Supporting information

for

Carbohydrate Catabolism
in *Phaeobacter inhibens* DSM 17395,
Member of the Marine *Roseobacter* Clade

Content

Proteomic and metabolomics datasets

Four different proteomic techniques were applied, resulting in the overall identification of 1351 different proteins: (i) 2D DIGE analysis revealed 369 regulated soluble proteins (fold change ≥|1.5|), which are represented by 409 spots. (ii) Shotgun analysis yielded 932 proteins. (iii) From the SDS PAGE-resolved, cytoplasmic membrane protein-enriched fractions, 614 proteins were identified; 245 of which contain at least one predicted transmembrane helix. (iv) The outer membrane protein-enriched fractions were also separated via SDS PAGE and yielded 141 identified proteins.

Furthermore, 116 intracellular metabolites were detected and identified (Table 2; Supplementary Table S5).

Growth of *P. inhibens* DSM 17395 with carbohydrates (TABLE S1, FIG S1)

TABLE S1 Growth of *P. inhibens* DSM 17395 with different carbohydrates.

Carbohydrate	**Concentration [mM] (1 mg/ml)**	**Growth observed**[a]
Arabinose	6.7	-
Cellobiose	2.9	+
Fructose	5.6	+
Fucose	6.1	-
Galactose	5.6	+
Glucose	5.6	+
Lactose	2.9	-
Maltose	2.9	+
Mannitol	5.5	+
Mannose	5.6	+
N-Acetylglucosamine	4.5	+
Rhamnose	6.1	-
Ribose	6.7	+
Sorbitol	5.5	+
Starch	6.1	-
Sucrose	2.9	+
Trehalose	2.9	+
Xylose	6.7	+

[a] The same results were observed in a Biolog experiment, except that results for L- and D-arabinose, as well as D-fucose were positive.

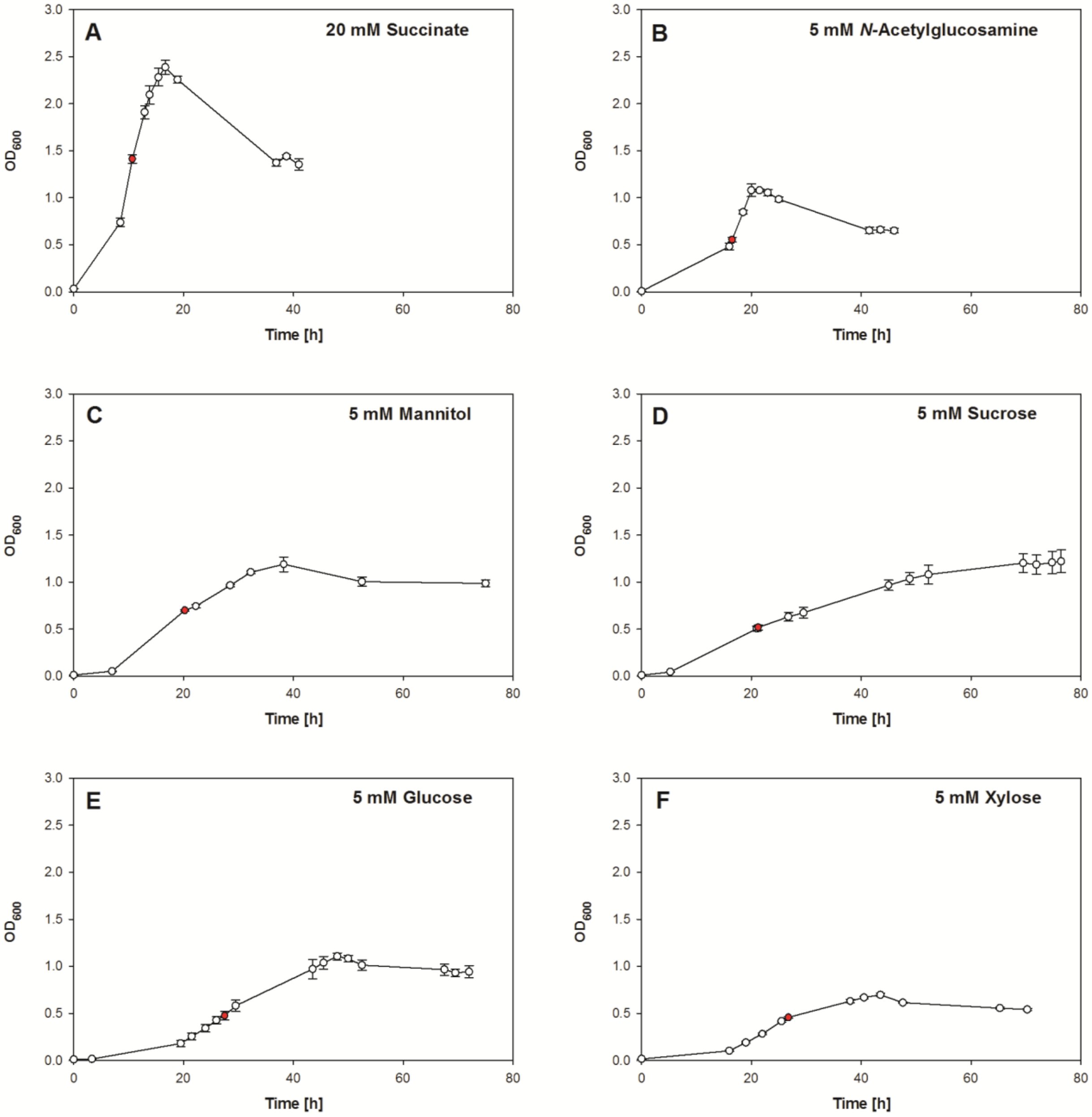

FIG S1 Growth curves of *P. inhibens* DSM 17395 with succinate and the five selected carbohydrates. Growth was monitored by measuring the optical density (OD) at 600 nm. Harvesting time points for proteomic and metabolomic analyses are marked in red.

Proteogenomics-based refinement of carbohydrate catabolism in *P. inhibens* DSM 17395 (TABLEs S2 & S3)

TABLE S2 Number of proteins assigned to the transport, regulation and degradation of the five selected carbohydrates in *P. inhibens* DSM 17395 based on the original genome annotation and their proteogenomics-based refinement in this study.

Degradation pathway for	Genome-based prediction[a]:	This study:				
	Sum of proteins	Predicted only[a]	Predicted[a] & identified	Newly predicted[b]	Newly predicted[b] & identified	Sum of proteins
N-Acetyl-glucosamine	**2**		2		7	**9**
Mannitol	**2**		2		5	**7**
Sucrose	**2**		2	1	5	**8**
Glucose	**3**		3	1	1	**5**
Xylose	**4**		4	1	1	**6**
All	**13**		13	3	19	**35**
All (non-redundant)	**9**		9	2	19	**30**

[a] Based on the original genome annotation: carbohydrate degradation to intermediates of central metabolism, i.e. glucose-6-phosphate, fructose-6-phosphate or xylulose-5-phosphate (Thole *et al.*, 2012).

[b] See Material and Methods.

TABLE S3 Compilation of genes of *P. inhibens* DSM 17395 with reannotated or refined functional prediction.

Acc. no.	Protein ID		Predicted protein function		Relevant	BLASTP hit used for reannotation		
	old	new	old	new	domains	Organism	E-value	Acc.no.
TRANSPORT								
N-*Acetylglucosamine*								
PGA1_c27930			Putative sugar ABC transporter, periplasmic binding protein	*N*-Acetylglucosamine ABC transporter, periplasmic sugar-binding protein	IPR006061	*Loktanella cinnabarina*	0.00E+00	WP_021694556
PGA1_c27940			ABC transporter permease protein	*N*- Acetylglucosamine ABC transporter, permease	IPR000515	*Loktanella cinnabarina*	0.00E+00	WP_021694555
PGA1_c27950			ABC transporter permease protein	*N*- Acetylglucosamine ABC transporter, permease	IPR000515	*Loktanella cinnabarina*	5.00E-172	WP_021694554
PGA1_c27970	SmoK		ATP-binding transport protein	*N*- Acetylglucosamine ABC transporter, ATP-binding protein	IPR003439, IPR003593, IPR008995, IPR012340, IPR013611, IPR017871, IPR027417	*Loktanella cinnabarina*	3.30E-160	WP_021694552
Mannitol								
PGA1_c13180		SmoK	ABC transporter, ATP binding protein	Mannitol ABC transporter, ATP-binding protein	IPR003439, IPR003593, IPR008995, IPR012340, IPR013611, IPR017871, IPR027417	*Roseobacter litoralis* Och 149	0.00E+00	YP_004691600
PGA1_c13190			ABC transporter permease protein	Mannitol ABC transporter, permease	IPR000515	*Roseobacter litoralis* Och 149	2.00E-173	YP_004691601
PGA1_c13200			ABC transporter permease protein	Mannitol ABC transporter, permease	IPR000515	*Roseobacter litoralis* Och 149	2.00E-174	YP_004691602
PGA1_c13210			Extracellular solute-binding protein	Mannitol ABC transporter, periplasmic sugar-binding protein	IPR006059	*Roseobacter litoralis* Och 149	0.00E+00	YP_004691603
Sucrose								
PGA1_c07860	AglE		Alpha-glucosides-binding periplasmic protein	Alpha-glucoside ABC transporter, periplasmic sugar-binding protein				
PGA1_c07870	AglF		Alpha-glucoside transport system permease protein	Alpha-glucoside ABC transporter, permease				
PGA1_c07880	AglG		Alpha-glucoside transport system permease protein	Alpha-glucoside ABC transporter, permease				
PGA1_c07900	AglK		Alpha-glucoside transport ATP-binding protein	Alpha-glucoside ABC transporter, ATP-binding protein				
Xylose, glucose, sucrose								
PGA1_262p00430	XylF		D-xylose-binding periplasmic prote	Xylose ABC transporter, periplasmic sugar-binding protein				
PGA1_262p00440	XylH		Xylose transport system permease protein	Xylose ABC transporter, permease				
PGA1_262p00450		XylG	Sugar ABC transporter ATP-binding protein	Xylose ABC transporter, ATP-binding protein	IPR003439, IPR003593, IPR027417	*Rhodobacter* sp. AKP1	1.00E-144	WP_009563216
Fructose								
PGA1_c28030		FrcK	Hypothetical protein	Fructose ABC transporter, kinase	IPR027417	*Ruegeria* sp. R11	6.00E-100	WP_008560036
PGA1_c28040		FrcA	ABC transporter ATP-binding protein	Fructose ABC transporter, ATP-binding protein	IPR003439, IPR003593, IPR027417	*Thalassobacter arenae*	5.00E-158	WP_021101677
PGA1_c28050		FrcC	Putative sugar transport system, permease protein	Fructose ABC transporter, permease	IPR018510	*Thalassobacter arenae*	5.00E-178	WP_021101676
PGA1_c28060		FrcB	Putative sugar transport system, periplasmic protein	Fructose ABC transporter, periplasmic sugar-binding protein	IPR025997, IPR028082	*Thalassobacter arenae*	0.00E+00	WP_021101675
Succinate								
PGA1_c20660	DctM5	DctM6	TRAP transporter, subunit DctM	TRAP transporter, transmembrane subunit				
PGA1_c20670	DctQ4	DctQ6	TRAP transporter, subunit	TRAP transporter, transmembrane subunit				
PGA1_c20680	DctP3	DctP6	C4-Dicarboxylate-binding periplasmic protein	TRAP transporter, periplasmic solute-binding protein				

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TABLE S3 continued

Acc. no.	Protein ID		Predicted protein function		Relevant	BLASTP hit used for reannotation		
	old	**new**	**old**	**new**	**domains**	**Organism**	**E-value**	**Acc.no.**
CARBOHYDRATE METABOLISM								
N-*Acetylglucosamine*								
PGA1_c27890		NagB	Putative hexosephosphate binding protein	Glucosamine-6-phosphate deaminase	IPR001347	*Ruegeria* sp. TW15	3.00E-148	WP_010439364
PGA1_c27910		NagK	Putative ATPase, BadF/BadG/BcrA/BcrD type	*N*-Acetylglucosamine kinase	IPR002731	*Roseobacter denitificans* OCh 114	2.00E-48	YP_682036
Sucrose								
PGA1_c111850		PspA	Hypothetical protein	Phage shock protein A	IPR007157	*Jannaschia* sp. CCS1	2.00E-67	YP_510935
CENTRAL METABOLISM								
Pyruvate dehydrogenase complex								
PGA1_c17550	PdhA		Pyruvate dehydrogenase, E1 component alpha subunit	Pyruvate dehydrogenase, E1 alpha subunit				
PGA1_c17560	PdhB		Pyruvate dehydrogenase, E1 component beta subunit	Pyruvate dehydrogenase, E1 beta subunit				
PGA1_c17570	PdhC		Pyruvate dehydrogenase, dihydrolipoyllysine-residue acetyltransferase component	Pyruvate dehydrogenase, E2				
PGA1_c17390	LpdA	LpdA1	Dihydrolipoyl dehydrogenase LpdA	Dihydrolipoyl dehydrogenase				
TCA cycle								
PGA1_c16970	GltA	GltA1	Citrate synthase GltA	Citrate synthase				
PGA1_c03600	SucA		2-Oxoglutarate dehydrogenase, E1 component SucA	2-Oxoglutarate dehydrogenase, E1				
PGA1_c03550	Lpd2		Dihydrolipoyl dehydrogenase 2	Dihydrolipoyl dehydrogenase				
SENSORY/REGULATORY PROTEINS								
N-*Acetylglucosamine*								
PGA1_c27900			Ligand-binding UTRA domain-containing protein	GntR family transcriptional regulator	IPR000524, IPR011663, IPR011991	*Ruegeria pomeroyi* DSS-3	5.00E-134	YP_167079
Sucrose								
PGA1_c07850		AlgR	Transcriptional regulator, lacI family		IPR000843, IPR010982, IPR028082	*Sinorhizobium fredii* USDA 257	3.00E-81	YP_006395698
Fructose								
PGA1_c28070		FcrR	MarR family transcriptional regulator		IPR027417	*Sinorhizobium meliloti* SM11	7.00E-115	YP_005718659

Proteins of *P. inhibens* DSM 17395 constituting the central metabolism, parts of amino acid biosynthesis and the urea cycle as schematically outlined in FIG 1 (TABLE S4)

