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The absence of SigX may result in a nutritional stress response in *Pseudomonas aeruginosa*.

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SigX is one of the 19 ECF sigma factor of *P. aeruginosa*. It is a master regulator of bacterial adaptation to osmotic stress that impacts more than 250 genes, among which genes involved in adaptation/protection, heat shock response, chemotaxis, motility/attachment, virulence and virulence-associated genes linked to the protein secretion/export apparatus or secreted factors. SigX was shown to be involved in modulation of fatty acid and phospholipid metabolisms and consequently in membrane lipidic composition. We have previously shown that a *sigX* mutant is severely altered during growth in LB rich broth but not in M9-glucose minimal medium. Since membrane homeostasis is required to fulfil important physiological functions including nutrients uptake, we wonder if the SigX mutant growth alterations observed in LB medium would be correlated to such metabolic pathways.

Microarrays (mRNA) and LTQ-orbitrap (Protein) assays were achieved on *P. aeruginosa* H103 and its isogenic *sigX* deletion mutant PA0SX grown in LB medium. A selection of the main dysregulated genes belonging to the "small molecules transport", "amino acids metabolism" and "carbon metabolism" Pseudocap functional classes, are presented, and the fold change between PA0SX and H103 is given in terms of mRNA and protein relative amounts (FC>2, Pvalue < 0.05).

Numerous genes transporters are dysregulated in the *sigX* mutant strain.

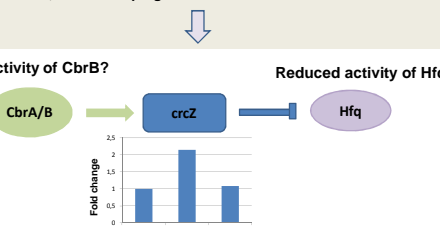
Locust	Gene	Product name	FC
Transport of small molecules			
PA0291	<i>oprB</i>	Amperically-induced outer membrane porin OprB precursor	-2.63
PA0292	<i>oprD</i>	probable periplasmic polysaccharide binding protein	-3.33
PA0958	<i>oprD</i>	basic amino acid, basic peptide and imipenem outer membrane porin (OprD)	-5.55
PA1178	<i>oprH</i>	"PspQ" and low Mg ²⁺ inducible outer membrane protein H1 precursor	-2.06
PA1183	<i>dctA</i>	c4-decarboxylase transport protein	-3.22
PA1863	<i>modA</i>	maltoheptaose-binding periplasmic protein precursor ModA	-3.45
PA2113	<i>oprB</i>	tryptophan/tryptamine porin (OprB)	-5.00
PA3186	<i>oprB</i>	thiosulfate/carbohydrate outer membrane porin OprB precursor	-2.70
PA3187	<i>oprB</i>	probable ATP-binding component of ABC transporter	-6.09
PA3188	<i>oprB</i>	probable periplasmic component of ABC transporter	-7.14
PA3189	<i>oprB</i>	probable periplasmic component of ABC transporter	-2.90
PA3190	<i>oprB</i>	probable binding protein component of ABC transporter	-2.00
PA3641	<i>oprB</i>	probable amino acid permease	-2.60
PA3790	<i>oprC</i>	putative copper transporter outer membrane porin OprC precursor	-4.35
PA3865	<i>oprC</i>	probable amino acid binding protein	-2.43
PA4067	<i>oprG</i>	outer membrane protein OprG precursor	-1.51
PA4616	<i>oprG</i>	probable c4-decarboxylase-binding protein	-2.41
PA4617	<i>dctA</i>	similar to E. coli dcaB, outer membrane receptor H4A	-2.37
PA4687	<i>hcr1</i>	iron-sulfur-binding periplasmic protein H4A	-2.27
PA5217	<i>oprC</i>	probable binding protein component of ABC transporter	-2.38
PA0215	<i>mauM</i>	malonate transporter MauM	15.91
PA0216	<i>mauM</i>	malonate transporter MauM	6.93
PA0280	<i>cysE</i>	half-cystine transport protein CysA	2.75
PA0603	<i>aprA</i>	ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-containing	8.66
PA0604	<i>aprB</i>	ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-containing	14.81
PA0755	<i>oprH</i>	cit-acetate porin (OprH)	1.58
PA0866	<i>amiE</i>	transmembrane amino acid transport protein AmiE	3.95
PA1074	<i>hcr1</i>	branchched chain amino acid transport protein H4A	2.78
PA1339	<i>oprD</i>	amino acid ABC transporter ATP binding protein	2.61
PA1342	<i>oprD</i>	ABC-type amino acid transport/transduction systems, periplasmic component/domain	2.45
PA1946	<i>rhlB</i>	binding protein component precursor of ABC ribose transporter	2.78
PA1947	<i>rhlB</i>	thiosulfate transport protein RhsA	4.75
PA1948	<i>rhlC</i>	membrane protein component of ABC ribose transporter	3.29
PA2329	<i>oprD</i>	probable ATP-binding component of ABC transporter	2.79
PA2338	<i>oprD</i>	probable binding protein component of ABC maltoheptaose/transport protein	4.35
PA2339	<i>oprD</i>	probable binding-protein-dependent maltoheptaose/transport protein	3.89
PA2340	<i>oprD</i>	probable binding-protein-dependent maltoheptaose/transport protein	4.42
PA2341	<i>oprD</i>	probable ATP-binding component of ABC maltoheptaose/transport protein	3.96
PA2711	<i>oprD</i>	probable periplasmic spermidine/potrescine-binding protein	2.14
PA3038	<i>oprD</i>	probable porin	14.88
PA3891	<i>oprC</i>	OprC ABC transporter, ATP-binding protein	2.10
PA4498	<i>oprC</i>	probable binding protein component of ABC transporter	5.29
PA4497	<i>oprC</i>	probable binding protein component of ABC transporter	5.69
PA4501	<i>oprD</i>	Dicaine-glutamate dipeptide porin OprD	3.28
PA4910	<i>oprD</i>	branched chain amino acid ABC transporter ATP binding protein	2.61
PA4913	<i>oprD</i>	probable binding protein component of ABC transporter	5.08
PA5094	<i>oprD</i>	probable ATP-binding component of ABC transporter	6.66
PA5096	<i>oprD</i>	probable binding protein component of ABC transporter	3.78
PA5153	<i>oprD</i>	amino acid (lysine/arginine/ornithine/histidine) ABC transporter periplasmic binding protein	2.44

