

Supplementary Materials

The onset of tacrolimus biosynthesis in *Streptomyces tsukubaensis* is dependent on the intracellular redox status

Sílvia D.S. Pires^{1,2,†,‡}, Rute Oliveira^{1,2,3,†}, Pedro Moradas-Ferreira^{1,2,4} and Marta V. Mendes^{1,2,4,*}

¹ i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal.

² IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal.

³ Programa doutoral em Biologia Molecular e Celular (MCBiology), ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

⁴ ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

* Correspondence: mvm@ibmc.up.pt;

† These authors contributed equally to this work.

‡ Present address: Jill Roberts Institute for IBD Research, Weill Cornell Medicine, New York, NY 10021, United States of America.

Table S1. Genes whose transcription is significantly affected ($pFDR \leq 0.05$; adjusted p value to control the false discovery rate between 80 and 89h in maltose-supplemented cultures but not in glucose cultures. Data retrieved from [1].

Gene	SCO Orthologue	Predicted product	$M_{c}^{Mal,89-80}$ ($=M_{g}^{Mal,89}-M_{g}^{Mal,80}$)
Oxidative stress response			
STSU_10876	SCO0379, katA1	Catalase	3,31
STSU_11535	SCO7590, katA2	Catalase	2,27
Regulatory proteins			
STSU_10741	SCO5240, wblC	WhiB-family transcriptional regulator	-2,30
STSU_14433	SCO3320, rex	Redox-sensing transcriptional repressor Rex	-2,86
STSU_16897	SCO5863, cutS	Integral membrane sensor signal transduction histidine kinase	2,29
Energy metabolism			
STSU_02385	SCO0923	Succinate dehydrogenase flavoprotein subunit (EC 1.3.5.1)	-2,66
STSU_02390	SCO0924	Cytochrome b subunit	-4,32
STSU_10194	SCO5366, atpI	ATP synthase protein I	-3,00
STSU_13933	SCO4575, nuoN	NADH-quinone oxidoreductase subunit N (EC 1.6.5.11) (NADH dehydrogenase I subunit N) (NDH-1 subunit N)	-3,08
STSU_13938	SCO4574, nuoM	NADH:ubiquinone oxidoreductase subunit M (EC 1.6.5.11)	-2,03
STSU_13948	SCO4572, nuoK2	NADH-quinone oxidoreductase subunit K (EC 1.6.5.11) (NADH dehydrogenase I subunit K) (NDH-1 subunit K)	-2,15
STSU_13953	SCO4571, nuoJ	NADH:ubiquinone oxidoreductase subunit J (EC 1.6.5.11)	-2,19
STSU_13958	SCO4570, nuoI1	NADH-quinone oxidoreductase subunit I (EC 1.6.5.11) (NADH dehydrogenase I subunit I) (NDH-1 subunit I)	-2,41
STSU_13963	SCO4569, nuoH	NADH-quinone oxidoreductase subunit H (EC 1.6.5.11) (NADH dehydrogenase I subunit H) (NDH-1 subunit H)	-3,59
STSU_13968	SCO4568, nuoG	NADH dehydrogenase subunit G (EC 1.6.5.11) (Fragment)	-2,22
STSU_13973	SCO4568, nuoG	NADH dehydrogenase subunit G (EC 1.6.5.11) (Fragment)	-2,97
STSU_13983	SCO4566, nuoE	NADH dehydrogenase subunit E (EC 1.6.5.11)	-2,56
STSU_13988	SCO4565, nuoD2	NADH-quinone oxidoreductase subunit D (EC 1.6.5.11) (NADH dehydrogenase I subunit D) (NDH-1 subunit D)	-2,97