TABLE S4 Proteins of *P. inhibens* DSM 17395 constituting the central metabolism, parts of amino acid biosynthesis and the urea cycle as schematically outlined in Fig. 1. Proteins were identified from 2DE gels (2D DIGE), by shotgun analysis (S), from SDS-PAGE resolved cytoplasmic membrane protein-enriched fractions (CM) and from SDS-PAGE resolved outer membrane protein-enriched fractions (OM). Abbreviations of substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose; Succ, succinate

Acc. No. (PGA1_)	Name	Predicted function	Fold change[a] in abundance (2D DIGE)					Mascot score (gel-free analysis)																	
								NAG			MTL			SUCR			GLC			XYL			Succ		
			NAG	MTL	SUCR	GLC	XYL	S	CM	OM	S	CM	OM	S	CM	OM	S	CM	OM	S	CM	OM	S	CM	OM
CENTRAL METABOLISM																									
Entner-Doudoroff pathway																									
c27980	Pgi	Glucose-6-phosphate isomerase	5.0	3.5	6.4	6.2	3.4	292						102			186			316					
c27990	Zwf2	Glucose-6-phosphate 1-dehydrogenase						713			894			693	59		995			760					
c15170	Pgl	6-Phosphogluconolactonase						142			177			133			171			124			166		
c28000	Edd[b]	Phosphogluconate dehydratase[b]	8.6	6.5	9.0	9.3	6.5	529			561			349			575			572					
c28010	Eda	KHG/KDPG aldolase	1.4	1.7	2.1	1.9	1.0	447			414			302			378			318			72		
Lower branch of Embden-Meyerhof Parnas pathway																									
c27490	Gap[b]	Glyceraldehyde-3-phosphate dehydrogenase[b]	10.7	13.6	14.7	16.6	16.6	519	75		763	68		546	75		840	120		818	67				
c17530	Pgk	Phosphoglycerate kinase						264			350			134			421			404			312		
c02770	GpmI	2,3-Bisphosphoglycerate-indep.phosphoglycerate mutase									137			74			159			77					
c11420	Eno[b]	Enolase[b]	2.0	2.4	2.6	2.3	3.1	1109			1409			949	36		1411	41		1541			711		
c06970	PykA	Pyruvate kinase						268	56		532			318			602			550			81		
Pentose phosphate pathway																									
c23740	RpiA	Ribose-5-phosphate isomerase A						97			96														
c17230	TktA[b]	Transketolase[b]	-1.3	-1.4	-1.7	-1.3	1.2	743			850			592			706			1024			759		
c05780	Tal	Transaldolase						788			814			752			696			762			767		
Pyruvate dehydrogenase complex																									
c17550	PdhA	Pyruvate dehydrogenase, E1 alpha subunit[c]	-1.3	-1.0	1.1	-1.1	-1.6	237			408			233			320			183			411		
c17560	PdhB[b,c]	Pyruvate dehydrogenase, E1 beta subunit[b,c]						344			642			580			670			516			607		
c17570	PdhC[b,c]	Pyruvate dehydrogenase, E2[b,c]						419			773			510			673			552			671		
c17390	LpdA1[b,c]	Dihydrolipoyl dehydrogenase[b,c]	-2.3	1.1	1.4	-1.1	-1.8	156			708			527	47		670	47		451			345		
TCA cycle																									
c16970	GltA1[c]	Citrate synthase						230			131			107			185			237			166		
c18830	AcnA	Aconitate hydratase	1.6	-1.3	1.2	-1.0	1.6	1061	58		895	33		595			897			982			878		
c28340	Icd[b]	Isocitrate dehydrogenase [NADP][b]	1.6	-1.3	-1.1	-1.4	-1.0	1645			1533			1194			1223			1585			1555		
c03590	SucB	2-Oxoglutarate dehydrogenase, E2						673			788	33		723			837	31		728			698		
c03600	SucA	2-Oxoglutarate dehydrogenase, E1						454			166	49		226			190						389		
c03550	Lpd2	Dihydrolipoyl dehydrogenase						280			500			340			502			315			318		
c03610	SucD	Succinyl-CoA ligase [ADP-forming], subunit alpha						598	51		568	49		543		54	445	44		431			637		
c03630	SucC	Succinyl-CoA ligase [ADP-forming], subunit beta	-1.3	-1.5	1.1	-1.6	-1.7	1000			847			746			944			968			882		
c03740	SdhC	Succinate dehydrogenase, cytochrome *b* 556 subunit							36			39			39			43			48				
c03760	SdhA	Succinate dehydrogenase, flavoprotein subunit						664	32		818			572			673			692			733		
c03780	SdhB	Succinate dehydrogenase, iron-sulfur subunit						234			155												122		
c13520	FumC	Fumarate hydratase class II						644			690			535			623			594			644		
c03650	Mdh	Malate dehydrogenase	-1.0	1.0	1.1	1.1	1.6	1300			1212	58		1091	104		1215	45		1386	40	40	1224		40

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TABLE S4 continued

Acc. No. (PGA1_)	Name	Predicted function	Fold change[a] in abundance (2D DIGE)					Mascot score (gel-free analysis)																	
								NAG			MTL			SUCR			GLC			XYL			Succ		
			NAG	MTL	SUCR	GLC	XYL	S	CM	OM	S	CM	OM	S	CM	OM	S	CM	OM	S	CM	OM	S	CM	OM
CENTRAL METABOLISM																									
Gluconeogenesis																									
c09420	Pyc[b]	Pyruvate carboxylase[b]	1.1	-1.3	-1.3	-1.5	-1.2	1487	1349		1815	1081		857	526		1365	881	186	1258	632	33	1248	508	33
c01140	PckA[b]	Phosphoenolpyruvate carboxykinase[b]	-4.0	-3.4	-3.0	-2.9	-3.6	93			203			484			266			172			722		
c11420	Eno[b]	Enolase[b]	2.0	2.4	2.6	2.3	3.1	1109			1409			949	36		1411	41		1541			711		
c02770	GpmI	2,3-Bisphosphoglycerate-independent phosphoglycerate mutase									137			74			159			77					
c17530	Pgk	Phosphoglycerate kinase						264			350			134			421			404			312		
c17250	GapB	Glyceraldehyde-3-phosphate dehydrogenase 2												242									436		
c20650	TpiA	Triosephosphate isomerase						193			344			249			357			260			359		
c23910	Fda	Fructose-bisphosphate aldolase class 1	-2.7	-3.3	-3.6	-3.4	-2.6	559			520			611			364			478			850		
c08640	GlpX	Fructose-1,6-bisphosphatase class 2						477			647			488			636			634			634		
c27980	Pgi	Glucose-6-phosphate isomerase	5.0	3.5	6.4	6.2	3.4	292						102			186			316					
c05420	Glk	Glucokinase						246			296			194	57		341			286			97		
AMINO SUGAR METABOLISM																									
c27920	MurQ	*N*-Acetylmuramate-6-phosphate etherase	4.0	1.1	1.1	1.0	1.1	445	71																
UDP- N-acetylglucosamine biosynthesis																									
c10230	GlmS	Glucosamine-fructose-6-phosphate aminotransferase	-1.5	1.6	2.0	1.6	2.0	196			654	123		324			460			624			191		
c06550	GlmM	Phosphoglucosamine mutase									223			134			133			91			169		
c10240	GlmU	Bifunctional enzyme							40		461	164		314			449			562			194		
AMINO ACID BIOSYNTHESIS																									
Cysteine																									
c17690	CysE	Serine acetyltransferase	-1.7	-1.6	1.3	-2.3	-2.0	311			324			463						183			471		
c13950	CysK	Cysteine synthase	1.1	-1.5	-1.5	-1.5	-1.6	441			828			641			805			684			707		
Arginine biosynthesis and urea cycle																									
c34600	ArgJ	Arginine biosynthesis bifunctional protein						159			139												151		
c02050	ArgB	Acetylglutamate kinase						253			146			206			219			221			187		
c17050	ArgC	*N*-Acetyl-gamma-glutamyl-phosphate reductase															111								
c24230	ArgD	Acetylornithine aminotransferase									163												74		
c24220	ArgF	Ornithine carbamoyltransferase						71			172			112						58			200		
c34920	ArgG	Argininosuccinate synthase						444			549			355			428			625			433		
c18720	GlnA	Glutamine synthetase						337	539	421	340	283	303	83	131	118	386	186	169	297	474	363	122	248	270
c06670	CarA	Carbamoyl-phosphate synthase, small chain									252			75			119			189			134		
c24560	CarB	Carbamoyl-phosphate synthase, large chain	-1.6	-1.4	-1.6	-1.5	-1.1	209			501			273			397			457			401		
SORBITOL DEGRADATION																									
c13170	PolS	Sorbitol dehydrogenase									365														
STRESS-ASSOCIATED																									
c11850	PspA[c]	Phage shock protein A[b,c]	-2.0	8.0	92.1	5.7	1.4				847	770	203	1009	1508	199	649	577	127	288	313		317	209	161
TRANSHYDROGENASE																									
c10030	PntA	NAD(P) transhydrogenase, subunit alpha							970			1205	220		674	171		859	224		1037	96		511	128
c10040	PntB	NAD(P) transhydrogenase, subunit beta							206	63		318	120		296	102		435	137		345	63		255	130

Fold change	n.d.	< -30.0	< -10.0	< -5.0	< -1.5	> - 1.5	> 1.5	> 5.0	> 10.0	> 35.0
Mascot score	n.d.	> 25	> 200	> 500	> 1000					

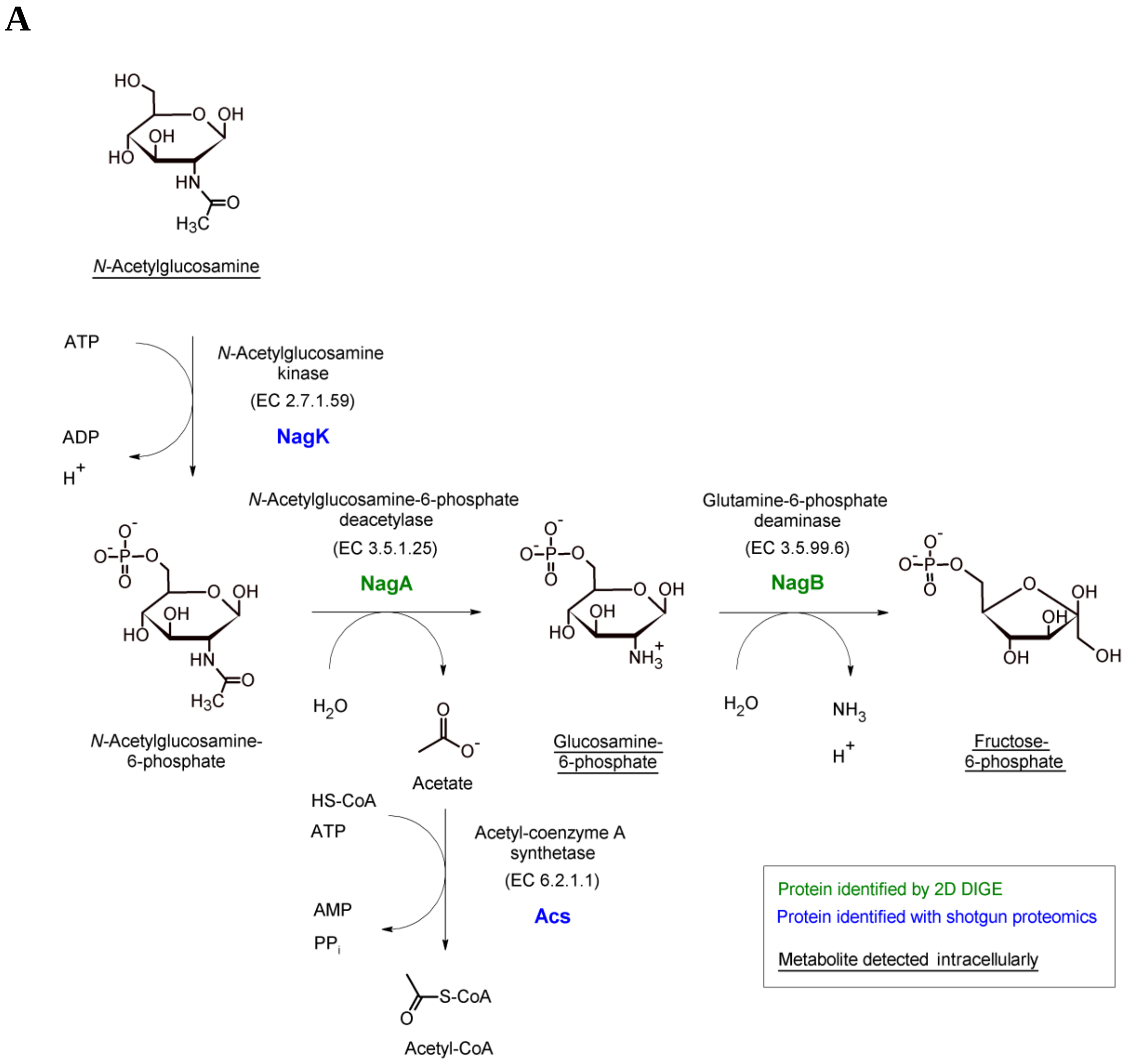
[a] As compared to succinate-adapted cells of *P. inhibens* DSM 17395.

[b] Identified from multiple spots on a single 2DE gel.

[c] For annotation refinement or full enzyme names see Supplementary TABLE S3.

