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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).

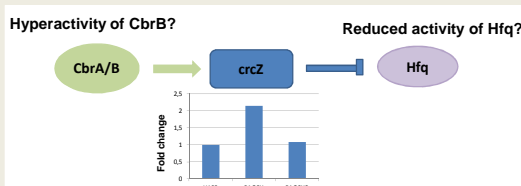
Numerous genes transporters are dysregulated in the *sigX* mutant strain.

Locus	Gene	Product name	FC
Transport of small molecules			
PA0291	<i>oprB</i>	Amperibically-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	-2.00
PA1183	<i>dctA</i>	c4-dicarboxylate 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 ABC sugar transporter	-7.14
PA3189	<i>oprB</i>	probable periplasmic ABC sugar transporter	-2.90
PA3190	<i>oprB</i>	probable binding protein component of ABC sugar transporter	-2.00
PA3641	<i>oprC</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-dicarboxylate-binding protein	-2.41
PA4617	<i>oprG</i>	similar to E. coli maltoheptaose outer membrane receptor H1A	-2.37
PA4687	<i>hcr1</i>	iron-iron-binding periplasmic protein H1A	-2.27
PA5217	<i>oprG</i>	probable binding protein component of ABC iron transporter	-2.38
PA0215	<i>malM</i>	maltoheptaose transporter MalM	15.91
PA0216	<i>malM</i>	maltoheptaose transporter MalM	6.93
PA0280	<i>cysE</i>	cellular 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>oprM</i>	cis-acconitate porin (OprM)	1.58
PA0866	<i>amiE</i>	aromatic amino acid transport protein AmiE	3.95
PA1074	<i>hcr1</i>	iron-iron-binding periplasmic protein H1A	2.79
PA1339	<i>amiE</i>	aromatic amino acid ABC transporter ATP binding protein	2.61
PA1342	<i>amiE</i>	ABC-type amino acid transport/ATP transduction systems, periplasmic component domain	2.45
PA1946	<i>rbcB</i>	binding protein component precursor of ABC ribose transporter	2.78
PA1947	<i>rbcB</i>	thiosulfate transport protein RbcA	4.75
PA1948	<i>rbcC</i>	membrane protein component of ABC ribose transporter	3.29
PA2329	<i>oprC</i>	probable ATP-binding component of ABC transporter	2.79
PA2338	<i>oprC</i>	probable binding protein component of ABC maltoheptaose/tryptamine transporter	4.35
PA2339	<i>oprC</i>	probable binding-protein-dependent maltoheptaose/tryptamine transport protein	3.89
PA2340	<i>oprC</i>	probable binding-protein-dependent maltoheptaose/tryptamine transport protein	4.42
PA2341	<i>oprC</i>	probable ATP-binding component of ABC maltoheptaose/tryptamine transporter	3.96
PA2711	<i>oprC</i>	probable periplasmic spermidine/putrescine-binding protein	2.14
PA3038	<i>oprC</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/orphanine/histidine) ABC transporter periplasmic binding protein	2.44

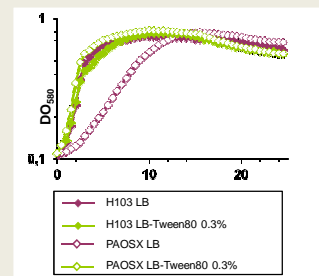
The absence of SigX leads to increased expression of many genes that are involved in amino acids and carbon metabolisms.