STSU_13993	SCO4564, nuoC	NADH-quinone oxidoreductase subunit C (EC 1.6.5.11) (NADH dehydrogenase I subunit C) (NDH-1 subunit C)	-3,75
STSU_13998	SCO4563, nuoB1	NADH-quinone oxidoreductase subunit B (EC 1.6.5.11) (NADH dehydrogenase I subunit B) (NDH-1 subunit B)	-4,00
STSU_14003	SCO4562, nuoA	NADH-quinone oxidoreductase subunit A (EC 1.6.5.11) (NADH dehydrogenase I subunit A) (NDH-1 subunit A) (NUO1)	-4,58
STSU_17808	SCO3945, cydA	Cytochrome oxidase subunit I	-3,25
STSU_17813	SCO3946, cydB	Cytochrome bd-I oxidase subunit II	-3,00
STSU_17818	SCO3947, cydCD	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydD	-3,20
STSU_21988	SCO3092	FAD-dependent pyridine nucleotide-disulfide oxidoreductase	-2,87
BCAA metabolism			
STSU_03489	SCO4913	Aldehyde dehydrogenase	2,03
STSU_09964	SCO5415, icmA	isobutyryl-CoA mutase A	2,47
STSU_23681	SCO2778, hmgL	hydroxymethylglutaryl-CoA lyase	3,08
STSU_23686	SCO2777, accC	Acetyl/propionyl CoA carboxylase subunit alpha	3,16
STSU_23691	SCO2776, accD1	Acetyl/propionyl CoA carboxylase subunit beta	2,71
STSU_23866	SCO2726, msdA	Methylmalonate-semialdehyde dehydrogenase	2,80
Amino acid metabolism			
STSU_08033	SCO5777, gluA	Phosphate ABC transporter ATP-binding protein	-2,60
STSU_08038	SCO5776, gluB	ABC transporter substrate-binding protein	-3,30
STSU_08043	SCO5775, gluC	Glutamate ABC transporter permease	-3,11
STSU_08048	SCO5774, gluD	Glutamate ABC transporter permease	-2,87
STSU_27064	SCO2026, gltB	Glutamate synthase (Ferredoxin)	2,24
STSU_27069	SCO2025, gltD	Glutamate synthase subunit beta	2,22
Nucleotide metabolism			
STSU_10791	SCO5226, nrdA	Ribonucleoside-diphosphate reductase (EC 1.17.4.1)	-2,47
STSU_10796	SCO5225, nrdB	Ribonucleoside-diphosphate reductase subunit beta (EC 1.17.4.1)	-3,03
STSU_12545	SCO4889	Cytidine deaminase (EC 3.5.4.5)	2,14
Biosynthesis of cofactors			
STSU_14188	SCO4475	Cytochrome C assembly protein	-2,10
STSU_14193	SCO4474	Cytochrome c biogenesis membrane protein	-3,98
STSU_14198	SCO4473	Cytochrome c-type biogenesis protein	-3,85

STSU_14203	SCO4472	Redoxin domain-containing protein	-3,94
STSU_14428	SCO3319, hemA	Glutamyl-tRNA reductase (GluTR) (EC 1.2.1.70)	-3,00
STSU_25889	SCO2256, panB	3-methyl-2-oxobutanoate hydroxymethyltransferase	3,19
STSU_27526	SCO1934, ctaB	Protoheme IX farnesyltransferase (Heme O synthase)	-2,03
STSU_27536	SCO1930	Cytochrome c oxidase subunit XV assembly protein	-3,62
Transport proteins			
STSU_05318	SCO6257	ABC transporter sugar-binding lipoprotein	2,09
STSU_11330	SCO5116, bldKE	Peptide ABC transporter	2,10
STSU_11335	SCO5115, bldKD	BldKD peptide ABC transporter	2,09
STSU_12550	SCO4888	Sugar ABC transporter integral membrane protein	2,75
STSU_27229	SCO2008	ABC transporter substrate-binding protein	2,40
Secondary metabolism			
STSU_31965	-	Thioesterase (FbkQ)	2,46
Hypothetical / uncharacterized proteins / not classified			
STSU_06598	-	Uncharacterized protein	2,13
STSU_09839	-	4-hydroxyphenylacetate 3-hydroxylase	3,46
STSU_09849	-	NmrA family protein	4,09
STSU_09979	SCO5409	Uncharacterized protein	-3,73
STSU_10871	SCO5207	Uncharacterized protein	2,20
STSU_11155	SCO5157	Mg2 transporter protein CorA family protein	2,10
STSU_12535	-	Uncharacterized protein	2,24
STSU_12540	SCO4890	Thymidine phosphorylase (EC 2.4.2.4)	2,52
STSU_19742	-	Putative Acetyltransferase	-2,09
STSU_21077	SCO4440	Uncharacterized protein	2,19
STSU_21641	-	Uncharacterized protein	4,01
STSU_21646	-	Uncharacterized protein	3,68
STSU_21938	SCO3101	putative lipoprotein	-2,86
STSU_23846	SCO6977	Uncharacterized protein	3,06
STSU_23851	SCO6976	conserved hypothetical protein, lolB protein	3,02
STSU_24016	SCO2682	Putative membrane protein	2,26
STSU_24671	SCO2556	Putative metallo-beta-lactamase	-2,32
STSU_28141	SCO1795	Hypothetical protein	2,24
STSU_29711	SCO1455	Hydrolase	2,49

STSU_29716	SCO1454	Putative amino oxidase	2,52
STSU_30440	SCO1296	Uncharacterized protein	2,46
STSU_33045	SCO6033	Uncharacterized protein	2,10

Table S2. Primers used in this study.

