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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 PAOSX 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 PAOSX 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.

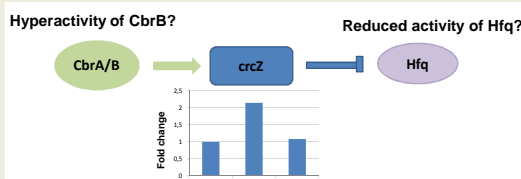
Locust	Gene	Product name	FC
Transport of small molecules			
PA0291	oprB	Amphotericin-induced outer membrane porin OprB precursor	-2.63
PA0292	oprD	probable periplasmic polysaccharide binding protein	-3.33
PA0958	oprD	basic amino acid, basic peptide and imipenem outer membrane porin OprD	-5.55
PA1178	oprH	"PspQ" and low Mg ²⁺ -inducible outer membrane protein H1 precursor	-2.00
PA1183	dctA	c4-dicarboxylate transport protein	-3.22
PA1863	modA	maltoheptaose-binding periplasmic protein precursor ModA	-3.45
PA2113	oprB	tryptophan/tryptamine porin OprB	-5.00
PA3186	oprB	thiosulfate/carbohydrate outer membrane porin OprB precursor	-2.70
PA3187	oprB	probable ATP-binding component of ABC transporter	-6.09
PA3188	oprB	probable periplasmic ABC sugar transporter	-7.14
PA3189	oprB	probable periplasmic ABC sugar transporter	-2.90
PA3190	oprB	probable binding protein component of ABC sugar transporter	-2.00
PA3641	oprC	probable amino acid permease	-2.60
PA3790	oprC	putative copper transporter outer membrane porin OprC precursor	-4.35
PA3865	oprC	probable amino acid binding protein	-2.43
PA4067	oprG	outer membrane protein OprG precursor	-1.51
PA4616	oprG	probable c4-dicarboxylate-binding protein	-2.41
PA4675	hslA	similar to E. coli autoinducer outer membrane receptor hslA	-2.37
PA4687	hslA	iron-iron-binding periplasmic protein HslA	-2.27
PA5217	oprB	probable binding protein component of ABC iron transporter	-2.38
PA0215	malM	malonate transporter MalM	15.91
PA0216	malM	malonate transporter MalM	6.93
PA0280	cysE	cellular transport protein CysA	2.75
PA0603	aprA	ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-containing	8.66
PA0604	aprB	ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-containing	14.81
PA0755	oprH	cis-acetamide porin OprH	1.58
PA0866	amiE	aromatic amino acid transport protein AmiE	3.95
PA1074	hcrC	branched chain amino acid transport protein HcrC	2.79
PA1339	amiE	aromatic amino acid ABC transporter ATP binding protein	2.61
PA1342	amiE	ABC-type amino acid transport/ATP transduction systems, periplasmic component/domain	2.45
PA1946	rhbB	binding protein component precursor of ABC ribose transporter	2.78
PA1947	rhbB	ribose transporter protein RhbA	4.75
PA1948	rhcC	membrane protein component of ABC ribose transporter	3.29
PA2329	oprB	probable ATP-binding component of ABC transporter	2.79
PA2338	oprB	probable binding protein component of ABC maltoheptaose/tryptamine transporter	4.35
PA2339	oprB	probable binding-protein-dependent maltoheptaose/tryptamine transporter	3.89
PA2340	oprB	probable binding-protein-dependent maltoheptaose/tryptamine transporter	4.42
PA2341	oprB	probable ATP-binding component of ABC maltoheptaose/tryptamine transporter	3.96
PA2711	oprB	probable periplasmic spermidine/potrescine-binding protein	2.14
PA3038	oprB	probable porin	14.88
PA3891	oprC	OprC ABC transporter, ATP-binding protein	2.10
PA4498	oprB	probable binding protein component of ABC transporter	5.29
PA4497	oprB	probable binding protein component of ABC transporter	5.69
PA4501	oprD	Dicaine-glutamate dipeptide porin OprD	3.28
PA4910	oprB	branched chain amino acid ABC transporter ATP binding protein	2.61
PA4913	oprB	probable binding protein component of ABC transporter	5.08
PA5094	oprB	probable ATP-binding component of ABC transporter	6.66
PA5096	oprB	probable binding protein component of ABC transporter	3.78
PA5153	oprB	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 PAOSX grown in LB medium compared to *P. aeruginosa* H103.

