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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 nutriments 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 functionnal 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
ansport o			
A0291	oprE	Anaerobically-induced outer membrane porin OprE precursor	-2,63
PA0295	/	probable periplasmic polyamine binding protein	-3,33
PA0958	oprD	basic amino acid , basic peptide and imipenem outer membrane porin OprD	-5,55
PA1178	oprH	PhoP/Q and low Mg2+ inducible outer membrane protein H1 precursor	-2,00
PA1183	dctA	C4-dicarboxylate transport protein	-3,22
PA1863	modA	molybdate-binding periplasmic protein precursor ModA	-3,45
PA2113	opdO	pyroglutamate porin OpdO	-5,00
PA3186	oprB	Glucose/carbohydrate outer membrane porin OprB precursor	-2,70
PA3187	1	probable ATP-binding component of ABC transporter	-9,09
PA3188	/	probable permease of ABC sugar transporter	-7,14
PA3189	/	probable permease of ABC sugar transporter	-2,90
PA3190	1	probable binding protein component of ABC sugar transporter	-2,00
PA3641	1	probable amino acid permease	-2,60
PA3790	oprC	Putative copper transport outer membrane porin OprC precursor	-4,35
PA3865	1	probable amino acid binding protein	-2,43
PA4067	oprG	Outer membrane protein OprG precursor	-1,51
PA4616	1	probable c4-dicarboxylate-binding protein	-2,44
PA4675	cht/4	similar to E. coli aerobactin outer membrane receptor lutA	-2.27
PA4687	hitA	ferric iron-binding periplasmic protein HitA	-2.27
PA5217	/	probable binding protein component of ABC iron transporter	-2.38
		process composition of case, som utilisposes	-2,00
PA0215		malonate transporter MadL	15,91
PA0216	madM*	malonate transporter MadM	6,93
PA0280	cysA	sulfate transport protein CysA	2,75
PA0603	agtA	ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-containing	8,66
PA0604	agtB	ATP-binding cassette (ABC) transporter complex, substrate-binding subunit-containing	14,81
PA0755	opdH	cis-aconitate porin OpdH	1.58
PA0866		aromatic amino acid transport protein AroP2	3.95
PA1074		branched-chain amino acid transport protein BraC	2 39
PA1339	aatP	amino acid ABC transporter ATP binding protein	2.61
		ABC-type amino acid transport/signal transduction systems,	
PA1342	aatJ	periplasmic component/domain	2,45
PA1946		binding protein component precursor of ABC ribose transporter	2,76
PA1947	rbsA	ribose transport protein RbsA	4,75
PA1948	rbsC	membrane protein component of ABC ribose transporter	3,29
PA2329	/	probable ATP-binding component of ABC transporter	2,79
PA2338	1	probable binding protein component of ABC maltose/mannitol transporter	4,35
PA2339	1	probable binding-protein-dependent maltose/mannitol transport protein	3,89
PA2340	1	probable binding-protein-dependent maltose/mannito1 transport protein	4,42
PA2341	1	probable ATP-binding component of ABC maltose/mannitol transporter	3,96
PA2711	1	probable periplasmic spermidine/putrescine-binding protein	2,14
PA3038	/	probable porin	14,88
PA3891	opuC	OpuC ABC transporter, ATP-binding protein,	2,10
PA4496	1	probable binding protein component of ABC transporter	5,29
PA4497	/	probable binding protein component of ABC transporter	5,69
PA4501	opdD	Glycine-glutamate dipeptide porin OpdP	3,28
PA4910	/	branched chain amino acid ABC transporter ATP binding protein	2,61
PA4913	/	probable binding protein component of ABC transporter	5.08
PA5094	1	probable ATP-binding component of ABC transporter	6.66
PA5096	/	probable binding protein component of ABC transporter	3,78
		amino acid (lysine/arginine/ornithine/histidine/octopine) ABC	
PA5153	/	transporter periplasmic binding protein	2,44

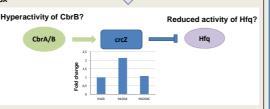
lany porins encoding genes were downregulated several amino acids transporters were upregulated in grown in LB medium compared to P. aeruginosa H103.

> Expression of CbrB direct target crcZ in H103, PAOSX and sigX complemented PAOSX mutant strain PAOSXC grown in LB broth (gRT-PCR).

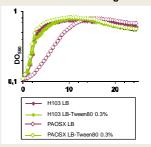
The absence of SigX leads to increased expression of many genes that are involved in amino acids and carbon metabolisms.