Primer	Sequence (5' → 3')	Use
RED_ahpC_F	TTGATCAATTTACCCGGCGATCGCGGGTATGACAGGGTGATTCCG	Redirect gene KO
	GGGATCCGTCGACC	
RED_ahpC_R	CGGTATGGCGGACTTCAGCGCGTCGAGCGACACGGGTCATGTAG	Redirect gene KO
	GCTGGAGCTGCTTC	
T_ahpC_F	GTGGATTTGTCCAACC	Redirect gene KO
T_ahpC_R	GGACTTGTGGTTGATG	Redirect gene KO
Tsu_hrdB_F	GCGGCACTGACCATCAGCGT	RT-qPCR analyses
Tsu_hrdB_R	GATTCCGCCAACCCAGTGGA	RT-qPCR analyses
Tsu_rpsP_F	GCGCCGACGGAAAGCCAGTA	RT-qPCR analyses
Tsu_rpsP_R	CCATCGAGGAGATCGGCCTG	RT-qPCR analyses
Tsu_fkbR_F	CTTGGGAACGTCCTGCCTTC	RT-qPCR analyses
Tsu_fkbR_R	GTCTTGACGTTGAAGTGCCG	RT-qPCR analyses
Tsu_fkbN_F	TGAACTGATCGCTCGTGACG	RT-qPCR analyses
Tsu_fkbN_R	CTCCAGCAGTGCCGTCTTGC	RT-qPCR analyses
RT_fkbL_F	TCAGCGCGATATCAAGCAGA	RT-qPCR analyses
RT_fkbL_R	ATGAACTCGATGACGCCGGG	RT-qPCR analyses
RT_fkbO_F	CGAACGCCTCCTCCTCGAAGGT	RT-qPCR analyses
RT_fkbO_R	TCGTGCCGTCGGACCTCGAA	RT-qPCR analyses
RT_fkbB_F	TCGGCCTGTGCACGAATTGCTG	RT-qPCR analyses
RT_fkbB_R	TCCGAGCAGCAGCGCGATAC	RT-qPCR analyses
Tsu_katA1_F	CTCCGGTCGCCGACAACCAGAA	RT-qPCR analyses
Tsu_katA1_R	CGGTTGAAGTGCGCGAGCTT	RT-qPCR analyses
Tsu_katA2_F	GTGTCGGCGGCGTTGACTGA	RT-qPCR analyses
Tsu_katA2_R	GCGTTGTTGGTGGTGTAGGGG	RT-qPCR analyses
Tsu_sodA_F	GCCATCTACACGCTGCCCCGA	RT-qPCR analyses
Tsu_sodA_R	TAGGCGGCGTGGTGCTTGTC	RT-qPCR analyses
Tsu_ahpC_F	TCAGCACCATGGAGTGACACTG	RT-qPCR analyses
Tsu_ahpC_R	AACTCCTTGCCGCTCTCGAG	RT-qPCR analyses