Carbohydrate-specific degradation pathways in *P. inhibens* DSM 17395 (FIGs. S2–S6)

1. *N*-Acetylglucosamine degradation (FIG S2)



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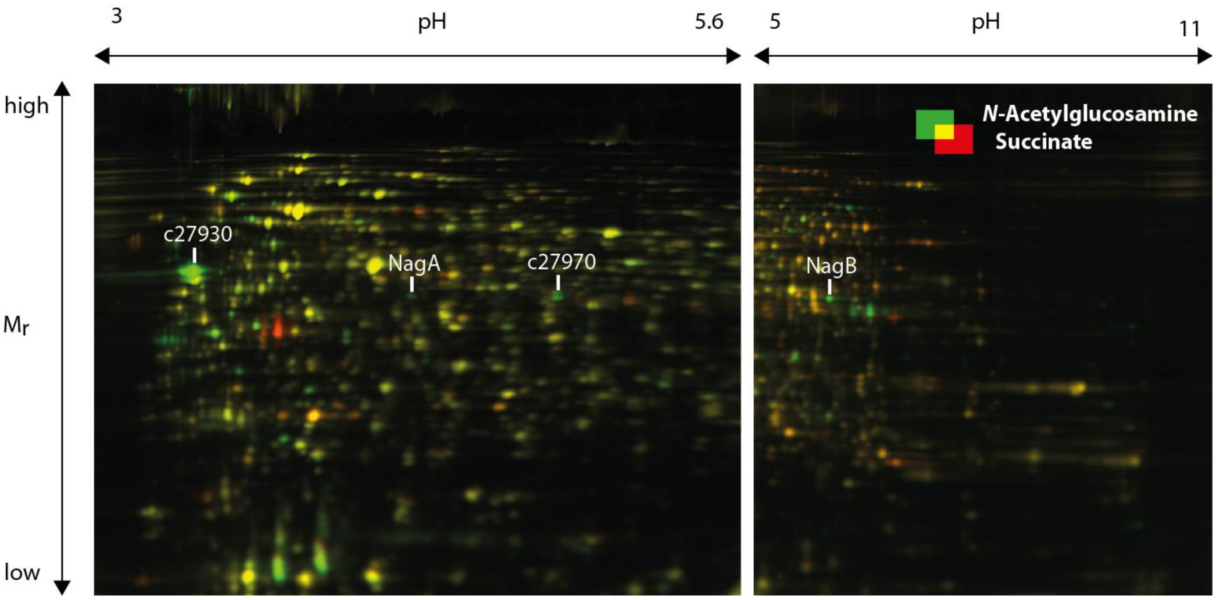


C

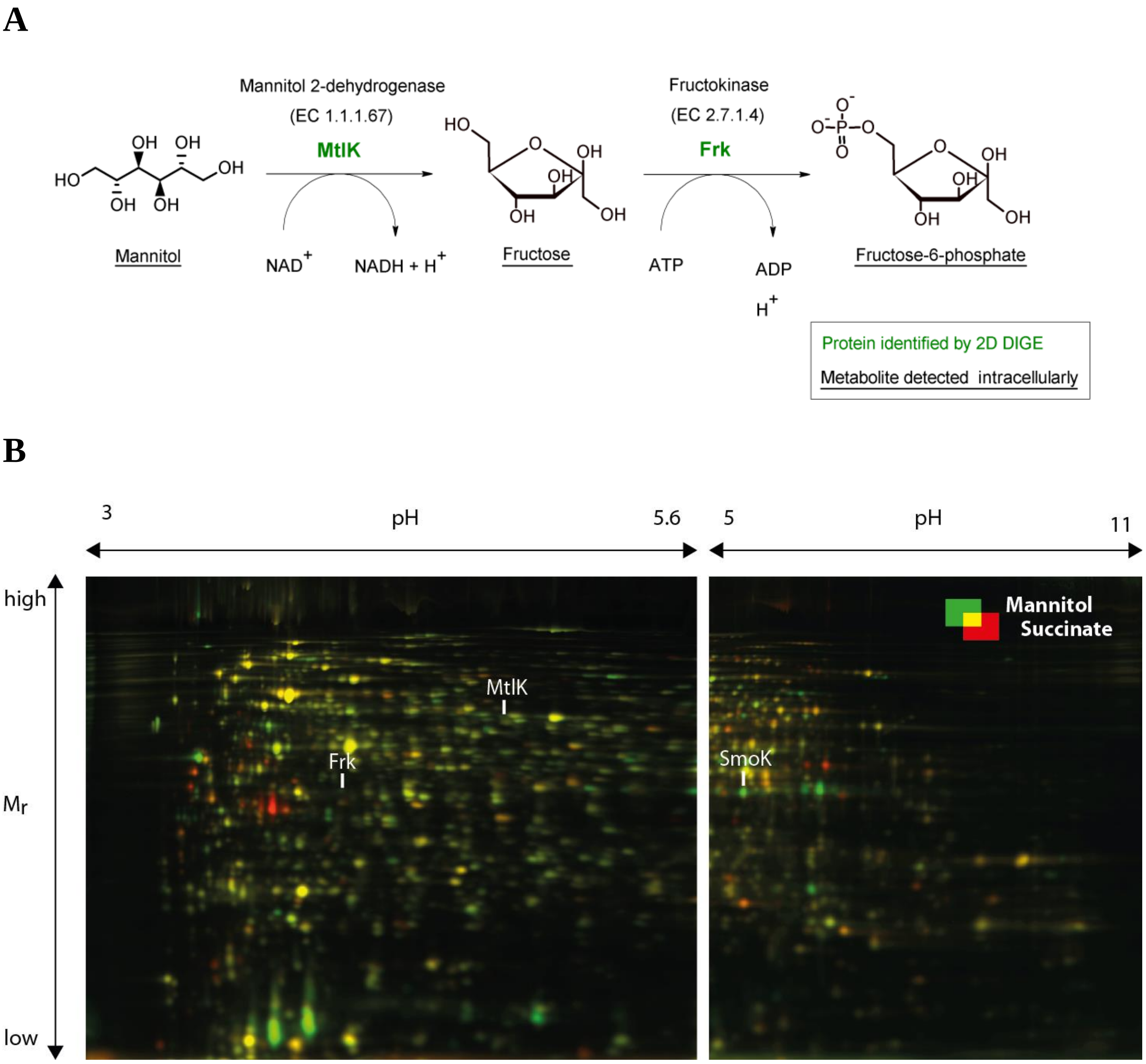
Acc. No. (PGA_)	Name	Predicted function	Fold change in abundance as compared to succinate-adapted cells (2D DIGE)				
			NAG	MTL	SUCR	GLC	XYL
CARBOHYDRATE METABOLISM							
N-*Acetylglucosamine*							
c27880	NagA	*N*-Acetylglucosamine-6-phosphate deacetylase	3.8	1.4	-1.0	1.4	-1.3
c27890	NagB[a]	Glucosamine-6-phosphate deaminase[a]	15.6	-1.1	-1.8	-2.1	-2.0
TRANSPORT							
N-*Acetylglucosamine*							
c27930		*N*-Acetylglucosamine ABC transporter, periplasmic sugar-binding protein[a]	26.1	-1.5	-4.1	-3.4	-3.6
c27970		*N*-Acetylglucosamine ABC transporter, ATP-binding protein[a]	2.4	-6.4	-5.9	-5.7	-11.7

Fold change					
		< -30.0	< -10.0	< -5.0	< -1.5
	> - 1.5	> 1.5	> 5.0	> 10.0	> 35.0

[a] Annotation refinement (see Supplementary TABLE S3).

FIG S2 Proteomic data and genomic predictions for *N*-acetylglucosamine degradation and transport in *P. inhibens* DSM 17395. (A) Proposed degradation pathway. (B) False-colour 2D DIGE gel image comparing the proteome profile of *N*-acetylglucosamine-adapted cells to that of succinate-adapted cells. Identified proteins involved in the degradation and transport of *N*-acetylglucosamine are marked. (C) Protein ID, predicted function and fold change in abundance of marked proteins. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose.

2. Mannitol degradation (FIG S3)



C

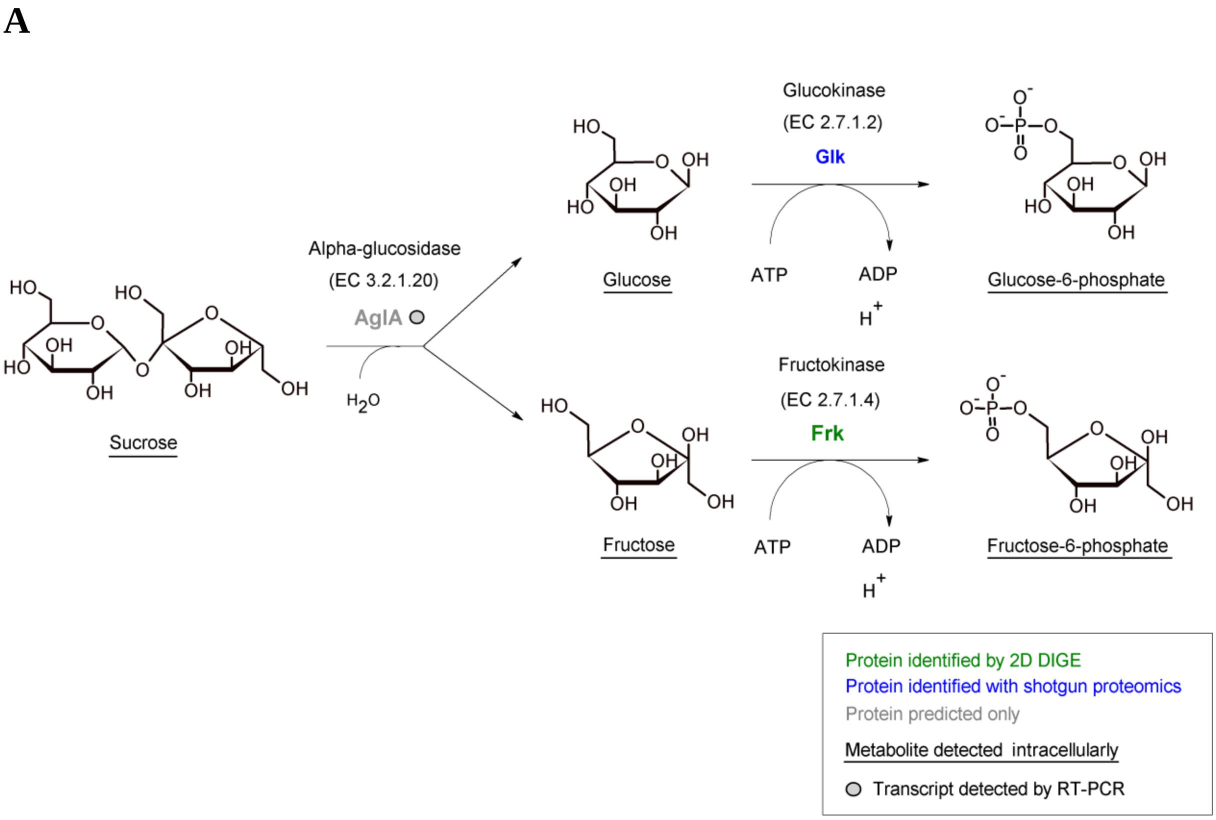
Acc. No. (PGA_)	Name	Predicted function	Fold change in abundance as compared to succinate-adapted cells (2D DIGE)				
			NAG	**MTL**	**SUCR**	**GLC**	**XYL**
CARBOHYDRATE METABOLISM							
Mannitol							
c13160	MtlK	Mannitol 2-dehydrogenase	**1.3**	**2.5**	**1.5**	**1.6**	**1.7**
c28020	Frk	Fructokinase	**1.6**	**1.5**	**2.2**	**-1.1**	**1.1**
TRANSPORT							
Mannitol							
c13180	SmoK[a]	Mannitol ABC transporter, ATP-binding protein[a]	**-1.3**	**4.9**	**-1.1**	**-1.1**	**-1.2**

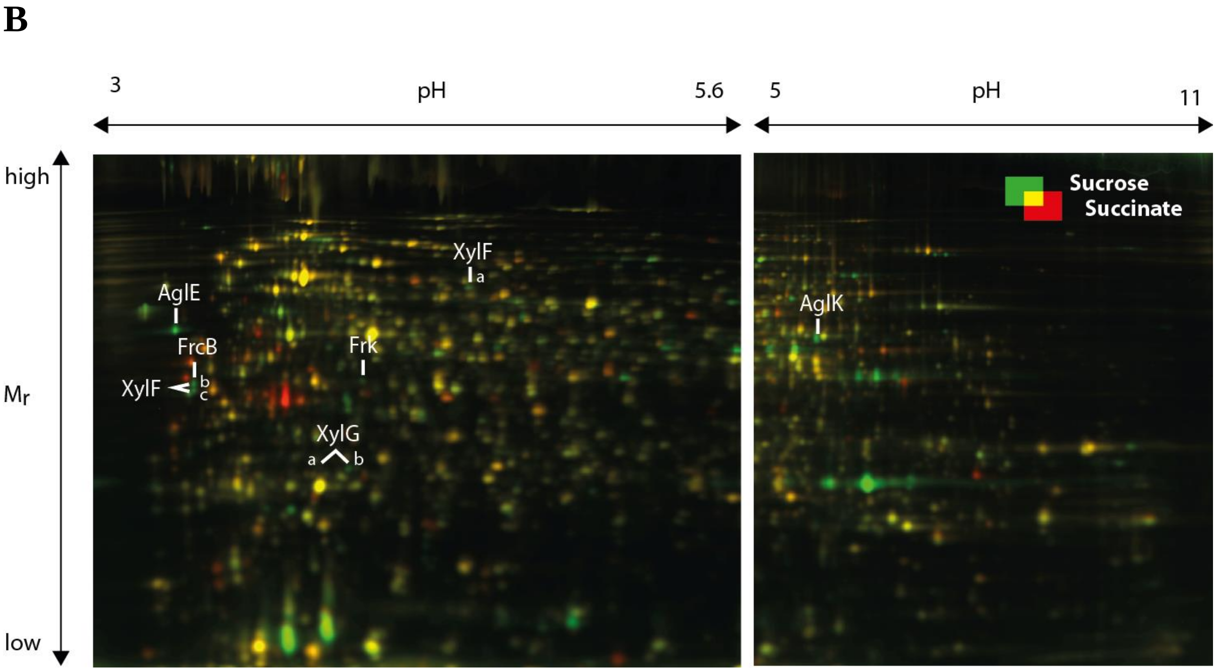
Fold change **> - 1.5** **> 1.5**

[a] Annotation refinement (see Supplementary TABLE S3).

FIG S3 Proteomic data and genomic predictions for mannitol degradation in *P. inhibens* DSM 17395. (A) Proposed degradation pathway. (B) False-colour 2D DIGE gel image comparing the proteome profile of mannitol-adapted cells to that of succinate-adapted cells. Identified proteins involved in the degradation and transport of mannitol are marked. (C) Protein ID, predicted function and fold change in abundance of marked proteins. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose.

