

# 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).

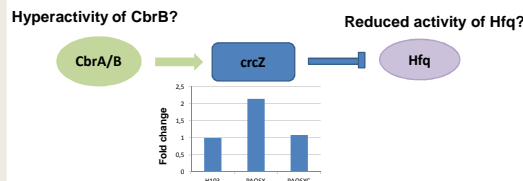
## The absence of SigX leads to increased expression of many genes that are involved in amino acids and carbon metabolisms.

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

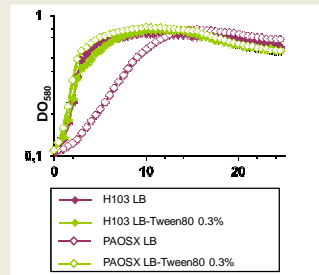
Locus	Gene	Product name	FC
<b>Transport of small molecules</b>			
PA0291	<i>oprB</i>	Amphoterically-induced outer membrane porin OprB precursor	-2.63
PA0292	<i>oprD</i>	probable periplasmic polysaccharide binding protein	-3.33
PA0958	<i>oprD</i>	basic amino acid, basic peptide and imipenem outer membrane porin OprD	-5.55
PA1178	<i>oprH</i>	[PspQ] and low Mg <sup>2+</sup> inducible outer membrane protein H1 precursor	-2.06
PA1183	<i>dcfA</i>	c3-dicarboxylate transport protein	-3.22
PA1863	<i>modA</i>	malbabin-binding periplasmic protein precursor ModA	-3.45
PA2113	<i>oprB</i>	bryostamine porin OprB	-5.00
PA3186	<i>oprB</i>	thiostone/carbohydrate outer membrane porin OprB precursor	-2.70
PA3187	<i>oprC</i>	probable ATP-binding component of ABC transporter	-4.09
PA3188	<i>oprC</i>	probable periplasmic ABC sugar transporter	-7.14
PA3189	<i>oprC</i>	probable periplasmic ABC sugar transporter	-2.90
PA3190	<i>oprC</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>	Panute copper transport 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 c3-dicarboxylate-binding protein	-2.41
PA4617	<i>oprH</i>	similar to E. coli malbabin outer membrane receptor H1A	-2.37
PA4687	<i>hcrI</i>	iron-sulfur-binding periplasmic protein H1A	-2.27
PA5217	<i>oprI</i>	probable binding protein component of ABC iron transporter	-2.38

PA0215	<i>malM</i>	malonate transporter MalM	15.91
PA0216	<i>malM</i>	malonate transporter MalM	6.93
PA0280	<i>cysE</i>	huller transport protein CysA	2.75
PA0603	<i>apcA</i>	ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-containing	8.66
PA0604	<i>apcB</i>	ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-containing	14.81
PA0755	<i>apcH</i>	cis-acconitate porin OprH1	1.58
PA0866	<i>amiE</i>	aromatic amino acid transport protein AmiE2	3.95
PA1104	<i>hcrC</i>	branched-chain amino acid transport protein H1C1	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/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>	ribose transport protein RbcA	4.75
PA1948	<i>rbcC</i>	membrane protein component of ABC ribose transporter	3.29
PA2329	<i>apcC</i>	probable ATP-binding component of ABC transporter	2.79
PA2338	<i>apcC</i>	probable binding protein component of ABC malhose/mannitol transporter	4.35
PA2339	<i>apcC</i>	probable binding-protein-dependent malhose/mannitol transport protein	3.89
PA2340	<i>apcC</i>	probable binding-protein-dependent malhose/mannitol transport protein	4.42
PA2341	<i>apcC</i>	probable ATP-binding component of ABC malhose/mannitol transporter	3.96
PA2711	<i>apcE</i>	probable periplasmic spermidine/putrescine-binding protein	2.14
PA3038	<i>apcC</i>	probable porin	14.88
PA3891	<i>apcC</i>	OprC ABC transporter, ATP-binding protein	2.10
PA4488	<i>apcC</i>	probable binding protein component of ABC transporter	5.29
PA4497	<i>apcC</i>	probable binding protein component of ABC transporter	5.69
PA4501	<i>apcD</i>	Dicaine-glutamate dipeptide porin OprD	3.28
PA4910	<i>apcC</i>	branched chain amino acid ABC transporter ATP binding protein	2.61
PA4913	<i>apcC</i>	probable binding protein component of ABC transporter	5.08
PA5094	<i>apcC</i>	probable ATP-binding component of ABC transporter	6.66
PA5096	<i>apcC</i>	probable binding protein component of ABC transporter	3.78
PA5153	<i>apcC</i>	amino acid (lysine/arginine/ornithine/histidine/octopine) 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.

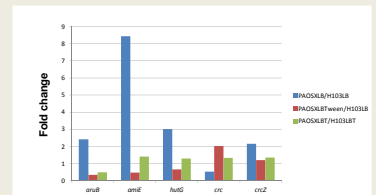


## The growth of the *sigX* mutant in LB broth is improved by a subinhibitory concentration of Tween 80 detergent.



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, *arc*, 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