Many porins encoding genes were downregulated while several amino acids transporters were upregulated in PA0SX grown in LB medium compared to *P. aeruginosa* H103.

The absence of SigX leads to increased expression of many genes that are involved in amino acids and carbon metabolisms.

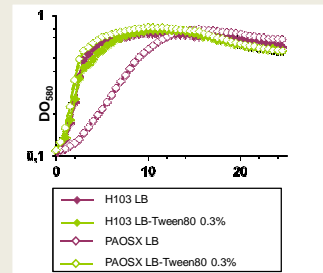
Gene	Gene name	Product name	PA0SX/mRNA	Protein	Chr/V	Hfq
Amino acid metabolism						
PA0189	<i>argD</i>	acetylglutamate 5-oxalaldehyde dehydrogenase	2.31	X	X	X
PA0909	<i>argD</i>	acetylglutamate dehydrogenase	2.42	X	X	X
PA0981	<i>argD</i>	acetylglutamate dehydrogenase	2.25	X	X	X
PA1338	<i>arg</i>	acetylglutamate:oxalaldehyde transaminase	2.09	X	X	X
PA1984	<i>argE</i>	NAD ⁺ -dependent aldehyde dehydrogenase E acyl	2.44	X	X	X
PA2247	<i>argH1</i>	oxalaldehyde dehydrogenase (alpha subunit)	4.39	2.3	X	X
PA2248	<i>argH2</i>	oxalaldehyde dehydrogenase (beta subunit)	5.81	X	X	X
PA2249	<i>argH3</i>	oxalaldehyde dehydrogenase (gamma subunit)	6.04	2.2	X	X
PA2250	<i>argH4</i>	oxalaldehyde dehydrogenase (delta subunit)	6.40	2.9	X	X
PA2251	<i>argH5</i>	oxalaldehyde dehydrogenase (epsilon subunit)	6.75	X	X	X
PA2309	<i>argM1</i>	oxalaldehyde dehydrogenase M1	29.66	X	X	X
PA2310	<i>argM2</i>	oxalaldehyde dehydrogenase M2	13.12	X	X	X
PA2311	<i>argM3</i>	oxalaldehyde dehydrogenase M3	2.49	X	X	X
PA2312	<i>argM4</i>	oxalaldehyde dehydrogenase M4	3.02	X	X	X
PA2313	<i>argM5</i>	oxalaldehyde dehydrogenase M5	2.39	X	X	X
PA2314	<i>argM6</i>	oxalaldehyde dehydrogenase M6	2.47	16.8	X	X
PA2315	<i>argM7</i>	oxalaldehyde dehydrogenase M7	12.74	15	X	X
PA2316	<i>argM8</i>	oxalaldehyde dehydrogenase M8	10.45	6.9	X	X
Carbon metabolism						
PA0133	<i>aceA</i>	isocitrate lyase 1 (4-dihydroxyhept-6-ylidene)-beta subunit	4.03	X	X	X
PA0134	<i>aceA</i>	isocitrate lyase 1 (4-dihydroxyhept-6-ylidene)-beta subunit	3.33	11.6	X	X
PA0211	<i>aceA</i>	isocitrate lyase 1 (4-dihydroxyhept-6-ylidene)-beta subunit	14.3	11.6	X	X
PA0212	<i>aceA</i>	isocitrate lyase 1 (4-dihydroxyhept-6-ylidene)-beta subunit	3.21	16.4	X	X
PA0213	<i>aceA</i>	isocitrate lyase 1 (4-dihydroxyhept-6-ylidene)-beta subunit	2.98	X	X	X
PA0214	<i>aceA</i>	isocitrate lyase 1 (4-dihydroxyhept-6-ylidene)-beta subunit	20.9	17.5	X	X
PA0228	<i>aceA</i>	isocitrate lyase 1 (4-dihydroxyhept-6-ylidene)-beta subunit	13.88	16.1	X	X
PA0807	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	2.88	X	X	X
PA1092	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	4.19	X	X	X
PA1093	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	4.04	X	X	X
PA1094	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	6.48	X	X	X
PA1095	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	7.32	X	X	X
PA1096	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	3.18	X	X	X
PA1097	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	4.47	X	X	X
PA1098	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	2.46	X	X	X
PA1099	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	4.34	X	X	X
PA1099	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	6.52	X	X	X
PA1099	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	7.46	X	X	X
PA1099	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	2.44	X	X	X
PA1099	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	2.97	5.4	X	X
PA2207	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	10.95	25.3	X	X