3. Sucrose degradation (FIG S4)





C

Acc. No. (PGA_)	Name	Predicted function	Spot	% of cumulative spot volume of single protein	Fold change in abundance as compared to succinate-adapted cells (2D DIGE)				
					NAG	MTL	SUCR	GLC	XYL
CARBOHYDRATE METABOLISM									
Sucrose									
c28020	Frk	Fructokinase			**1.6**	**1.5**	**2.2**	**-1.1**	**1.1**
TRANSPORT									
Sucrose									
c07860	AglE	Alpha-glucoside ABC transporter, periplasmic sugar-binding protein[a]			**1.3**	**-2.7**	**29.1**	**-1.0**	**-1.6**
c07900	AglK	Alpha-glucoside ABC transporter, ATP-binding protein[a]			**1.3**	**1.7**	**6.8**	**1.7**	**1.7**
Fructose									
c28060	FrcB[a]	Fructose ABC transporter, periplasmatic sugar-binding protein[a]			**1.8**	**2.3**	**6.8**	**1.3**	**1.2**
Xylose, glucose, sucrose									
262p00430	XylF	Xylose ABC transporter, periplasmic sugar-binding protein[a]	a	11.7	**2.1**	**2.0**	**6.4**	**1.9**	**2.8**
262p00430	XylF	Xylose ABC transporter, periplasmic sugar-binding protein[a]	b	13.9	**1.5**	**-1.1**	**3.7**	**4.7**	**7.4**
262p00430	XylF	Xylose ABC transporter, periplasmic sugar-binding protein[a]	c	74.4	**1.8**	**-3.8**	**3.2**	**7.3**	**11.8**
262p00450	XylG[a]	Xylose ABC transporter, ATP-binding protein[a]	a	16.1	**-1.5**	**-3.0**	**4.9**	**20.8**	**21.6**
262p00450	XylG[a]	Xylose ABC transporter, ATP-binding protein[a]	b	83.9	**-1.5**	**-1.2**	**1.6**	**3.5**	**3.4**

Fold change			
	< -5.0	**< -1.5**	**> - 1.5**
	> 1.5	**> 5.0**	**> 10.0**

---

[a] Annotation refinement (see Supplementary TABLE S3).

D

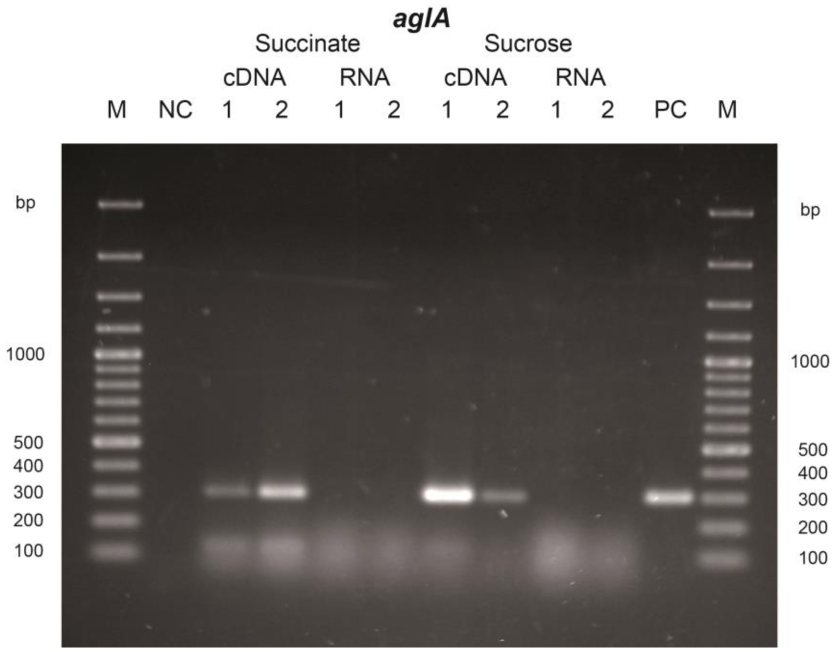
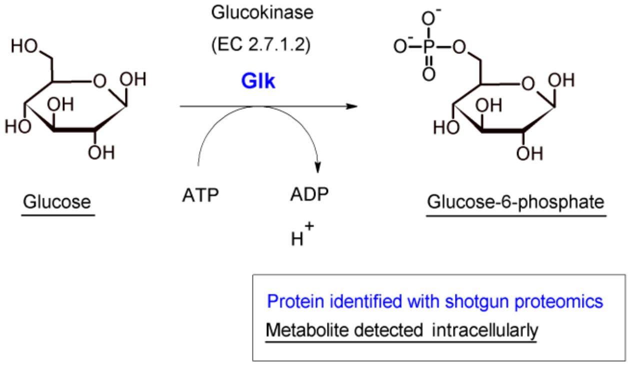


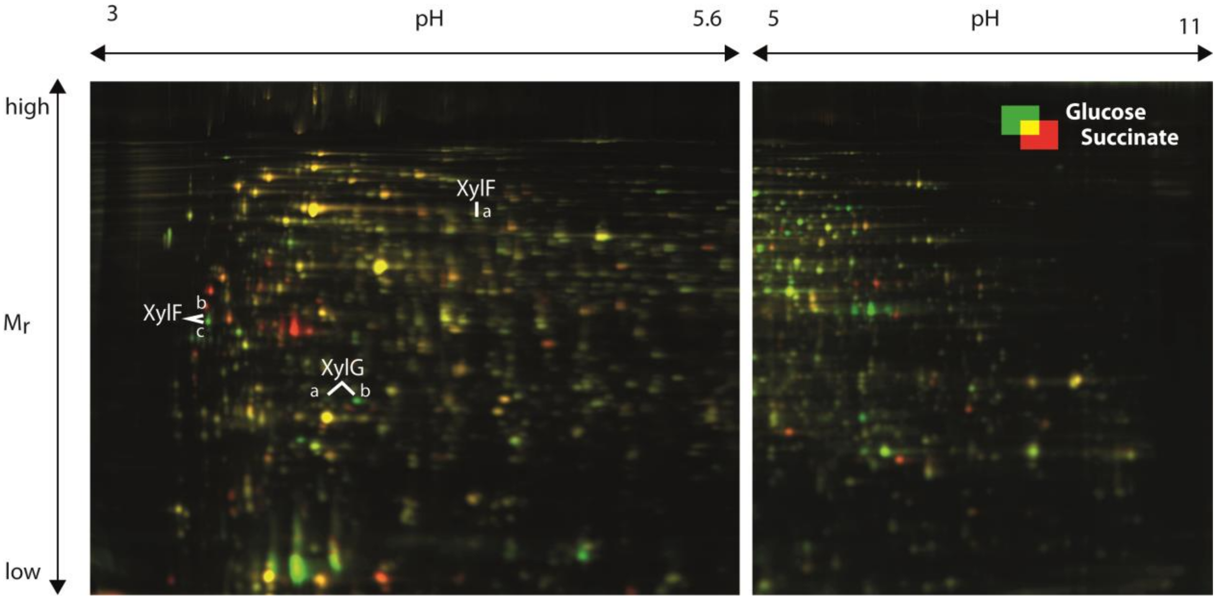
FIG S4 Proteomic data, RT-PCR data and genomic predictions for sucrose degradation and transport in *P. inhibens* DSM 17395. (A) Proposed degradation pathway. (B) False-colour 2D DIGE gel image comparing the proteome profile of sucrose-adapted cells to that of succinate-adapted cells. Identified proteins involved in the degradation and transport of sucrose are marked. (C) Protein ID, predicted function and fold change in abundance of marked proteins. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose. (D) Expression of α-glucosidase *aglA* (not captured by proteomics) analysed by RT-PCR. M, marker; NC, negative control; PC, positive control; 1, 2, biological replicates.

4. Glucose degradation (FIG S5)

A



B



C

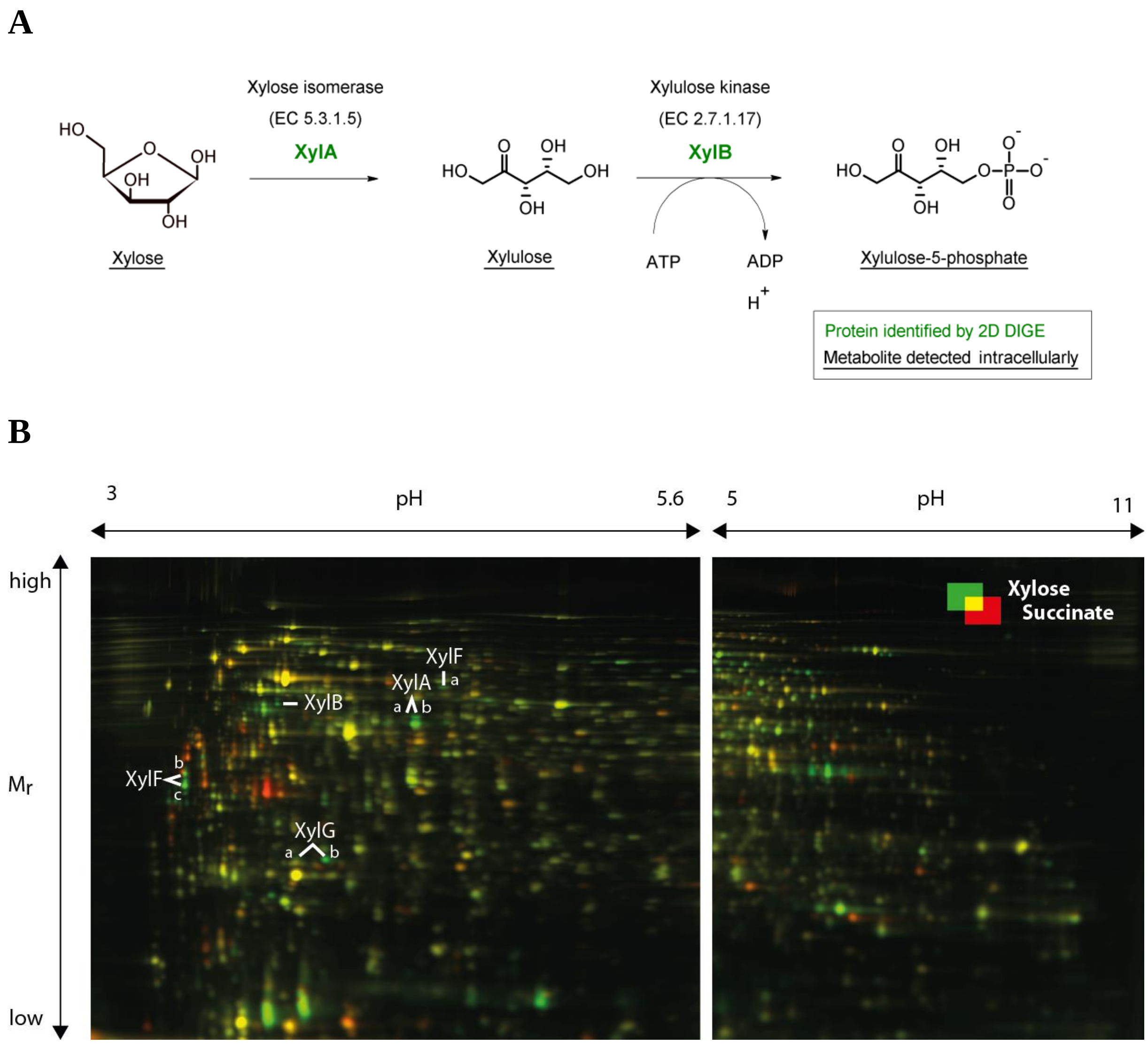
Acc. No. (PGA_)	Name	Predicted function	Spot	% of cumulative spot volume of single protein	Fold change in abundance as compared to succinate-adapted cells (2D DIGE)				
					NAG	**MTL**	**SUCR**	**GLC**	**XYL**
TRANSPORT									
Xylose, glucose, sucrose									
262p00430	XylF	Xylose ABC transporter, periplasmic sugar-binding protein[a]	a	11.7	**2.1**	**2.0**	**6.4**	**1.9**	**2.8**
262p00430	XylF	Xylose ABC transporter, periplasmic sugar-binding protein[a]	b	13.9	**1.5**	**-1.1**	**3.7**	**4.7**	**7.4**
262p00430	XylF	Xylose ABC transporter, periplasmic sugar-binding protein[a]	c	74.4	**1.8**	**-3.8**	**3.2**	**7.3**	**11.8**
262p00450	XylG[a]	Xylose ABC transporter, ATP-binding protein[a]	a	16.1	**-1.5**	**-3.0**	**4.9**	**20.8**	**21.6**
262p00450	XylG[a]	Xylose ABC transporter, ATP-binding protein[a]	b	83.9	**-1.5**	**-1.2**	**1.6**	**3.5**	**3.4**

Fold change < -1.5 | > - 1.5 | > 1.5 | > 5.0 | > 10.0

[a] Annotation refinement (see Supplementary TABLE S3).

FIG S5 Proteomic data and genomic predictions for glucose degradation and transport in *P. inhibens* DSM 17395. (A) Proposed degradation pathway. (B) False-colour 2D DIGE gel image comparing the proteome profile of glucose-adapted cells to that of succinate-adapted cells. (C) Protein ID, predicted function and fold change in abundance of marked proteins. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose.

5. Xylose degradation (FIG S6)



C

Acc. No. (PGA_)	Name	Predicted function	Spot	% of cumulative spot volume of single protein	Fold change in abundance as compared to succinate-adapted cells (2D DIGE)				
					NAG	**MTL**	**SUCR**	**GLC**	**XYL**
CARBOHYDRATE METABOLISM									
Xylose									
c14000	XylA	Xylose isomerase	b	75.3	**1.0**	**-1.2**	**1.0**	**-1.2**	**5.0**
c14000	XylA	Xylose isomerase	a	24.7	**1.5**	**1.4**	**1.4**	**1.4**	**1.8**
c14010	XylB	Xylulose kinase			**-1.4**	**1.4**	**1.3**	**1.9**	**17.2**
TRANSPORT									
Xylose, glucose, sucrose									
262p00430	XylF	Xylose ABC transporter, periplasmic sugar-binding protein[a]	a	11.7	**2.1**	**2.0**	**6.4**	**1.9**	**2.8**
262p00430	XylF	Xylose ABC transporter, periplasmic sugar-binding protein[a]	b	13.9	**1.5**	**-1.1**	**3.7**	**4.7**	**7.4**
262p00430	XylF	Xylose ABC transporter, periplasmic sugar-binding protein[a]	c	74.4	**1.8**	**-3.8**	**3.2**	**7.3**	**11.8**
262p00450	XylG[a]	Xylose ABC transporter, ATP-binding protein[a]	a	16.1	**-1.5**	**-3.0**	**4.9**	**20.8**	**21.6**
262p00450	XylG[a]	Xylose ABC transporter, ATP-binding protein[a]	b	83.9	**-1.5**	**-1.2**	**1.6**	**3.5**	**3.4**

Fold change	**< -1.5**	**> - 1.5**	**> 1.5**	**> 5.0**	**> 10.0**

[a] Annotation refinement (see Supplementary TABLE S3).