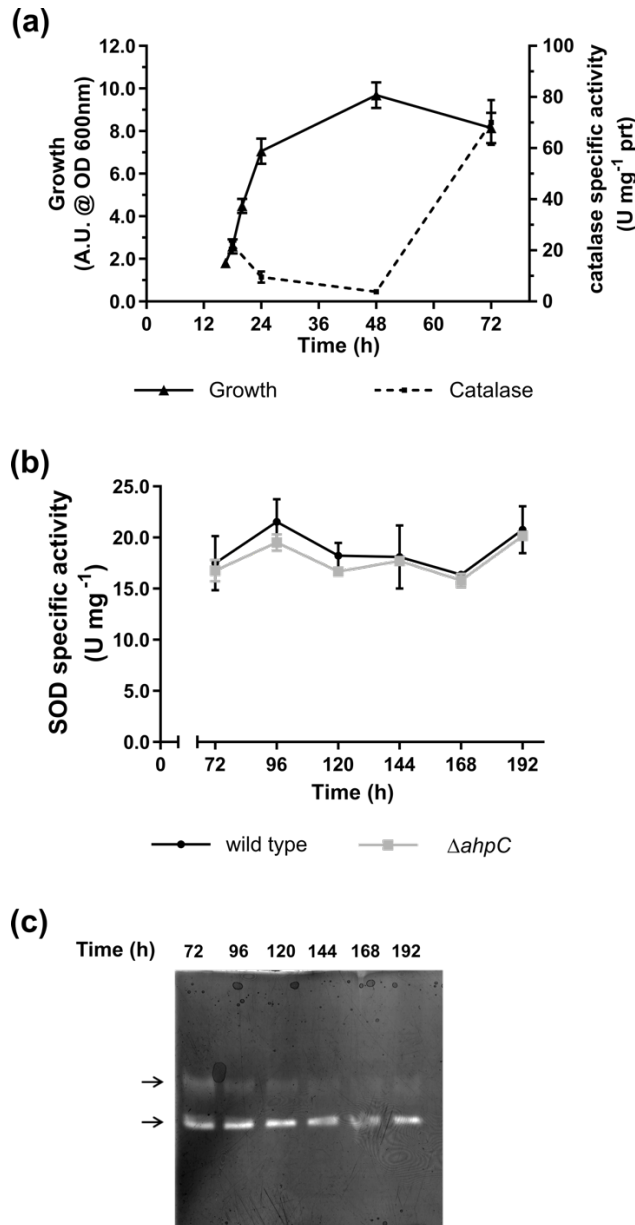


Figure S1. Characterization of *S. tsukubaensis* cultures. **(a)** Growth curve (solid line) and catalase specific activity (dashed line) of *S. tsukubaensis* wild type grown in YEME medium. Note that growth in YEME medium is faster than in MGM-2.5 medium (see Figure 1a) and that stationary phase occurs earlier. **(b)** SOD specific activity of *S. tsukubaensis* wild type (black line) and *S. tsukubaensis* $\Delta ahpC$ (grey line) grown in MGM-2.5 medium. Vertical bars indicate standard deviation of the mean values. No significant differences ($p > 0.05$) between the wild type and $\Delta ahpC$ were identified (t-test with Holm-Sidak correction for multiple comparisons). Results are the average of at least three independent experiments. **(c)** Native-PAGE of *S. tsukubaensis* cell extracts (50 μ g total protein per lane) stained for SOD activity. Arrows indicate the two protein bands that display SOD activity.

