

Table SI 1 List of identified p-peptides and their corresponding p-proteins detected on glucose or tryptone grown *S. solfataricus* P2 cells. Shown are all detected p-peptides from both biological replicates and under both conditions (A.), all unique p-proteins (B.) and all p-proteins identified in glucose grown (C.) or tryptone grown (D.) cells.

Table SI 2. Identified archaeal target p-proteins.

Accession Nr.	Target protein	Predicted function	Organism	Information	Reference
Q973R9	ST0829	FHA-domain containing protein	<i>Sulfolobus tokodaii</i>	Phosphorylated by ST1565	1
-	-	Methyltransferase activating protein	<i>Methanosarcina barkerii</i>	Autophosphorylation with ³² P	2
Q76KA7	TK0925	Phenylalanyl-tRNA synthase subunit β-chain	<i>Thermococcus kodakaraensis</i>	Antiphospho-Tyr antibody	3
Q5JH20	TK1404	Phosphomannomutase	<i>Thermococcus kodakaraensis</i>	Antiphospho-Tyr antibody	3
Q980S1	SSO0207	(*) Hexosephosphate mutase	<i>Sulfolobus solfataricus</i>	Phosphorylated on a Ser-residue	4
D4GYZ1	HVO_1562	β-subunit of 20S proteasome	<i>Haloferax volcanii</i>	Phosphorylated on Ser ¹²⁹	5
B0R4J9	CheA	Taxis sensor His kinase cheA	<i>Halobacterium salinarum</i>	Phosphorylated with [γ- ³² P]ATP & Mg ²⁺	6
B0R4K1	CheY	Response regulator cheY	<i>Halobacterium salinarum</i>	Phosphorylated on Asp-residue	6
D9PYR8	MTH1412	Cell division control protein 6 homolog 1	<i>Methanobacterium thermoautotrophicum</i>	Autophosphorylation with ³² Pon Ser-residue	7
D9PU97	MTH1599	Cell division control protein 6 homolog 2	<i>Methanobacterium thermoautotrophicum</i>	Autophosphorylation with ³² Pon Ser-residue	7
Q980N4	SSO0257	Cell division control protein 6 homolog 1	<i>Sulfolobus solfataricus</i>	Autophosphorylation with ³² Pon Ser-residue	8
Q8ZYK1	PAE0737	Cell division control protein 6	<i>Pyrobaculum aerophilum</i>	Phosphorylated on Ser-residue	7
O58655	PH0961	Translation initiation factor 2 subunit α	<i>Pyrococcus horikoshii</i>	Phosphorylated by hPKR from human	9
P95928	SSO2154	Zn-dependent aminopeptidase	<i>Sulfolobus solfataricus</i>	Phosphorylated on Ser- or Thr-residue	10
-	-	(*) α-subunit of succinyl-CoA synthetase	<i>Sulfolobus solfataricus</i>	³² P incorporation	4a
-	-	Glycogen synthase	<i>Sulfolobus acidocaldarius</i>	³² P incorporation	11
Q97U27	SSO3198	Gluconate dehydratase	<i>Sulfolobus solfataricus</i>	³² P-labelled, no enzyme activity after incubation with phosphatase	12
Q980A0	SSO0417	(*) Phosphoglycerate mutase	<i>Sulfolobus solfataricus</i>	Phosphorylation at Ser ⁵⁹	13
Q9HLV3	Ta0122	2-keto-3deoxy-gluconate kinase	<i>Thermoplasma acidophilum</i>	, No enzyme activity after incubation with phosphatase	14

(*) possible phospho-enzyme intermediate.

Table SI 3. Comparison of genome and p-proteome features of available prokaryotic p-proteome analyses.

Organism	D.o.l. (*)	Genome size [bp]	Gene count	P-proteins [Nr.]	Phosphorylation [%]
<i>E. coli</i> ¹⁵	Bacteria	4,639,675	4497	69	4.5
<i>B. subtilis</i> ¹⁶	Bacteria	4,215,606	4354	78	1.8
<i>L. lactis</i> ¹⁷	Bacteria	2,365,589	2434	63	2.6
<i>H. salinarium</i> ¹⁸	Archaea	2,668,776	2801	79	2.8
<i>S. solfataricus</i> ¹⁹	Archaea	2,992,245	3141	542	17.3

(*)D.o.l = domain of life

Table SI 4. Comparison of archaeal and bacterial p-proteome analyses. The comparisons were performed with BLAST-P analyses (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Only hits with E-values lower than E^{-14} are shown.