FIG S6 Proteomic data and genomic predictions for xylose degradation and transport in *P. inhibens* DSM 17395. (A) Proposed degradation pathway. (B) False-colour 2D DIGE gel image comparing the proteome profile of xylose-adapted cells to that of succinate-adapted cells. Identified proteins involved in the degradation and transport of xylose are marked. (C) Protein ID, predicted function, fold change in abundance of marked proteins and percentage share of each protein spot from the cumulative spot volume of a single protein. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose.

Intra- and extracellular metabolites of *P. inhibens* DSM 17395 identified in this study (TABLEs S5 & S6)

TABLE S5 Intracellular metabolites of *P. inhibens* DSM 17395 identified in this study. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose.

Metabolites	**Fold change**[a]				
	NAG	**MTL**	**SUCR**	**GLC**	**XYL**
Sugars and derivatives					
Hexoses					
1,6-Anhydro-glucose	**1.46**	**1.71**	**0.84**	**1.07**	**1.23**
6-Deoxy-mannose	**13.04**	**15.60**	**4.07**	**4.64**	**14.99**
Fructose	**0.73**	**8.27**	**17.29**	**2.21**	**1.05**
Fructose-6-phosphate	**0.07**	**0.02**	**4.28**	**4.30**	**0.44**
Glucose	**1.59**	**2.00**	**0.78**	**1.04**	**0.86**
Glucose/Galactose/Mannose-6-phosphate	**0.03**	**0.04**	**0.16**	**0.30**	**0.14**
Glucose/Glucosamine/Sorbitol-6-phosphate	**0.03**	**0.12**	**0.66**	**47.27**	**0.33**
Gluconate-1,4-lactone	**0.91**	**1.97**	**1.44**	**0.81**	**1.13**
Glucoheptonate-1,4-lactone	**1.53**	**0.70**	**0.69**	**0.56**	**2.18**
Glucosamine	**0.49**		**0.12**		**0.12**
Glucosaminate	**##**	**#**	**#**	**#**	**#**
N-Acetylglucosamine	**12.02**	**1.33**	**1.69**	**2.65**	**1.36**
N-Acetylmannosamine	**#**		**#**	**#**	
Glucuronate			**3.35**	**87.00**	
Mannitol	**0.76**	**19.86**	**1.02**	**0.47**	**0.43**
Pentoses / Pentose phosphate pathway					
Arabinonate	**9.34**	**11.24**	**4.25**	**21.62**	**109.52**
Erythrose-4-phosphate	**3.74**	**2.75**	**5.45**	**0.48**	**0.99**
Ribonate	**5.71**	**13.57**	**5.49**	**8.90**	**69.55**
Ribose	**1.02**	**2.30**	**0.81**	**0.89**	**2.45**
Xylose			**#**		**##**
Xylulose	**0.81**	**0.76**	**0.52**	**0.87**	**0.87**
Xylulose-/ Ribulose-5-phosphate	**0.09**		**0.05**	**0.17**	**1.00**
Di-Saccharides					
Melibiose	**#**	**#**	**#**	**#**	**#**
Sucrose			**#**		
Alpha-D-Glucopyranosyl-(1,6)-mannitol		**#**			
Alpha-D-Mannopyranosyl-(1,2)-D-mannopyranose		**##**	**#**		
Entner-Doudoroff pathway					
Glucono-1,5-lactone *	**4.70**	**8.99**	**9.83**	**22.36**	**4.99**
Galactonate / Gluconate *	**11.38**	**4.62**	**8.06**	**25.95**	**6.58**
6-Phosphogluconate	**0.24**	**0.96**	**5.05**	**15.77**	**0.17**
2-Keto-3-deoxy-D-gluconate (KDG) *	**30.50**	**4.66**	**1.87**	**3.18**	**2.05**
Glyceraldehyde *	**1.05**	**0.75**	**0.50**	**0.64**	**0.76**
Dihydroxyacetonephosphate	**1.68**	**0.31**	**0.39**	**0.63**	**0.60**
1,3-Dihydroxyacetone *	**0.77**	**0.95**	**1.00**	**1.12**	**0.98**
3-Phosphoglycerate	**3.77**	**1.58**	**1.66**	**8.75**	**2.60**
2-Phosphoglycerate	**1.64**	**0.42**	**0.80**	**2.70**	
Phosphoenolpyruvate	**1.95**	**1.77**	**1.27**	**4.43**	**1.84**
Pyruvate	**7.71**	**3.62**	**3.11**	**3.84**	**5.35**
TCA cycle					
Citrate	**4.00**	**1.28**	**0.89**	**5.06**	**4.39**
2-Oxoglutarate	**1.90**	**0.73**	**0.60**	**1.10**	**1.31**
Succinate	**0.60**	**0.30**	**0.07**	**0.13**	**0.24**
Fumarate	**2.11**	**0.71**	**0.35**	**0.37**	**0.52**
Malate	**3.45**	**0.48**	**0.28**	**0.34**	**0.40**

TABLE S5 continued

Metabolites	Fold change[a]				
	NAG	MTL	SUCR	GLC	XYL
Amino acids and derivatives					
Alanine	4.21	0.47	0.05	0.16	0.27
Aspartate	0.13	0.08	0.05	0.06	0.03
Beta-Alanine	0.55	0.44	0.24	0.28	0.42
Glutamate	0.89	0.75	0.43	0.70	0.66
Glutamine	1.12	1.48	1.41	1.33	1.09
Glycine	0.76	0.36	0.20	0.18	0.23
Homoserine	1.16	1.39	1.23	1.21	3.02
Isoleucine	1.20	1.27	0.16	0.77	0.58
Leucine	1.05	1.43	0.24	0.74	0.56
Lysine	0.19	0.42	0.08	0.21	0.17
Methionine	2.53	0.31	0.12	0.26	0.47
N,N -Dimethylglycine	1.02	0.73	1.38	2.23	1.29
N -Acetylglutamate	6.77	0.78		0.87	1.08
O -Acetylserine	18.63	11.83	0.91	3.08	11.50
Ornithine	1.46	0.41	0.22	0.38	1.04
Phenylalanine	0.91	0.35	0.13	0.24	0.37
Proline	0.57	0.39	0.06	0.13	0.27
Pyroglutamate	0.64	0.91	0.55	0.70	0.70
Serine	1.15	0.41	0.25	0.33	0.42
Threonine	1.17	1.01	0.31	0.42	0.74
Tyrosine	0.93	0.72	0.17	0.43	0.48
Valine	1.99	1.64	0.54	0.62	0.66
Polyamines and dipeptides					
Cadaverine	0.93	1.17	0.73	1.36	1.06
Glycylglycine	0.92	0.46	0.06	0.18	0.71
Putrescine	0.99	0.52	0.12	0.13	0.53
Organic acids					
2-Aminoadipate	0.36	1.36	0.08	0.29	0.71
Benzoate	1.35	1.74	1.45	1.76	1.91
Erythronate	8.23	2.70	1.61	2.90	1.91
Glycerate	0.99	1.98	1.86	2.21	1.34
Glyoxylate	1.72	1.31	2.20	5.33	3.03
2-Hydroxyglutarate	2.03	0.44	0.29	0.19	0.63
Iminodiacetate	31.08	1.90	0.77	5.64	2.13
Lactate	1.30	0.33	0.51	0.42	0.30
Maleate	2.53	0.64	0.42	0.69	0.52
Malonate	#	#	#	#	#
2-Methyl-malate	4.02	2.59	2.29	4.20	2.88
2-Methyl-serine	1.83	2.81	3.29	1.27	0.91
Nicotinamide	0.91	1.03	0.72	0.86	0.85
Nicotinate	0.39	0.71	0.58	0.66	0.42
Phenylacetate	0.39	0.79	0.88	0.81	0.59
Succinate-methylester	0.96	1.34	1.04	1.29	1.05
Tartrate	0.68	1.59	10.82	168.67	3.06
Threonate	2.59	4.20	29.65	13.08	2.64
Urea	0.98	0.80	0.26	0.69	0.27
Butanoates					
2-Aminobutanoate	0.42	0.16	0.12	0.19	0.19
4-Aminobutanoate	0.81	0.55	0.21	0.19	0.46
3-Aminoisobutanoate	3.03	0.29	0.09	0.20	0.50
2-Hydroxybutanoate	0.82	1.25	1.16	1.29	1.06
3-Hydroxybutanoate	0.19	0.29	0.34	0.29	0.18
4-Oxobutanoate	0.80	0.51	0.36	0.48	0.59

TABLE S5 continued

Metabolites	Fold change[a]				
	NAG	MTL	SUCR	GLC	XYL
Lipid and phospholipid headgroup components					
Ethanolamine	0.41	0.23	0.12	0.19	0.18
Ethanolaminephosphate	1.01	0.09	0.23	0.36	0.36
Diethanolamine	6.30	8.24	2.19	5.14	7.85
Glycerol-3-phosphate	0.18	0.11	0.05	0.12	0.07
Glycerophosphoglycerol	0.05	0.03	0.09	0.89	0.07
Octanoate	0.67	0.29	0.27	0.31	0.25
3-Hydroxydecanoate	0.57	0.60	0.67	0.63	0.52
Dodecanoate	0.59	1.13	1.52	1.52	0.95
Dodecanol	0.44	13.58	11.49	5.63	4.75
Tetradecanoate	0.36	0.44	0.25	0.28	0.41
Tetradecanol	0.79	1.84	1.69	1.61	1.28
Hexadecanoate	0.71	1.02	0.83	0.96	1.12
Octadecanoate	0.51	1.11	1.11	0.80	0.93
Octadecenoate	0.29	0.22	0.80	0.47	0.20
Nucleosides and nucleobases					
Cytosine	0.35	0.30	0.06	0.05	0.33
Thymine	1.20	0.94	0.16	0.22	0.99
Uracil	0.66	0.74	0.66	0.31	0.55
Uridine	0.28	2.11	1.03	0.27	18.28
Others					
Borate	0.67	0.54	2.18	7.71	1.75
Carbodiimide	0.69	1.20	0.85	0.84	0.90
Hydrogen sulfide	0.78	0.82	0.80	0.51	0.84
Phosphate	0.79	0.88	0.67	0.69	0.81
Phosphate monomethyl ester	0.88	1.29	1.36	1.34	1.11
Toluate	0.94	1.41	1.30	1.41	1.42

n.d	
🟥	< 0.1
🟥	< 0.34
🟧	< 0.66
🟨	0.67 - 1.5
🟩	> 1.5
🟩	> 2.5
🟩	> 5.0
🟩	> 10.0

#	compound not detected in reference
##	compound not detected in reference, but intense peak in corresponding cultivation condition
*****	probably dephosphorylated during extraction

---

[a], Fold change in metabolite abundance as compared to reference condition (succinate as sole carbon source)

Sensory/regulatory proteins related to carbohydrate catabolism in *P. inhibens* DSM 17395 (TABLE S6)

TABLE S6 Genes for sensory/regulatory proteins of *P. inhibens* DSM 17395 affiliating with the degradation of the five studied carbohydrates. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose; Succ, succinate.

Acc. No. (PGA1_)	**Name**	**Predicted function**	**Catabolic enzymes specifically formed**[a]	**Fold change**[b] **in abundance (2D DIGE)**					**Mascot score (gel-free analysis)**					
				NAG	**MTL**	**SUCR**	**GLC**	**XYL**	**NAG**	**MTL**	**SUCR**	**GLC**	**XYL**	**Succ**
N-*Acetylglucosamine*														
Nag-proteins (NagKBA) and ABC transporter c27930-60														
c27900		GntR family transcriptional regulator[c]	+						779					
Mannitol														
Mannitol 2-dehydrogenase (MtlK) and ABC transporter c13180-210														
c13220		Putative transcriptional regulator, lacI family	(+)							103				
Sucrose														
Agl-proteins (AglEFGAK)														
c07850	AglR[c]	Transcriptional regulator, lacI family	+	-1.1	-1.1	1.8	-1.1	1.3						
Xylose														
Xylose isomerase (XylA), xylulose kinase (XylB)														
c13990		Putative transcriptional regulator, lacI family	+										91	
Fructose														
Fructokinase Frk and ABC transporter FrcBCAK														
c28070	FrcR[c]	MarR family transcriptional regulator	(+)											
Xylose, Glucose, Sucrose														
ABC transporter XylFGH														
262p00460		Transcriptional regulator, MerR family	+											

[a] +, Catabolic enzymes identified at specifically increased abundance; (+), rather specific increase in abundance; –, non-specific identification.

[b] As compared to succinate-adapted cells of *P. inhibens* DSM 17395.

[c] For annotation refinement or full enzyme names see Supplementary Table S3.

Fold change	n.d.	> -1.5	> 1.5
Mascot score	n.d.	> 25	> 500

Comparative genome analysis to study the relevance of the studied carbohydrate degradation pathways within *Rhodobacterales* and other heterotrophic bacteria (TABLE S7, FIG S7)

TABLE S7 Genes of *P. inhibens* DSM 17395 used for comparative genomics are compiled together with their locus tags, EC numbers and corresponding enzyme names.

