( $\triangle$ ). All data are average of at least three samples with standard deviation (error bar).



**Fig. S2. The deletion of *sorA* does not affect sulfide oxidation to sulfate.**

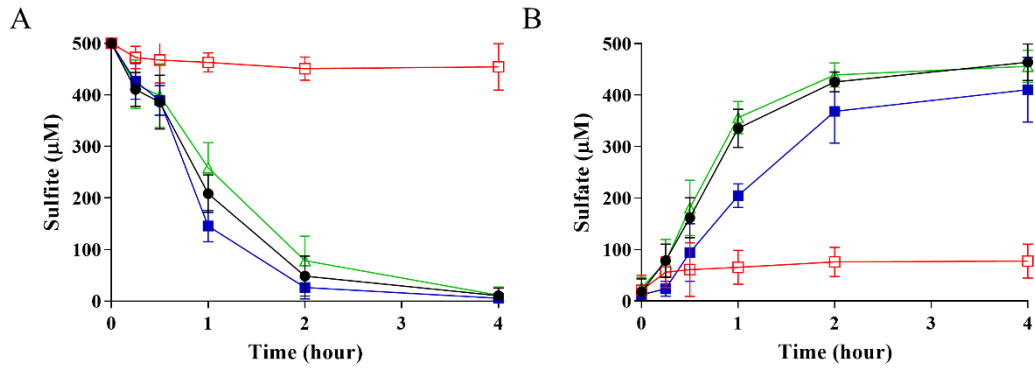
Cells were harvested, washed, and re-suspended at  $OD_{600}$  of 2.0 in 100 mM HEPES

buffer, pH 7.4. Sulfide was added to 500  $\mu\text{M}$  to initiate the reaction. Sulfide (A),

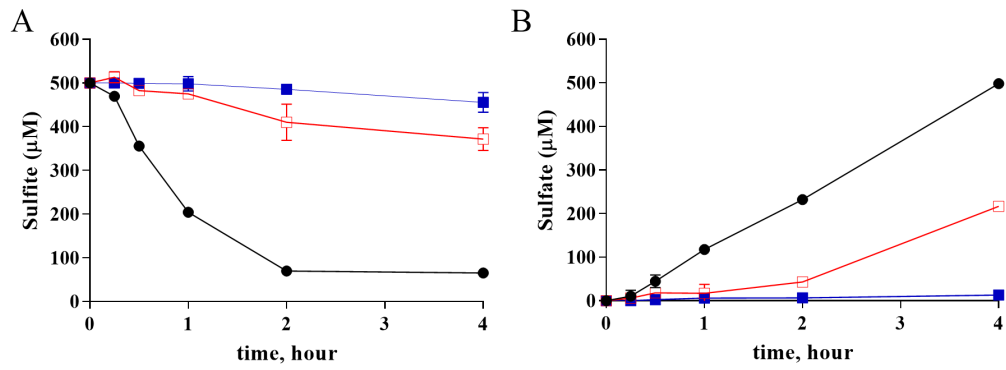
polysulfides (B), thiosulfate (C), and sulfate (D) in cell suspensions of  $\Delta sorA$

(■) and  $\Delta sorA::sorA$  (●) were determined at different times. All data are average of at

least three samples with standard deviation (error bar).



**Fig. S3. SorA is responsible for the oxidation of added sulfite.** Sulfite-induced cells were harvested, washed, and re-suspended at  $OD_{600}$  of 2.0 in 100 mM HEPES buffer, pH 7.4. Sulfite was added to 500  $\mu\text{M}$  to initiate the reaction. Sulfite (A) and sulfate (B) in cell suspensions of *C. pinatubonensis* JMP134 wild type (●),  $\Delta sorA$  (□),  $\Delta sorA::sorA$  (■), and  $\Delta soxYZ$  (△) were determined at different times. All data are average of at least three samples with standard deviation (error bar).