Comparison of phospho-proteome studies - The comparison of p-proteins identified from prokaryotic p-proteome studies (*E. coli*, *B. subtilis*, *L. lactis*, *H. salinarium*) revealed 29 significant matches ($e\text{-value} \leq e^{-14}$). According with their phylogenetic distance Bacteria comprise only 3-5 phosphorylated orthologs, whereas the Archaeon *H. salinarium* possesses 15 phosphorylated orthologs. The majority (six) of the orthologs found in *H. salinarium* belongs to the arCOG J (translation) category; the remaining orthologs are distributed in different arCOG categories. Disregarding different experimental set ups and growth conditions isocitrate dehydrogenase (IDH) is phosphorylated in three species (*S. solfataricus*, *H. salinarium* and *E. coli*) highlighting its important function in the regulation of carbon flux into the glyoxylate and TCA cycle (see also TCA cycle).

Organism	Gen	Predicted function	Orthologs	arCOG annotation	arCOG functional code	E-value
<i>H. salinarium</i> strain R1 & $\Delta serB$ ²⁰	OE4169F	Conserved hypothetical protein	SSO0258	Predicted DNA-binding protein	R	2.00E-40
	OE4671R	Conserved hypothetical protein	SSO0572	ATPase (Pit family)	R	3.00E-95
	OE3943R	Conserved hypothetical protein	SSO0408	Ribosomal protein S15P	J	1.00E-22
	OE2629F/rps11	Ribosomal protein S11	SSO0069	Ribosomal protein L13	J	3.00E-19
	OE3388F/rpl3	Ribosomal protein L3	SSO0719	Ribosomal protein L3	J	7.00E-54
	OE3412F/rpl32R (*)	Ribosomal protein L32.eR	SSO0701	Ribosomal protein L32E	J	8.00E-14
	OE4101R/trpS1 (*)	Tryptophanyl-tRNA synthetase	SSO0452	Tryptophanyl-tRNA synthetase	J	2.00E-17
	OE4736R/rps12 (*)	Ribosomal protein S12	SSO0219	Ribosomal protein S12	J	3.00E-46
	OE2866R/sdhB	Succinate dehydrogenase subunit B	SSO2357	Succinate dehydrogenase /fumarate reductase	C	2.00E-32
	OE3634F/icd (*)	Isocitrate dehydrogenase	SSO2182	Isocitrate dehydrogenase	C	1.00E-92
	OE2458R/guaB (*)	IMP dehydrogenase	SSO0613	Aspartate carbamoyltransferase	F	8.00E-37
	OE4301R	ABC-type transport system ATP-binding protein	SSO2615	ABC-type oligopeptide transport system	E	3.00E-70
	OE4230F/yuxL (*)	Probable acylaminoacyl-peptidase	SSO2693	Dipeptidyl aminopeptidase /acylaminoacyl-peptidase	E	2.00E-47
	OE1385F/thiN (*)	Thiamine biosynthesis protein thiN	SSO0468	Predicted transcriptional regulator fused phosphor-methylpyrimidine kinase	H	2.00E-15
	OE5243F/car, htr11, htrXI (*)	Transducer protein car	SSO0669	Mn-dependent transcriptional regulator	K	2.00E-18
<i>E. coli</i> K12 isolate: MG1665 ²¹	icd	Isocitrate dehydrogenase	SSO2182	Isocitrate dehydrogenase	C	1.00E-108
	lysS	Lysine tRNA synthetase,	SSO0090	Lysyl-tRNA synthetase (class II)	J	8.00E-103

	lysU	Lysyl-tRNA synthetase	SSO0090	Lysyl-tRNA synthetase (class II)	J	4.00E-107
	tpx	Heat shock protein, integral membrane protein	SSO2613	Peroxiredoxin	O	7.00E-14
<i>L. lactis</i> IL1403 ²²	dltA	D-alanine activating enzyme	SSO2523	Acyl-CoA synthetase/AMP-acid ligase II	I, Q	3.00E-15
	hisS	Histidyl-tRNA synthetase	SSO0279	Histidyl-tRNA synthetase	J	3.00E-48
	mleS	Malolactic enzyme	SSO2869	Malic enzyme	C	4.00E-13
	optC	Oligopeptide ABC transporter permease protein	SSO1283	ABC-type dipeptide/oligopeptide/nickel transport system	E, P	3.00E-16
	serS	Seryl-tRNA synthetase	SSO0602	Seryl-tRNA synthetase	J	2.00E-67
<i>B. subtilis</i> strain 168 ²³	BSU1242	Unknown	SSO0176	ATPase of the AAA+ class	O	4.00E-23
	BSU0094	Cysteinyl-tRNA synthase	SSO2280	Cysteinyl-tRNA synthase	J	1.00E-98
	BSU1322	Related to the activation of sigma-H	SSO1893	ABC-type cobalt transport system, ATPase component	P	2.00E-74
	BSU1676	Aspartate-semialdehyde dehydrogenase	SSO0876	Aspartokinase	E	3.00E-24

(*) *H. salinarium* ΔserB

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