Gene name	Accesion No.	Predicted function	EC number
CARBOHYDRARTE TRANSPORT AND METABOLISM			
N-*Acetylglucosamine*			
acs	PGA1_c12950	Acetyl-coenzyme A synthetase	6.2.1.1
nagA	PGA1_c27880	*N*-Acetylglucosamine-6-phosphate deacetylase	3.5.1.25
nagB	PGA1_c27890	Glucosamine-6-phosphate deaminase	3.5.99.6
	PGA1_c27900	GntR family transcriptional regulator	
nagK	PGA1_c27910	*N*-Acetylglucosamine kinase	2.7.1.59
murQ	PGA1_c27920	*N*-Acetylmuramic acid 6-phosphate etherase	4.2.1.126
	PGA1_c27930	*N*-Acetylglucosamine ABC transporter, periplasmic sugar-binding protein	
	PGA1_c27940	*N*-Acetylglucosamine ABC transporter, permease	
	PGA1_c27950	*N*-Acetylglucosamine ABC transporter, permease	
	PGA1_c27970	*N*-Acetylglucosamine ABC transporter, ATP-binding protein	
Mannitol			
mtlK	PGA1_c13160	Mannitol 2-dehydrogenase	1.1.1.67
polS	PGA1_c13170	Sorbitol dehydrogenase	1.1.1.14
smoK	PGA1_c13180	Mannitol ABC transporter, ATP-binding transport protein	
	PGA1_c13190	Mannitol ABC transporter, permease	
	PGA1_c13200	Mannitol ABC transporter, permease	
	PGA1_c13210	Mannitol ABC transporter, periplasmic sugar-binding protein	
	PGA1_c13220	Putative transcriptional regulator, lacI family	
Sucrose			
aglR	PGA1_c07850	Transcriptional regulator, lacI family	
aglE	PGA1_c07860	Alpha-glucosides ABC transporter, periplasmic sugar-binding protein	
aglF	PGA1_c07870	Alpha-glucoside ABC transporter, permease	
aglG	PGA1_c07880	Alpha-glucoside ABC transporter, permease	
aglA	PGA1_c07890	Alpha-glucosidase	3.2.1.20
aglK	PGA1_c07900	Alpha-glucoside transport ATP-binding protein	
Fructose			
frk	PGA1_c28020	Fructokinase	2.7.1.4
frcK	PGA1_c28030	Fructose ABC transporter, kinase	
frcA	PGA1_c28040	Fructose ABC transporter, ATP-binding protein	
frcC	PGA1_c28050	Fructose ABC transporter, permease	
frcB	PGA1_c28060	Fructose ABC transporter, periplasmic sugar-binding protein	
frcR	PGA1_c28070	MarR family transcriptional regulator	
Xylose, glucose, sucrose			
xylF	PGA1_262p00430	Xylose ABC transporter, periplasmic sugar-binding protein	
xylH	PGA1_262p00440	Xylose ABC transporter, permease	
xylG	PGA1_262p00450	Xylose ABC transporter, ATP-binding protein	
	PGA1_262p00460	Transcriptional regulator, MerR family	
Glucose, sucrose			
glk	PGA1_c05420	Glucokinase	2.7.1.2
Xylose			
	PGA1_c13990	Putative transcriptional regulator, lacl family	
xylA	PGA1_c14000	Xylose isomerase	5.3.1.5
xylB	PGA1_c14010	Xylolose kinase	2.7.1.17

Gene name	Accesion No.	Predicted function	EC number
CENTRAL METABOLISM			
Entner-Doudoroff pathway			
pgl	PGA1_c15170	6-Phosphogluconolactonase	3.1.1.31
pgi	PGA1_c27980	Glucose-6-phosphate isomerase	5.3.1.9
zwf2	PGA1_c27990	Glucose-6-phosphate 1-dehydrogenase	1.1.1.49
edd	PGA1_c28000	Phosphogluconate dehydratase	4.2.1.12
eda	PGA1_c28010	KHG/KDPG aldolase	4.1.3.16/ 4.1.2.14
Lower branch of Embden-Meyerhof Parnas pathway and pyruvate dehydrogenase complex			
gpmI	PGA1_c02770	2,3-Bisphosphoglycerate-independent phosphoglycerate mutase	5.4.2.1
pykA	PGA1_c06970	Pyruvate kinase	2.7.1.40
eno	PGA1_c11420	Enolase	4.2.1.11
lpdA1	PGA1_c17390	Dihydrolipoyl dehydrogenase	1.8.1.4
pgk	PGA1_c17530	Phosphoglycerate kinase	2.7.2.3
	PGA1_c17540	Septum formation initiator-like protein	
pdhA	PGA1_c17550	Pyruvate dehydrogenase, E1 alpha subunit	1.2.4.1
pdhB	PGA1_c17560	Pyruvate dehydrogenase, E1 beta subunit	1.2.4.1
pdhC	PGA1_c17570	Pyruvate dehydrogenase, E2	2.3.1.12
gap	PGA1_c27490	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12
Pentose phosphate pathway			
rpe	PGA1_c04170	Ribulose-phosphate 3-epimerase	5.1.3.1
tal	PGA1_c05780	Transaldolase	2.2.1.2
rpiB	PGA1_c10730	Ribose-5-phosphate isomerase B	5.3.1.6
tktA	PGA1_c17230	Transketolase	2.2.1.1
rpiA	PGA1_c23740	Ribose-5-phosphate isomerase A	5.3.1.6
TCA cycle			
lpd2	PGA1_c03550	Dihydrolipoyl dehydrogenase	1.8.1.4
sucB	PGA1_c03590	2-Oxoglutarate dehydrogenase complex, E2	2.3.1.61
sucA	PGA1_c03600	2-Oxoglutarate dehydrogenase, E1	1.2.4.2
sucD	PGA1_c03610	Succinyl-CoA ligase [ADP-forming], subunit alpha	6.2.1.5
	PGA1_c03620	Hypothetical protein	
sucC	PGA1_c03630	Succinyl-CoA ligase [ADP-forming], subunit beta	6.2.1.5
mdh	PGA1_c03650	Malate dehydrogenase	1.1.1.37
sdhC	PGA1_c03740	Succinate dehydrogenase, cytochrome b_{556} subunit	1.3.99.1
sdhD	PGA1_c03750	Succinate dehydrogenase, hydrophobic membrane anchor subunit	1.3.99.1
sdhA	PGA1_c03760	Succinate dehydrogenase, flavoprotein subunit	1.3.99.1
	PGA1_c03770	Hypothetical protein	
sdhB	PGA1_c03780	Succinate dehydrogenase, iron-sulfur subunit	1.3.99.1
fumC	PGA1_c13520	Fumarate hydratase class II	4.2.1.2
gltA1	PGA1_c16970	Citrate synthase	2.3.3.1
acnA	PGA1_c18830	Aconitate hydratase	4.2.1.3
icd	PGA1_c28340	Isocitrate dehydrogenase [NADP]	1.1.1.42

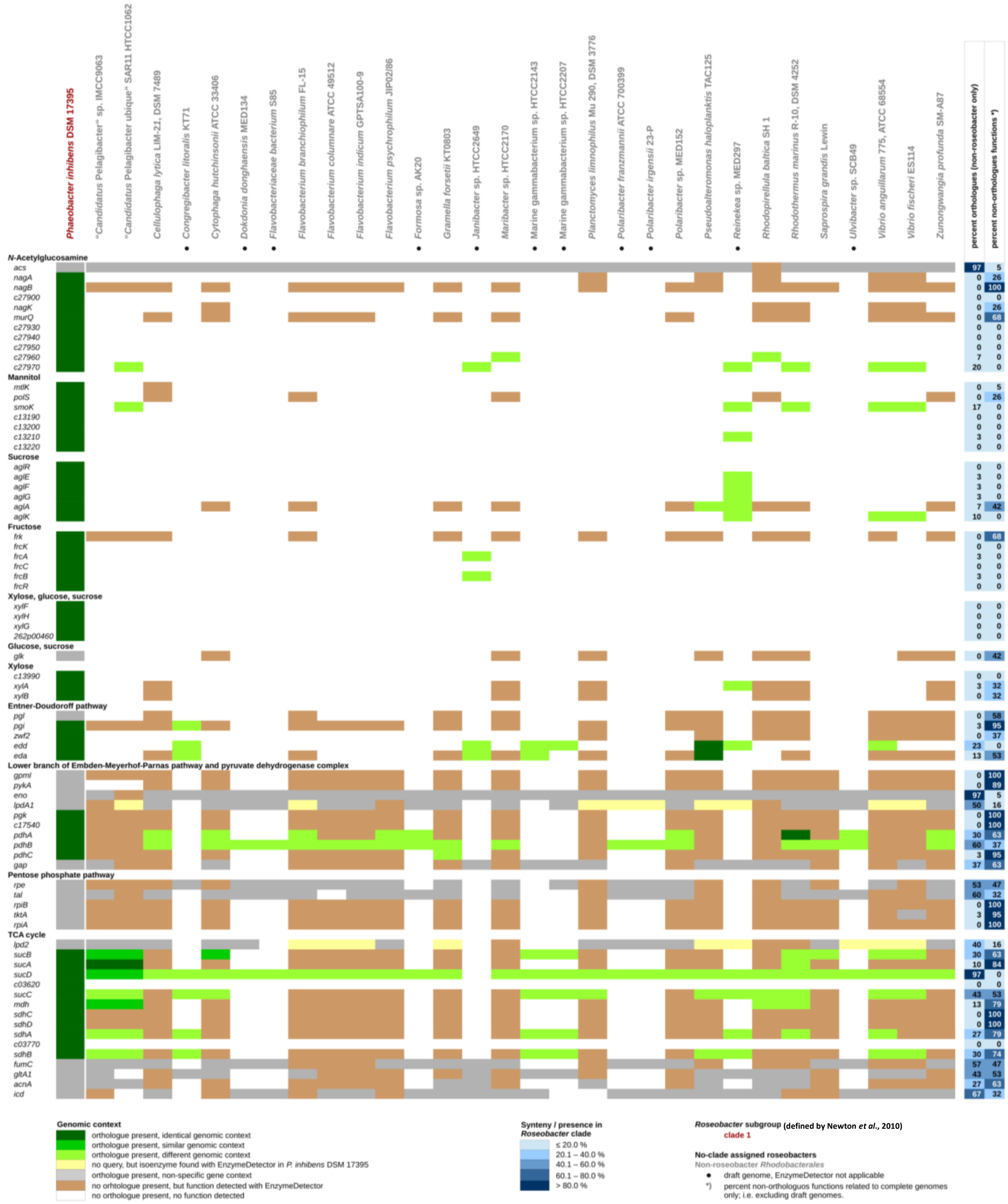


FIG S7 Genome comparison of 30 selected heterotrophic bacteria. Shown are genes of *P. inhibens* DSM 17395 related to the investigated carbohydrate transport, degradation and regulation, as well as central metabolism (gene names as shown in Table S7) with their respective orthologues in the other species. Where applicable, the genomic context of the involved genes was investigated, leading to a categorization, with *P. inhibens* DSM 17395 set as reference (see Material and Methods).

SDS-PAGE gels of outer and cytoplasmic membrane protein-enriched and soluble fractions (FIG S8)

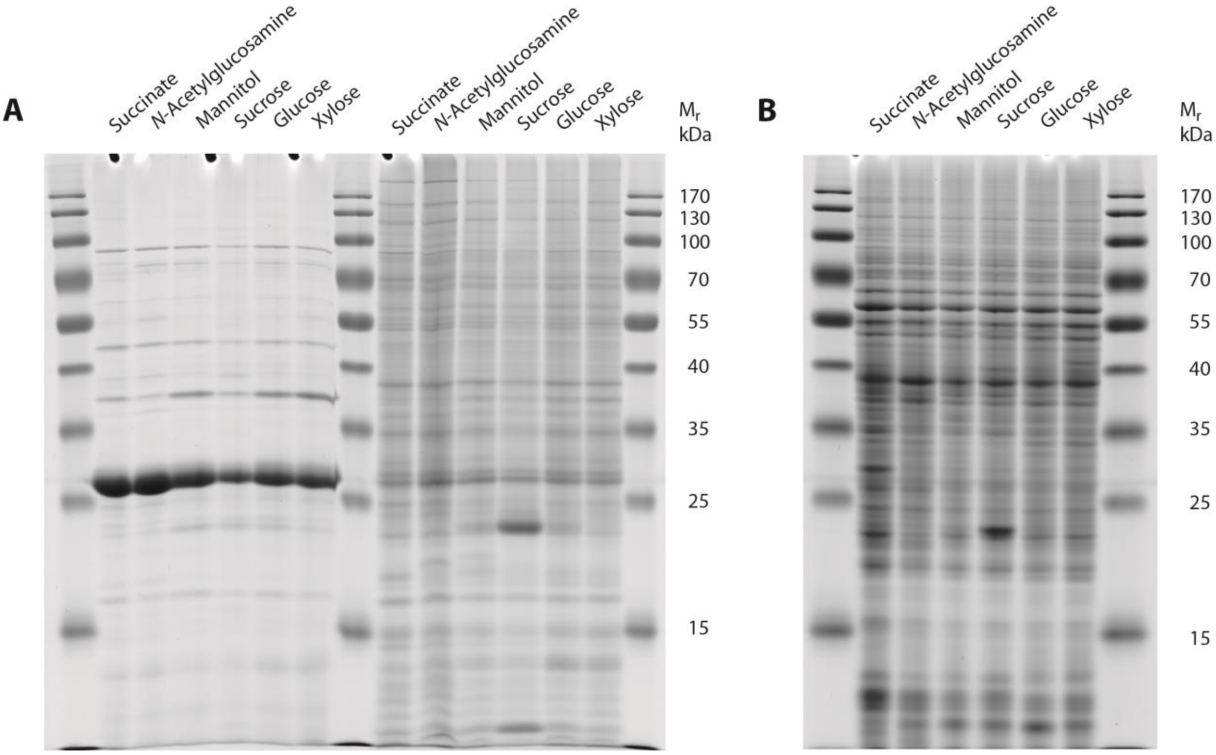
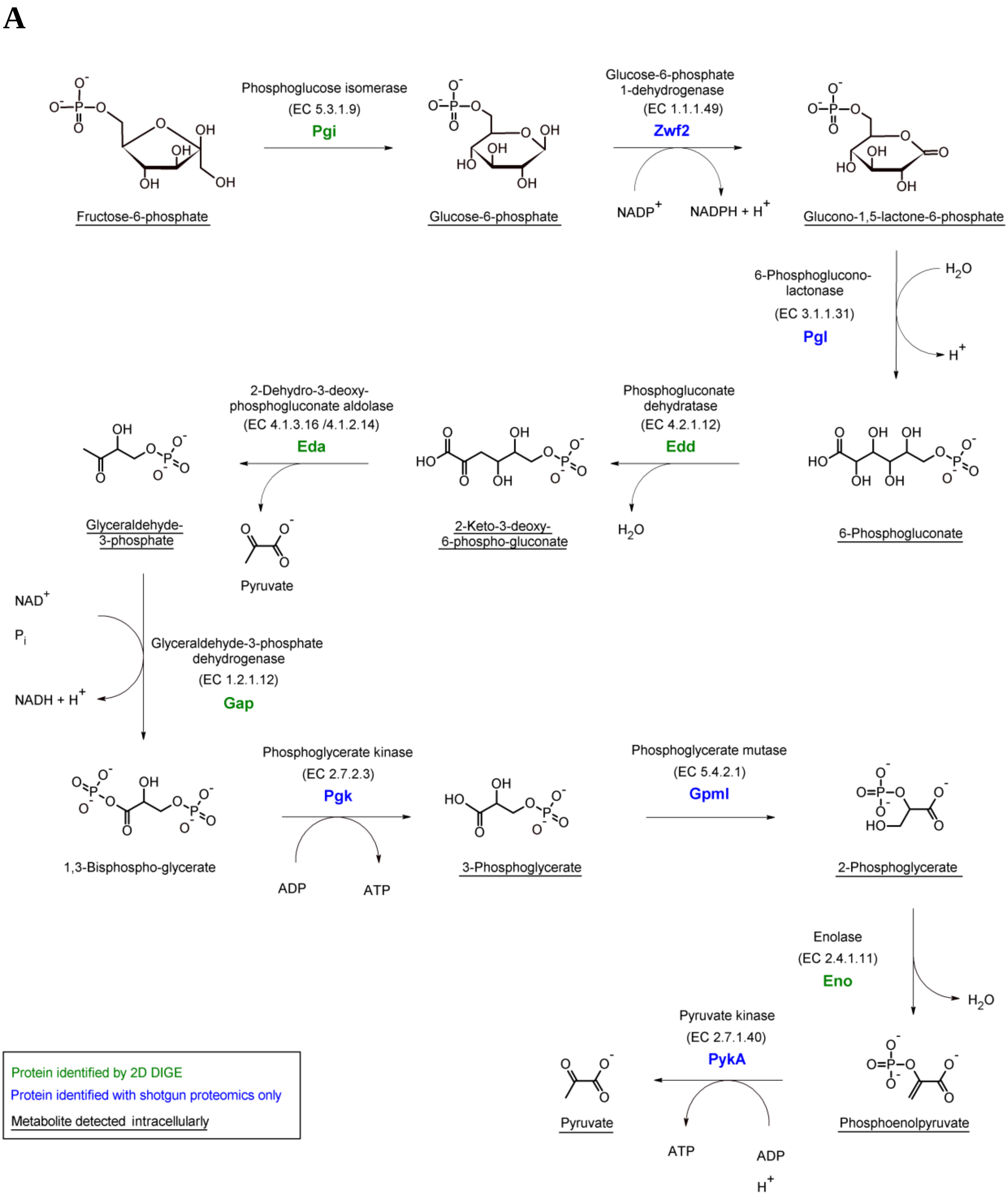