ocus	Gene name	Product name		PAOSNH103		Hfq
			mRNA	Pretein	ChrA/B	
nino acid me		,				
PA0898	aruD	raccinylghtamate 5-semialdehyde dehydrogenase	2.31		X	
PA0899	araß	saccinylarginine dihydrolase	2.42		X	
PA0901	araf	succinylghatamate dessectinylase	2.22		X	
PA1338	881	garrens-gluturnyltranspeptidase precursor	2.69			X
PA1984	exaC	NAD+ dependent alldehyde dehydrogenase ExaC	2.44		X	
PA2247	bkd41	2-cooisovalerate dehydrogenase (alpha subunit)	4.39	2,7	X	X
PA2248	bluit42	2-exxisovalerate dehydrogenase (beta subunit)	5.61		X	X
PA2249	Hulli	tranched-chain alpha-keto acid dehydrogenase	6.04	2,2	X	X
PA2250	lpdV	ipoamide dehydrogenase-Val	6.40	2,9	X	X
PA2862	Lip4	factorizing lipase precursor	4.72		X	
PA3569	nones.4	transhed chain aminoucid assimilation	29.96			X
PA3570	лини В	tranched chain aminoacid assimilation	33.12			X
PA4588	gali4	glutamate dehydrogenase	2.40			X
PA5091	hwG	N-formylglutamate amidohydrolase	3.02		X	
PA5092	hud	imidanolone-S-propionate hydrolase Hutl	2.90		X	
PA5096		probable ATB binding cassette	3,8	18,8		
PA5098	kwtH	tistidine armonia-lyase	32.74	15	X	
PA5100	kwtU	stocatase	14.45	5,9	X	
arbon metab		,				
PA0153	pcaH	protocatechuate 3,4-dioxygenase, beta subunit	4.03			X
PA0154	pcaG	protocatechuate 3,4-dioxygenuse, alpha subunit	3.31			X
PA0211	mdcD	scetyl-CoA carboxylase beta subunit	14,3	31,6		
PA0212	mdcE	tralonate decarboxylase garmu subanit	5,21	44,4		
PA0226		catechol degradation	9.83			X
PA0227		rulonate decarboxylase garmu subunit	20.6	17,5		X
PA0228	pcaF	probable CoA transferase, subunit B	13.88	16,1		X
PA0887	ecs4	scetyl-coerayme A synthetase	2.89		X	
PA1892		hypothetical protein	4.19			X
PA1893		typothetical protein	4.04			X
PA1894		rypothetical protein	9.45			X
PA1895		typothetical protein	7.32			X
PA1896 PA1897		typothetical protein	5.18 4.47			X
		typothetical protein				
PA1978 PA1981	erbR	glycerol metabolism activator inpothetical protein	2.40			X
PA1981			4.51			- X
PA1982 PA1983	exad exaB	quinoprotein ethanol dehydrogenase cytochrome e550	7.46			X
PA1984	exac	NAD+ dependent aldehyde dehydrogenase ExaC	2.44			X
PA2003	hdh4	4-bydroxybatyrate dehydrogenase Exac.	5.87	5.4	-	X
PA2507	caté	r-nyeronymayrase denyerogenise catechol I ,2-dioxygenise	159.95	25.5		- X
PA2508	cate	maconolacione delta-isomerase	139.93	23,3		X
PA2509	cetti	nuconate cycloisomerase I	7.30	8.5	-	Ŷ
PA2512	enti	onthranilate dioxygenase large subunit	163.16	10.4		Ŷ
PA2513	ensil	enthranilate dioxygenase small subunit	502.10	13		X
PA2514	antC	enthranilate dioxygenuse reductase	140.44	5.4		Ŷ
				-7		
PA2515	xyll.	cis-1,2-dihydroxycyclohexa-3,4-diene carboxylate dehydrogenase	4.62			Х
PA2516	xylZ	oluste 1,2-dioxygenuse electron transfer component	3.11			X
PA2517	xylY	oluste 1,2-dioxygerase beta subunit	5.47			X
PA2518	xylX	oluate 1,2-dioxygenase alpha subunit	4.92			X
PA2519	xylS	ranscriptional regulator	3.14			X
PA3366	атаЕ	aliphatic amidase	8.43	2,6		X
PA3367	атій	hypothetical protein	4.67			X
PA3368	awS	probable acetyltrans ferase	3.22			X
PA3709		probable major facilitator superfamily (MFS) transporter	2.32			X
PA4209	pksM	phenazine specific methyltransferase	10.17			X
PA4496		putative binding protein component of ABC transporter	5.29			X
PA4733	acsB	scetyl-coensyme B synthetase	2.69		X	
PA4910		putative ABC transporter proteins	2.61			X
PA4913		probable binding protein component of ABC transporter	5.08			X
PA5380	ghdit	franscriptional regulator for glycine betaine catabolism	2.88			X
PA5332	ere	catabolite repression control protein	-2,17			
PA5543 PA5545		spothetical protein	2.54			X
		inpothetical protein	2.34			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 upregulated in Moreover several genes that were shown to upregulated in a hfq mutant, were also upregulated in PAOSX mutant strain.

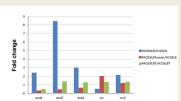


The growth of the sigX mutant in LB broth is improved by a subinhibitory concentration of Tween 80 detergent.



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, crcZ) or Hfg (amiE) were assayed by qRT-PCR in PAOSX grown in LB or LBT compared to H103 grown in LB or LBT. Expression of aruls, hurg, crcZ and arniE was lowered when PAOSX was grown in LB or LBT compared to LB. Inversely, crc, whose expression was reduced in PAOSX grown in LB, was increased when grown in LB.

Conclusions

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

The absence of SigX leads to strong alterations at the nutritionnal 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