PA2208	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	10.02	8.5	X	X
PA2209	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	7.39	8.5	X	X
PA2210	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	10.16	10.4	X	X
PA2211	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	102.10	11	X	X
PA2212	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	10.94	2.4	X	X
PA2315	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	4.42	X	X	X
PA2316	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	3.11	X	X	X
PA2317	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	3.41	X	X	X
PA2318	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	4.92	X	X	X
PA2319	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	3.14	X	X	X
PA2320	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	3.41	2.6	X	X
PA2321	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	4.67	X	X	X
PA2322	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	3.22	X	X	X
PA2323	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	2.32	X	X	X
PA2324	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	10.17	X	X	X
PA4496	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	3.27	X	X	X
PA4913	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	2.66	X	X	X
PA4914	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	2.53	X	X	X
PA4915	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	3.08	X	X	X
PA4916	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	2.18	X	X	X
PA4917	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	2.17	X	X	X
PA4918	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	2.54	X	X	X
PA4919	<i>aceB</i>	isocitrate lyase 2 (4-dihydroxyhept-6-ylidene)-beta subunit	3.34	X	X	X

As indicated in this table, amino acids genes that were downregulated in a CbrB mutant, were upregulated in PA0SX mutant, suggesting that CbrB might be overactivated in this strain. Moreover several genes that were shown to be upregulated in a *hfq* mutant, were also upregulated in PA0SX mutant strain.



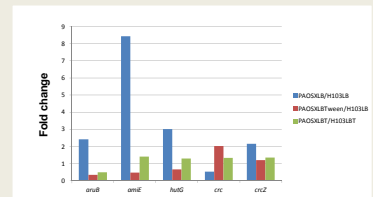
Expression of CbrB direct target *crzC* in H103, PA0SX and *sigX* complemented PA0SX mutant strain PA0SXC grown in LB broth (qRT-PCR).

The growth of the *sigX* mutant in LB broth is improved by a subinhibitory concentration of Tween 80 detergent.



The growth of PA0SX is strongly affected in LB medium, and can be fully restored when adding Tween 80 at 0.3%. A similar effect was observed when SDS was added in case of Tween80.

Expression of selected genes is partly restored when PA0SX is grown in LB containing Tween 80 (LBT)



Some genes that are regulated by CbrB (*aruB*, *hutG*, *crzC*) or Hfq (*amiE*) were assayed by qRT-PCR in PA0SX grown in LB or LBT compared to H103 grown in LB or LBT. Expression of *aruB*, *hutG*, *crzC* and *amiE* was lowered when PA0SX was grown in LBT compared to LB. Inversely, *crzC*, whose expression was reduced in PA0SX grown in LB, was increased when grown in LBT.

Conclusions

Most of the effects of SigX on these selected gene expression are indirect.

The absence of SigX leads to strong alterations at the nutritional stress response level suggesting that PA0SX encounters a high C/N ratio.

The question is now to better understand the role of Tween 80 on the mutant strain