FIG S8. Separation of (A) outer (left) and cytoplasmic (right) membrane protein-enriched and (B) soluble fractions of *P. inhibens* DSM 17395 by SDS-PAGE. Substrate-adaptation conditions are indicated at the top of each gel lane.

Central metabolism in *P. inhibens* DSM 17395 (FIGs. S8–S11)

1. Entner-Doudoroff pathway and lower branch of Embden-Meyerhof-Parnas pathway (FIG S9)



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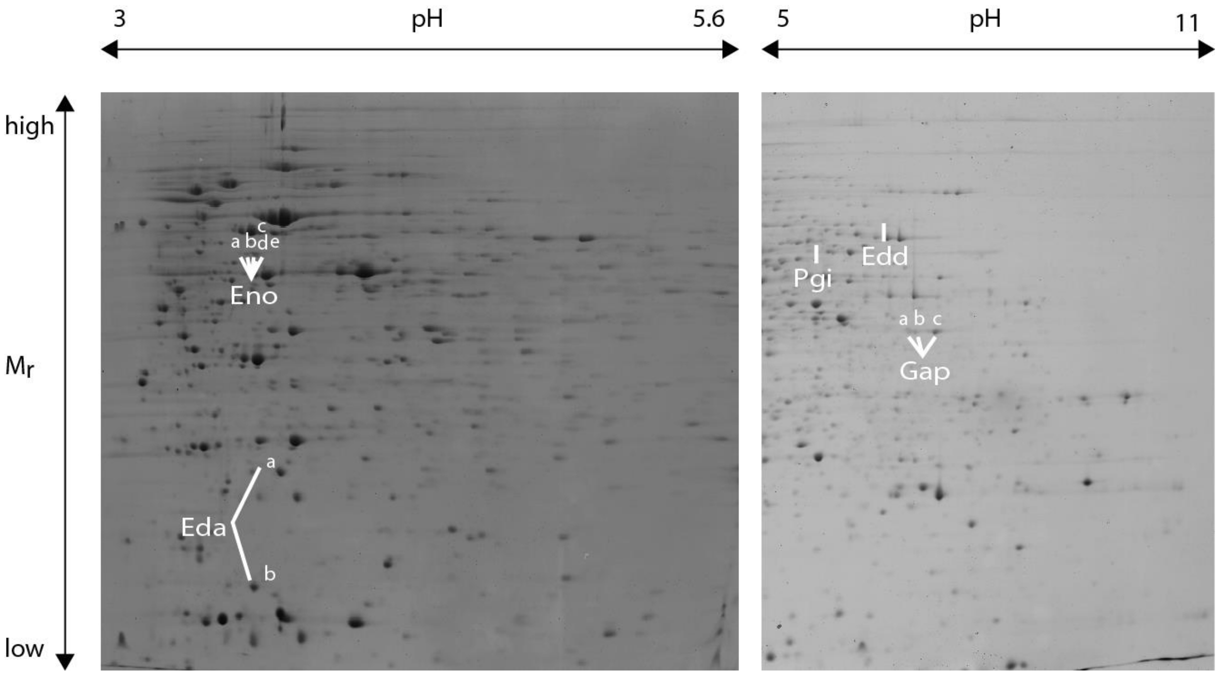


C

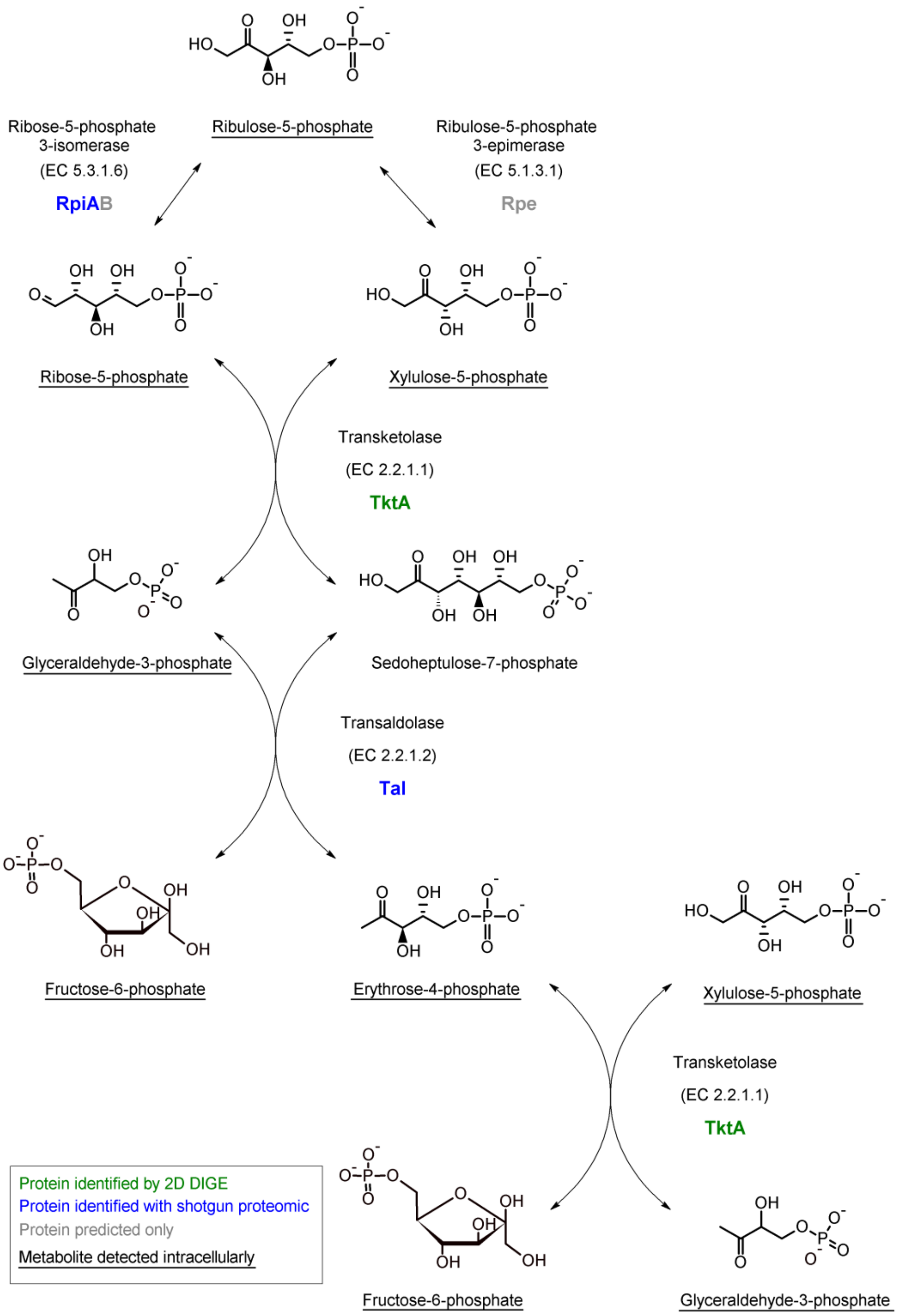
Acc. No. (PGA_)	Name	Predicted function	Spot	% of cumulative spot volume of single protein	Fold change in abundance as compared to succinate-adapted cells (2D DIGE)				
					NAG	**MTL**	**SUCR**	**GLC**	**XYL**
CARBOHYDRATE METABOLISM									
Entner-Doudoroff pathway									
c27980	Pgi	Glucose-6-phosphate isomerase			**5.0**	**3.5**	**6.4**	**6.2**	**3.4**
c28000	Edd	Phosphogluconate dehydratase			**8.6**	**6.5**	**9.0**	**9.3**	**6.5**
c28010	Eda	KHG/KDPG aldolase	a	23.1	**6.6**	**4.1**	**5.3**	**6.3**	**3.9**
c28010	Eda	KHG/KDPG aldolase	b	76.9	**1.4**	**1.7**	**2.1**	**1.9**	**1.0**
Lower branch of Emden-Meyerhof-Parnas pathway									
c27490	Gap	Glyceraldehyde-3-phosphate dehydrogenase	a	7.8	**1.4**	**1.9**	**2.3**	**2.2**	**2.6**
c27490	Gap	Glyceraldehyde-3-phosphate dehydrogenase	b	23.5	**9.2**	**11.5**	**13.9**	**14.4**	**15.1**
c27490	Gap	Glyceraldehyde-3-phosphate dehydrogenase	c	68.7	**10.7**	**13.6**	**14.7**	**16.6**	**16.6**
c11420	Eno	Enolase	a	3.8	**1.5**	**2.5**	**3.4**	**2.2**	**4.1**
c11420	Eno	Enolase	b	13.5	**2.1**	**2.6**	**3.4**	**2.5**	**3.9**
c11420	Eno	Enolase	c	59.0	**2.0**	**2.4**	**2.6**	**2.3**	**3.1**
c11420	Eno	Enolase	d	9.8	**2.0**	**2.3**	**2.6**	**2.2**	**3.1**
c11420	Eno	Enolase	e	13.8	**1.9**	**2.3**	**2.6**	**2.0**	**3.1**

Fold change **> - 1.5** **> 1.5** **> 5.0** **> 10.0**

FIG S9 Proteomic data and genomic predictions for the Entner-Doudoroff and lower branch of the Embden-Meyerhof-Parnas pathway of *P. inhibens* DSM 17395. (A) Enzymatic reactions of the Entner-Doudoroff and lower branch of Emden-Meyerhof-Parnas pathway. (B) 2DE gel image of succinate-adapted cells. Identified proteins of the Entner-Doudoroff and lower Emden-Meyerhof-Parnas pathway are marked. (C) Protein ID, predicted function, fold change in abundance of marked proteins and percentage share of each protein spot from the cumulative spot volume of a single protein. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose.