**Fig. S4. Thiosulfate-induced  $\Delta\text{sorA}$  cells slowly oxidized sulfite to sulfate.** Cells were harvested, washed, and re-suspended at  $OD_{600}$  of 2.0 in 100 mM HEPES buffer, pH 7.4. Sulfite was added to 500  $\mu\text{M}$  to initiate the reaction. Sulfite (A) and sulfate (B) of in cell suspensions of *C. pinatubonensis* JMP134 wild type (●),  $\Delta\text{sorA}$  (□), and  $\Delta\text{sorAsoxYZ}$  (■) were determined at different times. All data are average of at least three samples with standard deviation (error bar).

**Table S1.** Sulfur-oxidizing enzymes in *C. pinatubonensis* JMP134

<b>Locus_tag</b>	<b>Accession Number</b>	<b>Protein</b>	<b>Size (aa)</b>	<b>Putative function</b>
Reut_B3588	AAZ62946	SQR	558	sulfide dehydrogenase
Reut_B3589	AAZ62947	PDO2	298	persulfide dioxygenase
Reut_B3590	AAZ62948	FisR	436	transcriptional regulator
Reut_B3591	AAZ62949	TauE	273	sulfite exporter
Reut_A3082	AAZ62442	SorB	208	sulfite:cytochrome <i>c</i> oxidoreductase subunit B
Reut_A3083	AAZ62443	SorA	417	SO family, molybdopterin binding domain
Reut_A3249	AAZ62608	SoxB	573	sulfate thio-hydrolase
Reut_A3250	AAZ62609	TlpA_like	165	disulfide reductases in cytochrome maturation
Reut_A3251	AAZ62610	SoxX	224	monoheme subunit cytochrome
Reut_A3252	AAZ62611	SoxA	275	diheme subunit cytochrome
Reut_A3253	AAZ62612	DsrE-like	152	Proposed thiosulfate transfer
Reut_A3254	AAZ62613	SoxZ	103	sulfur compound chelating protein
Reut_A3255	AAZ62614	SoxY	151	sulfur covalently binding protein
Reut_A3256	AAZ62615	cyt <i>c</i>	108	cytochrome <i>c</i>
Reut_A3257	AAZ62616	SoxD	344	diheme <i>c</i> -type cytochrome
Reut_A3258	AAZ62617	SoxC	449	molybdenum cofactor-containing subunit
Reut_A3259	AAZ62618	ArsR	138	transcriptional regulator
Reut_A3260	AAZ62619	EamA-like	297	O-acetylserine/cysteine export protein
Reut_A3261	AAZ62620	FccB	419	monomeric flavoprotein
Reut_A3262	AAZ62621	Cyt <i>c</i>	108	cytochrome <i>c</i> <sub>553</sub>
Reut_A3334	AAZ62692	PDO1	245	persulfide dioxygenase

**Table S2.** Sulfur-oxidizing enzymes in GB17, OCh 114, and DSS-3<sup>a</sup>

Enzymes	GB17	OCh 114	DSS-3
SQR	QFG36295/36% <sup>b</sup>	ABG30463/67%	AAV94899/39%
PDO <sup>c</sup>	QFG35975/51%	ABG30464/57%	AAV94334/59%
	QFG36611/51%	ABG30238/56%	
SoxX	QFG36009/31%	ABG31144/31%	AAV94297/52%
SoxY	QFG35251/40%	ABG31145/37%	AAV94298/37%
SoxZ	QFG35250/37%	ABG31146/33%	AAV94299/33%
SoxA	QFG35249/27%	ABG31147/26%	AAV94300/26%
SoxB	QFG35248/49%	ABG31148/48%	AAV94301/49%
SoxC	QFG35247/51%	ABG31149/50%	AAV94302/50%
SoxD	QFG35246/34%	ABG31150/31%	AAV94303/34%
FccA (SoxE)	QFG35245/40%	ABG31151/36%	AAV94304/37%
FccB (SoxF)	QFG35244/41%	ABG31152/38%	AAV94305/42%

<sup>a</sup> Use the enzymes in *C. pinatubonensis* JMP134 (Table S1) as the query sequences. <sup>b</sup> Percentage of identities with the query protein. <sup>c</sup> PDO2 (AAZ62947) of *C. pinatubonensis* JMP134. The strains are *P. pantotrophus* GB17, *Roseobacter denitrificans* OCh 114, and *Ruegeria pomeroyi* DSS-3.