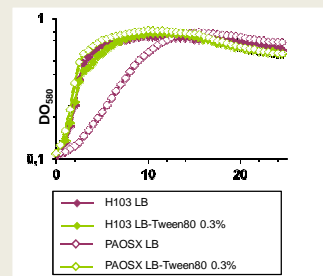
The absence of SigX leads to increased expression of many genes that are involved in amino acids and carbon metabolisms.

Gene	Gene name	Product name	PAOSX/mRNA	Protein	Chr/Pl	Hfq
Amino acid metabolism						
PA0166	argD	acetylglutamate 5-semialdehyde dehydrogenase	2.31	X	X	X
PA0999	argA	acetylglutamate dehydrogenase	2.42	X	X	X
PA0981	argA	acetylglutamate dehydrogenase	2.25	X	X	X
PA1338	arg	acetylglutamate/tryptophan transporter	2.09	X	X	X
PA1984	argA	NAD-dependent aldehyde dehydrogenase C-act	2.44	X	X	X
PA2247	hcrC	branched-chain amino acid dehydrogenase (beta subunit)	4.39	2.17	X	X
PA2248	hcrC	branched-chain amino acid dehydrogenase (beta subunit)	5.94	2.2	X	X
PA2249	hcrC	branched-chain amino acid dehydrogenase	6.04	2.2	X	X
PA2250	hcrC	branched-chain amino acid dehydrogenase-VII	6.40	2.0	X	X
PA2251	hcrC	branched-chain amino acid dehydrogenase	6.72	2.0	X	X
PA2308	amiE	branched-chain amino acid permease	20.66	X	X	X
PA2370	amiE	branched-chain amino acid permease	13.12	X	X	X
PA2371	amiE	branched-chain amino acid permease	21.49	X	X	X
PA2391	hcrC	branched-chain amino acid dehydrogenase	3.02	X	X	X
PA2692	amiE	branched-chain amino acid permease	2.39	X	X	X
PA2693	amiE	branched-chain amino acid permease	2.49	18.8	X	X
PA2694	amiE	branched-chain amino acid permease	12.74	15	X	X
PA2695	amiE	branched-chain amino acid permease	10.45	15.9	X	X
Carbon metabolism						
PA0153	argD	acetylglutamate 5-d-aminopentanoate, beta subunit	4.03	X	X	X
PA0154	argD	acetylglutamate 5-d-aminopentanoate, alpha subunit	3.51	11.6	X	X
PA0211	modD	maltoheptaose-binding periplasmic protein	14.3	11.6	X	X
PA0212	modD	maltoheptaose-binding periplasmic protein	3.21	14.4	X	X
PA0213	modD	maltoheptaose-binding periplasmic protein	2.88	X	X	X
PA0217	modD	maltoheptaose-binding periplasmic protein	20.9	17.5	X	X
PA0228	argD	probable CoA-transferase, subunit B	13.88	16.1	X	X
PA0807	argD	acetylglutamate 5-oxalopentanoate	2.88	X	X	X
PA1092	oprB	hypothetical protein	4.19	X	X	X
PA1093	oprB	hypothetical protein	4.04	X	X	X
PA1094	oprB	hypothetical protein	6.48	X	X	X
PA1095	oprB	hypothetical protein	7.32	X	X	X
PA1096	oprB	hypothetical protein	3.18	X	X	X
PA1097	oprB	hypothetical protein	4.47	X	X	X
PA1098	oprB	hypothetical protein	4.24	X	X	X
PA1099	oprB	hypothetical protein	4.52	X	X	X
PA1099	oprB	hypothetical protein	4.40	X	X	X
PA1100	oprB	hypothetical protein	4.34	X	X	X
PA1101	oprB	hypothetical protein	4.52	X	X	X
PA1102	oprB	hypothetical protein	4.46	X	X	X
PA1103	oprB	hypothetical protein	4.46	X	X	X
PA1104	oprB	NAD-dependent aldehyde dehydrogenase C-act	2.44	X	X	X
PA1105	hcrC	branched-chain amino acid dehydrogenase	2.97	5.4	X	X
PA1207	argD	branched-chain amino acid dehydrogenase (beta subunit)	10.95	25.5	X	X