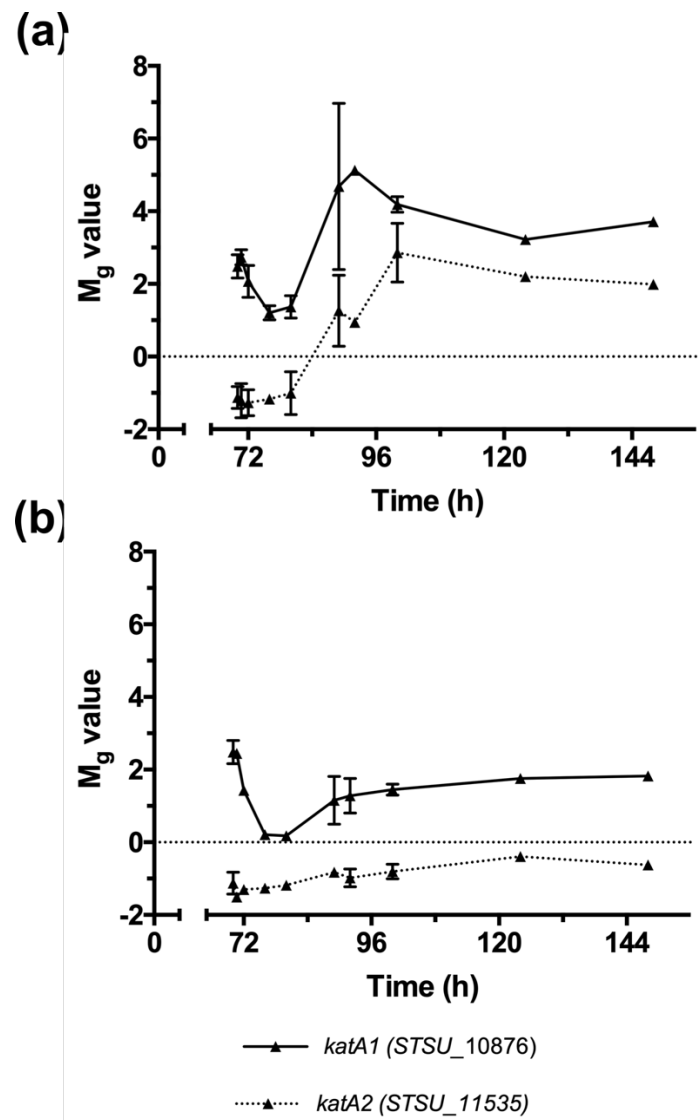


Figure S2. Transcriptional profiles of the catalase encoding genes. M_g values ($\log_2$ transcription) of *katA1* (solid line) and *katA2* (dotted line) genes in *S. tsukubaensis* wild type grown in **(a)** tacrolimus producing conditions (maltose added cultures) and **(b)** tacrolimus non-producing conditions (glucose added cultures). Data was retrieved from the GSE99752 dataset described in detail in [1].