Gene	Gene name	Product name	PAOSX/mRNA	Protein	Chr/Pl	Hfq
Amino acid metabolism						
PA0169	<i>argD</i>	acetylornithine 5-oxalylate dehydratase	2.31	X	X	
PA0199	<i>argA</i>	acetylornithine dehydratase	2.42	X	X	
PA0198	<i>argA</i>	acetylornithine dehydratase	2.25	X	X	
PA1138	<i>arg</i>	acetylornithine/ornithine dehydratase	2.09	X	X	
PA1984	<i>argA</i>	NAD-dependent aldehyde dehydrogenase C-act	2.44	X	X	
PA2247	<i>hcr1</i>	iron-iron-binding periplasmic protein H1A	4.39	2.7	X	X
PA2248	<i>hcr1</i>	iron-iron-binding periplasmic protein H1A	5.81	X	X	X
PA2249	<i>hcr1</i>	iron-iron-binding periplasmic protein H1A	6.04	2.2	X	X
PA2250	<i>hcr1</i>	iron-iron-binding periplasmic protein H1A	6.40	2.9	X	X
PA2251	<i>hcr1</i>	iron-iron-binding periplasmic protein H1A	6.72	X	X	X
PA2308	<i>amiE</i>	aromatic amino acid permease	29.66	X	X	
PA2370	<i>amiE</i>	aromatic amino acid permease	13.12	X	X	
PA2371	<i>amiE</i>	aromatic amino acid permease	2.49	X	X	
PA2391	<i>amiE</i>	aromatic amino acid permease	3.02	X	X	
PA2392	<i>amiE</i>	aromatic amino acid permease	2.39	X	X	
PA2393	<i>amiE</i>	aromatic amino acid permease	2.47	18.8	X	X
PA2394	<i>amiE</i>	aromatic amino acid permease	32.74	15	X	X
PA2395	<i>amiE</i>	aromatic amino acid permease	19.45	6.9	X	X
Carbon metabolism						
PA0153	<i>argD</i>	acetylornithine 5-oxalylate dehydratase	4.03	X	X	
PA0154	<i>argD</i>	acetylornithine 5-oxalylate dehydratase	3.33	11.6	X	
PA0211	<i>malM</i>	maltoheptaose transporter MalM	14.3	11.6	X	
PA0212	<i>malM</i>	maltoheptaose transporter MalM	3.21	14.4	X	
PA0213	<i>malM</i>	maltoheptaose transporter MalM	2.88	X	X	
PA0217	<i>malM</i>	maltoheptaose transporter MalM	20.9	17.5	X	
PA0228	<i>aprA</i>	ATP-binding cassette (ABC) transporter complex, substrate-binding subunit	13.88	16.1	X	
PA0807	<i>aprA</i>	ATP-binding cassette (ABC) transporter complex, substrate-binding subunit	2.88	X	X	
PA1092	<i>oprC</i>	probable binding protein component of ABC transporter	4.19	X	X	
PA1093	<i>oprC</i>	probable binding protein component of ABC transporter	4.04	X	X	
PA1094	<i>oprC</i>	probable binding protein component of ABC transporter	4.48	X	X	
PA1095	<i>oprC</i>	probable binding protein component of ABC transporter	7.32	X	X	
PA1096	<i>oprC</i>	probable binding protein component of ABC transporter	3.18	X	X	
PA1097	<i>oprC</i>	probable binding protein component of ABC transporter	4.47	X	X	
PA1098	<i>oprC</i>	probable binding protein component of ABC transporter	4.47	X	X	
PA1099	<i>oprC</i>	probable binding protein component of ABC transporter	2.40	X	X	
PA1100	<i>oprC</i>	probable binding protein component of ABC transporter	4.34	X	X	
PA1102	<i>oprC</i>	probable binding protein component of ABC transporter	6.52	X	X	
PA1103	<i>oprC</i>	probable binding protein component of ABC transporter	7.46	X	X	
PA1104	<i>oprC</i>	probable binding protein component of ABC transporter	2.44	X	X	
PA1105	<i>oprC</i>	probable binding protein component of ABC transporter	2.97	5.4	X	
PA1207	<i>oprC</i>	probable binding protein component of ABC transporter	159.95	25.5	X	
PA2208	<i>oprC</i>	probable binding protein component of ABC transporter	100.10	8.5	X	
PA2209	<i>oprC</i>	probable binding protein component of ABC transporter	2.39	8.5	X	
PA2210	<i>oprC</i>	probable binding protein component of ABC transporter	103.16	10.4	X	
PA2211	<i>oprC</i>	probable binding protein component of ABC transporter	102.10	11	X	
PA2212	<i>oprC</i>	probable binding protein component of ABC transporter	159.94	2.4	X	
PA2515	<i>crz</i>	1,3-dihydroxycyclohexane-1,4-dione carboxylate dehydratase	4.42	X	X	
PA2516	<i>crz</i>	1,3-dihydroxycyclohexane-1,4-dione carboxylate dehydratase	3.11	X	X	
PA2517	<i>crz</i>	1,3-dihydroxycyclohexane-1,4-dione carboxylate dehydratase	3.41	X	X	
PA2518	<i>crz</i>	1,3-dihydroxycyclohexane-1,4-dione carboxylate dehydratase	4.92	X	X	
PA2519	<i>crz</i>	1,3-dihydroxycyclohexane-1,4-dione carboxylate dehydratase	3.14	X	X	
PA3106	<i>amiE</i>	aromatic amino acid permease	3.43	2.6	X	
PA3107	<i>amiE</i>	aromatic amino acid permease	4.67	X	X	
PA3108	<i>amiE</i>	aromatic amino acid permease	3.22	X	X	
PA3709	<i>hcr1</i>	iron-iron-binding periplasmic protein H1A	2.32	X	X	
PA4209	<i>hcr1</i>	iron-iron-binding periplasmic protein H1A	13.17	X	X	
PA4496	<i>oprC</i>	probable binding protein component of ABC transporter	2.28	X	X	
PA4497	<i>oprC</i>	probable binding protein component of ABC transporter	2.28	X	X	
PA4498	<i>oprC</i>	probable binding protein component of ABC transporter	2.53	X	X	
PA4499	<i>oprC</i>	probable binding protein component of ABC transporter	2.53	X	X	
PA4499	<i>oprC</i>	probable binding protein component of ABC transporter	3.08	X	X	
PA4500	<i>oprD</i>	Dicaine-glutamate dipeptide porin OprD	2.18	X	X	
PA4502	<i>oprD</i>	Dicaine-glutamate dipeptide porin OprD	-2.17	X	X	
PA4503	<i>oprD</i>	Dicaine-glutamate dipeptide porin OprD	2.54	X	X	
PA4504	<i>oprD</i>	Dicaine-glutamate dipeptide porin OprD	2.54	X	X	

As indicated in this table, amino acids 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 *hfr* mutant, were also upregulated in PAOSX mutant strain.

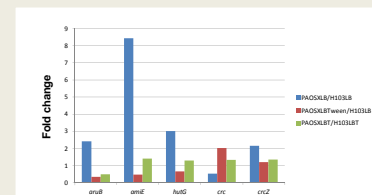


The growth of the *sigX* mutant in LB broth is improved by a subinhibitory concentration of Tween 80 detergent.



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*, *crz*) 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*, *crz* and *amiE* was lowered when PAOSX was grown in LBT compared to LB. Inversely, *crz*, 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



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