PA2208	argD	branched-chain amino acid dehydrogenase	10.02	8.5	X	X
PA2209	argD	branched-chain amino acid dehydrogenase	7.39	8.5	X	X
PA2210	argD	branched-chain amino acid dehydrogenase	10.16	10.4	X	X
PA2211	argD	branched-chain amino acid dehydrogenase	10.02	11	X	X
PA2212	argD	branched-chain amino acid dehydrogenase	10.94	5.4	X	X
PA2515	argD	lysine 1,2-diaminocyclohexane-3,4-diamine carboxylate dehydrogenase	4.42	X	X	X
PA2516	argD	lysine 1,2-diaminocyclohexane-3,4-diamine carboxylate dehydrogenase	4.92	X	X	X
PA2517	argD	lysine 1,2-diaminocyclohexane-3,4-diamine carboxylate dehydrogenase	3.11	X	X	X
PA2518	argD	lysine 1,2-diaminocyclohexane-3,4-diamine carboxylate dehydrogenase	3.41	X	X	X
PA2519	argD	lysine 1,2-diaminocyclohexane-3,4-diamine carboxylate dehydrogenase	4.92	X	X	X
PA2520	argD	lysine 1,2-diaminocyclohexane-3,4-diamine carboxylate dehydrogenase	3.14	X	X	X
PA3306	amiE	aromatic amino acid permease	3.41	2.6	X	X
PA3307	amiE	aromatic amino acid permease	4.67	X	X	X
PA3308	amiE	aromatic amino acid permease	3.22	X	X	X
PA3709	argD	tryptophan/tryptamine transporter	2.32	X	X	X
PA4209	hcrC	branched-chain amino acid dehydrogenase	13.17	X	X	X
PA4496	oprB	probable binding protein component of ABC transporter	2.28	X	X	X
PA4973	oprB	probable binding protein component of ABC transporter	2.56	X	X	X
PA4974	oprB	probable binding protein component of ABC transporter	2.53	X	X	X
PA4975	oprB	probable binding protein component of ABC transporter	3.08	X	X	X
PA4976	oprB	probable binding protein component of ABC transporter	2.18	X	X	X
PA4977	oprB	probable binding protein component of ABC transporter	2.17	X	X	X
PA4978	oprB	probable binding protein component of ABC transporter	2.17	X	X	X
PA4979	oprB	probable binding protein component of ABC transporter	2.14	X	X	X
PA4980	oprB	probable binding protein component of ABC transporter	2.14	X	X	X

As indicated in this table, some genes that were downregulated in a CbrB mutant, were upregulated in PAOSX 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 PAOSX mutant strain.

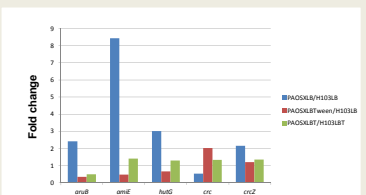


The growth of the sigX mutant in LB broth is improved by a subinhibitory concentration of Tween 80 detergent.



The growth of PAOSX 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 PAOSX 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 PAOSX grown in LB or LBT compared to H103 grown in LB or LBT. Expression of *aruB*, *hutG*, *crzC* and *amiE* was lowered when PAOSX was grown in LBT compared to LB. Inversely, *crzC*, whose expression was reduced in PAOSX 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 PAOSX encounters a high C/N ratio.

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