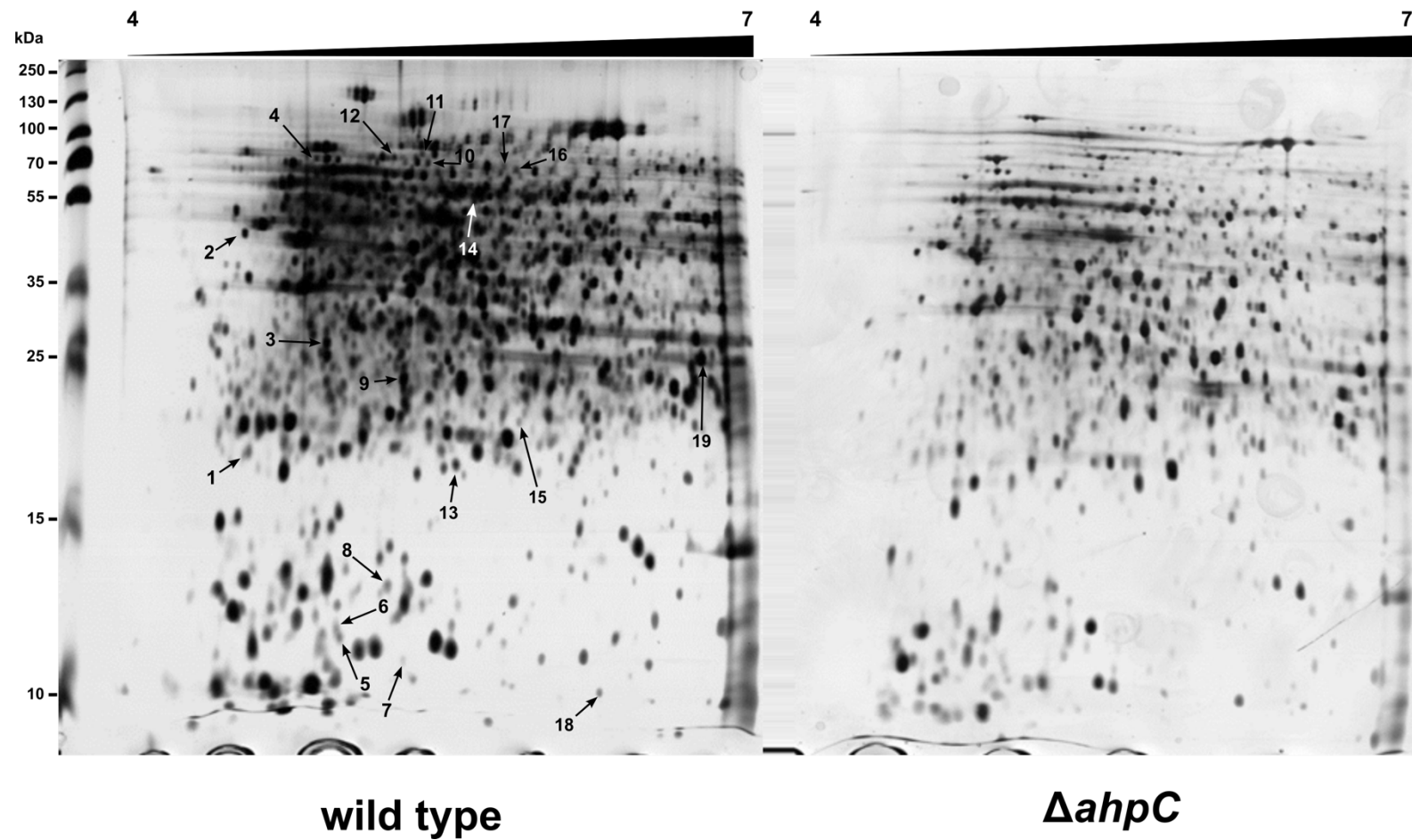


Figure S3. Comparative 2D gel electrophoresis of protein extracts of *S. tsukubaensis* wild type and $\Delta ahpC$ strains at 72h of growth. Protein spots identified by PMF-MS/MS are indicated by an arrow. pH range is indicated on top of the gel. M - molecular weight. Fold variations in Table 1 reflect the 2D-gel analysis of three independent experiments.

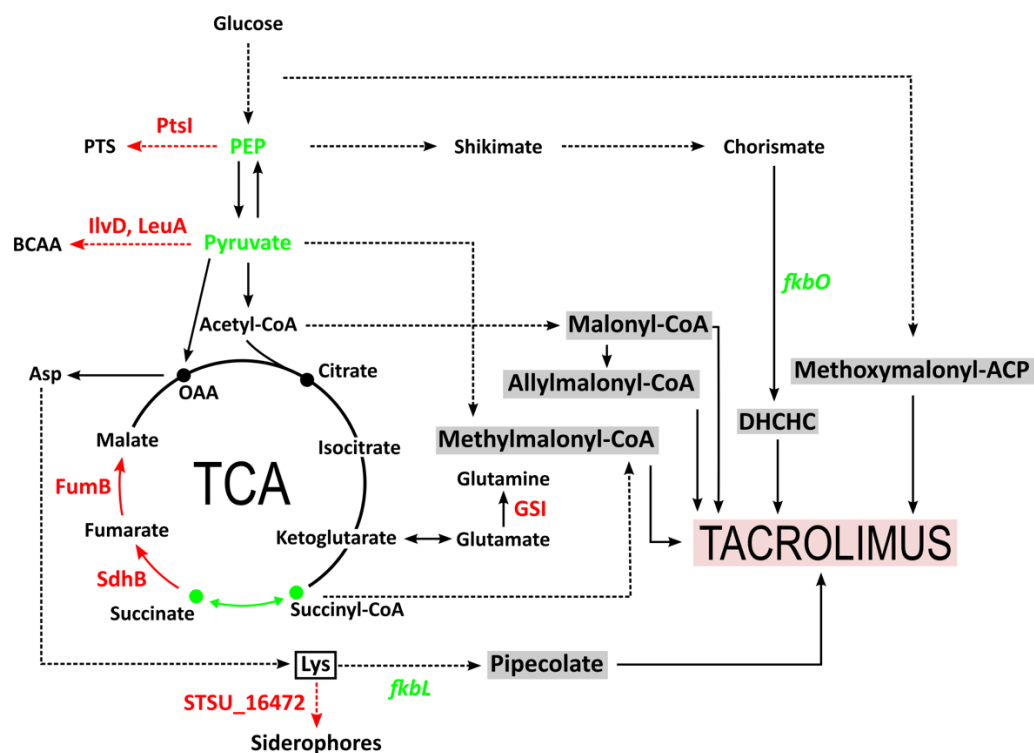


Figure S4. Schematic representation of the pathways affected in *S. tsukubaensis* Δ *ahpC* when compared to the wild type strain. Combined, the proteomic and RT-qPCR results suggest an increased availability of tacrolimus biosynthetic precursors in *S. tsukubaensis* Δ *ahpC* strain. Red - down-regulation; green - up-regulation.

References:

1. Ordonez-Robles, M.; Santos-Beneit, F.; Albillos, S.M.; Liras, P.; Martin, J.F.; Rodriguez-Garcia, A. *Streptomyces tsukubaensis* as a new model for carbon repression: transcriptomic response to tacrolimus repressing carbon sources. *Appl Microbiol Biotechnol* **2017**, *101*, 8181-8195, doi:10.1007/s00253-017-8545-5.