2. Pentose phosphate pathway (FIG S10)

A



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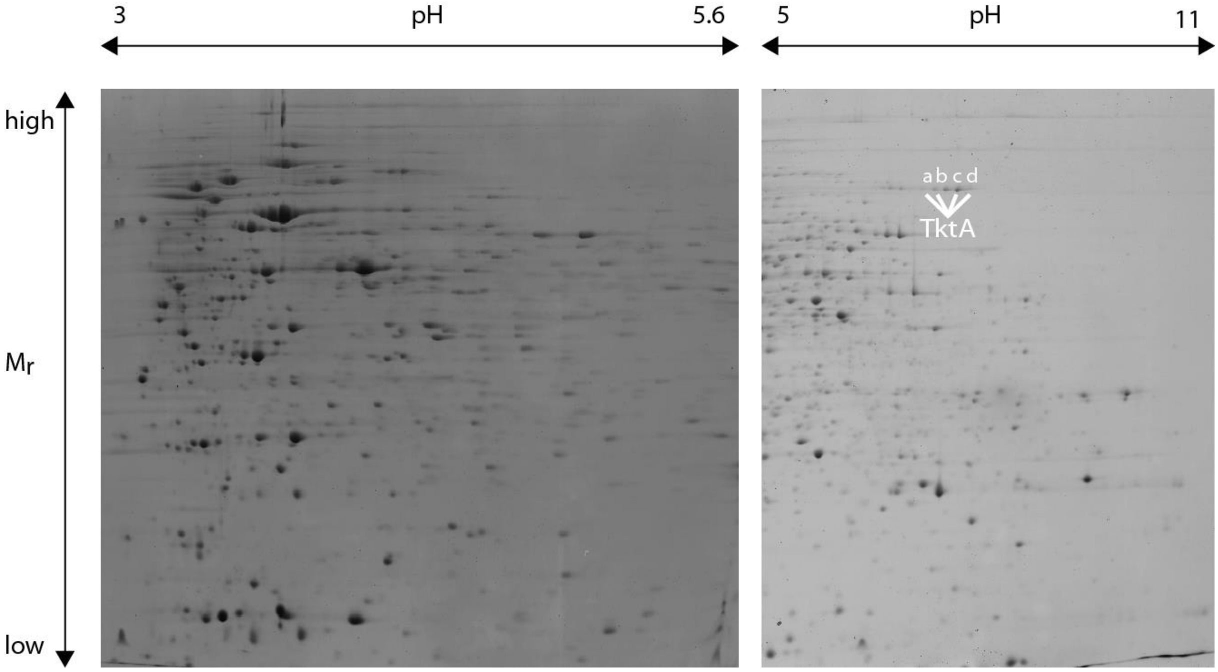


C

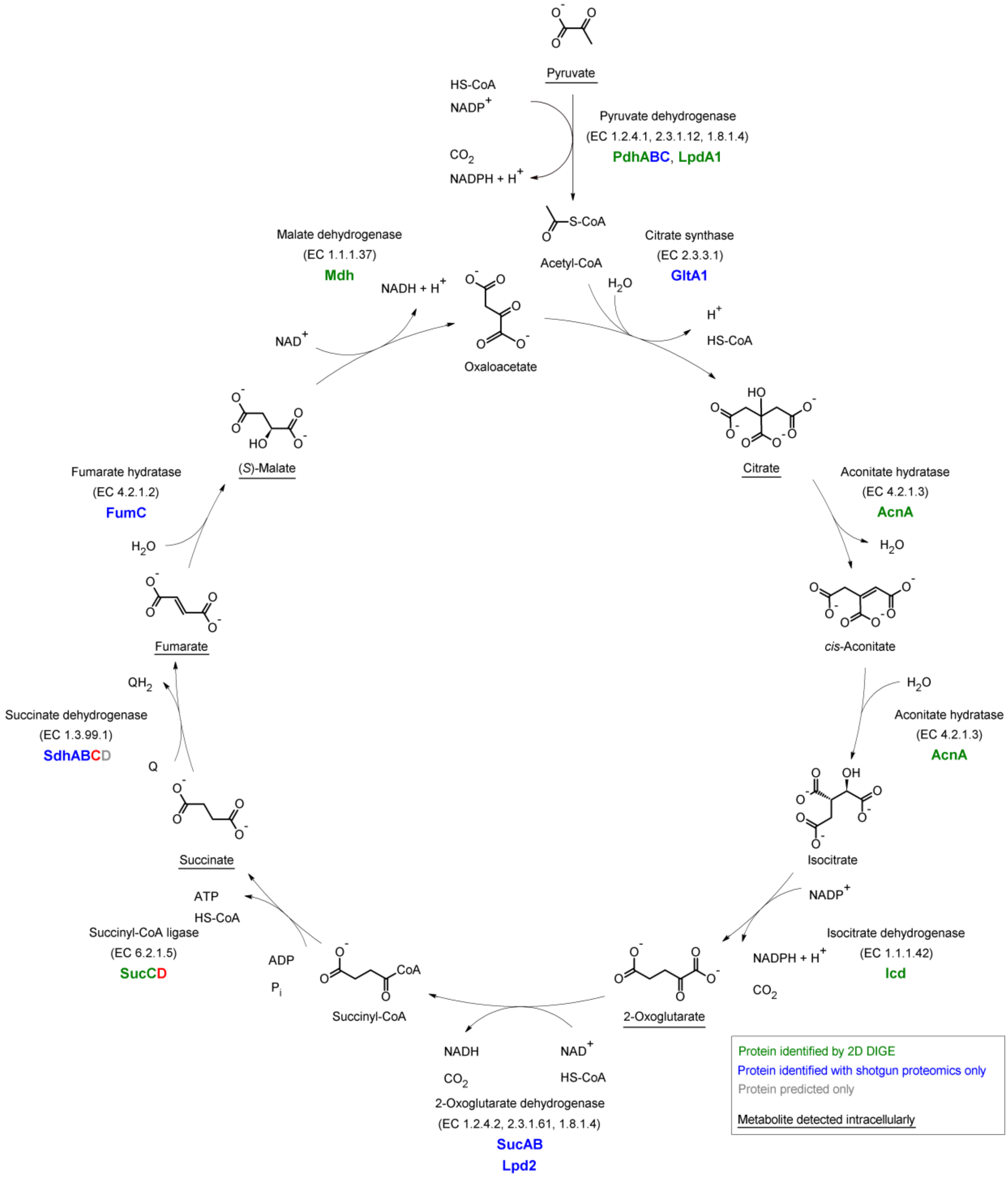
Acc. No. (PGA_)	Name	Predicted function	Spot	% of cumulative spot volume of single protein	Fold change in abundance as compared to succinate-adapted cells (2D DIGE)				
					NAG	MTL	SUCR	GLC	XYL
CARBOHYDRATE METABOLISM									
Pentose phosphate pathway									
c17230	TktA	Transketolase	a	5.1	-1.1	-1.2	1.1	-1.1	1.7
c17230	TktA	Transketolase	b	9.0	-1.2	-1.2	-1.0	-1.1	1.7
c17230	TktA	Transketolase	c	74.9	-1.3	-1.4	-1.7	-1.3	1.2
c17230	TktA	Transketolase	d	11.1	-1.2	-1.3	-1.5	-1.2	1.2

Fold change < -1.5 | > - 1.5 | > 1.5

FIG S10 Proteomic data and genomic predictions for the pentose phosphate pathway of *P. inhibens* DSM 17395. (A) Enzymatic reactions of the pentose phosphate pathway. (B) 2DE gel image of succinate-adapted cells. Identified proteins of the pentose phosphate pathway are marked. (C) Protein ID, predicted function, fold change in abundance of marked proteins and percentage share of each protein spot from the cumulative spot volume of a single protein. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose.

3. Pyruvate dehydrogenase complex and TCA cycle (FIG S11)

A



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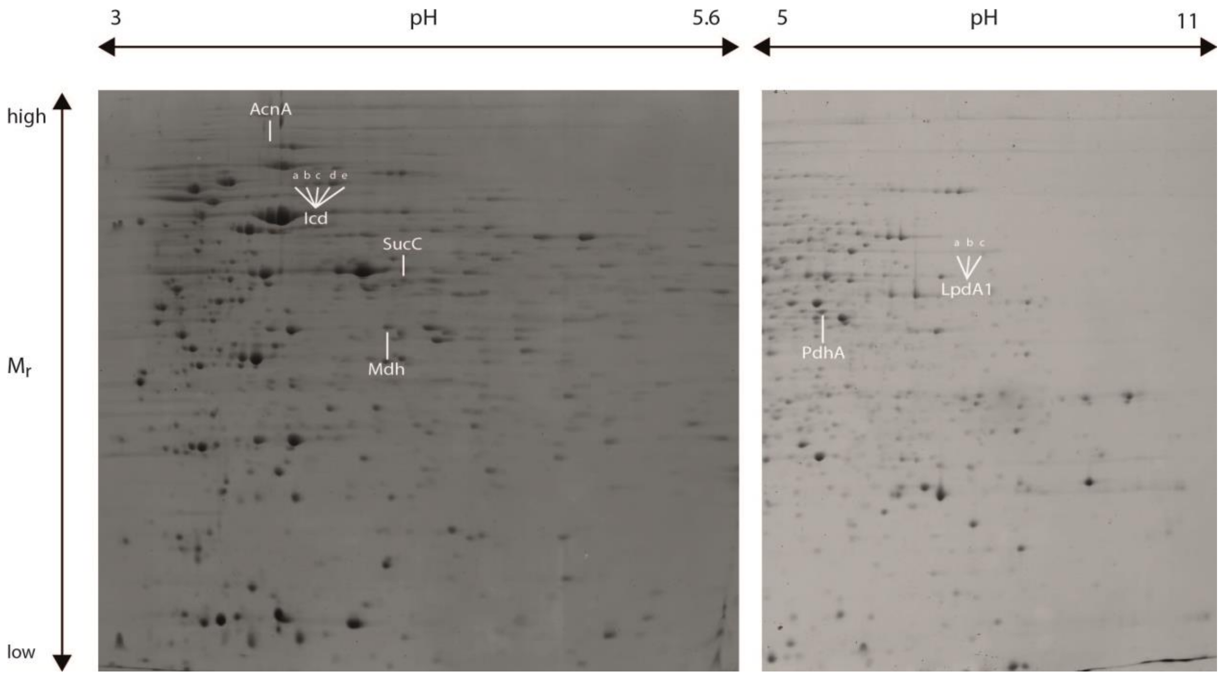

C

Acc. No. (PGA_)	Name	Predicted function	Spot	% of cumulative spot volume of single protein	Fold change in abundance as compared to succinate-adapted cells (2D DIGE)				
					NAG	MTL	SUCR	GLC	XYL
CARBOHYDRATE METABOLISM									
Pyruvate dehydrogenase complex									
c17550	PdhA	Pyruvate dehydrogenase, E1 alpha subunit[a]			-1.3	-1.0	1.1	-1.1	-1.6
c17390	LpdA1[a]	Dihydrolipoyl dehydrogenase[a]	a	8.3	-2.0	1.2	2.0	1.1	-1.0
c17390	LpdA1[a]	Dihydrolipoyl dehydrogenase[a]	b	24.0	-2.3	1.1	1.6	-1.1	-1.5
c17390	LpdA1[a]	Dihydrolipoyl dehydrogenase[a]	c	67.7	-2.3	1.1	1.4	-1.1	-1.8
TCA cycle									
c18830	AcnA	Aconitate hydratase			1.6	-1.3	1.2	-1.0	1.6
c28340	Icd	Isocitrate dehydrogenase [NADP]	a	2.0	1.4	-1.4	1.6	-1.1	1.3
c28340	Icd	Isocitrate dehydrogenase [NADP]	b	4.9	1.6	-1.1	1.8	-1.1	1.5
c28340	Icd	Isocitrate dehydrogenase [NADP]	c	18.1	1.5	-1.3	1.1	-1.3	1.1
c28340	Icd	Isocitrate dehydrogenase [NADP]	d	63.1	1.6	-1.3	-1.1	-1.4	-1.0
c28340	Icd	Isocitrate dehydrogenase [NADP]	e	11.9	1.6	-1.4	-1.2	-1.4	-1.1
c03630	SucC	Succinyl-CoA ligase [ADP-forming], subunit beta			-1.3	-1.5	1.1	-1.6	-1.7
c03650	Mdh	Malate dehydrogenase			-1.0	1.0	1.1	1.1	1.6

Fold change < -1.5 | > - 1.5 | > 1.5

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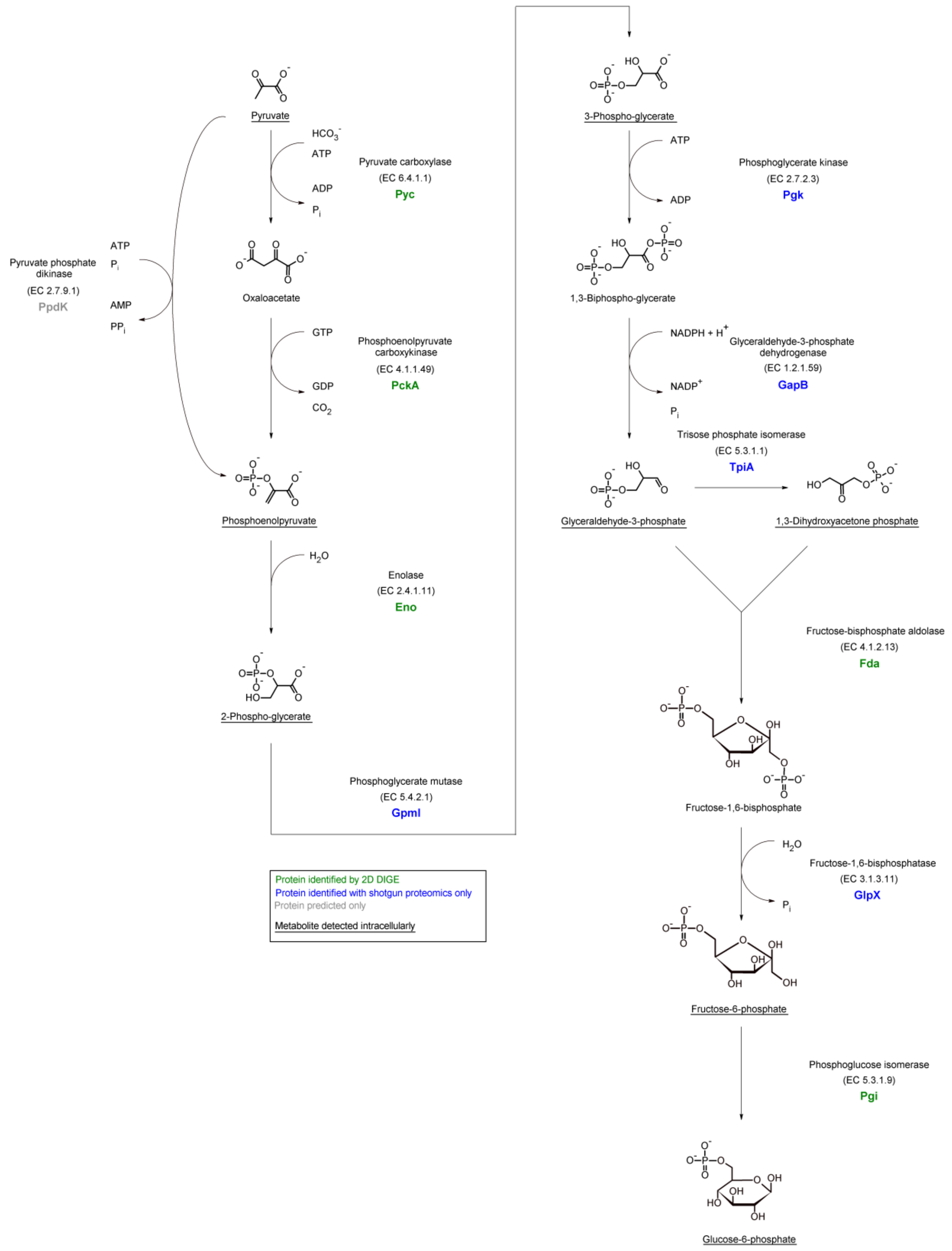
[a] Annotation refinement (see Supplementary TABLE S3).

FIG S11 Proteomic data and genomic predictions for the pyruvate dehydrogenase complex and TCA cycle of *P. inhibens* DSM 17395. (A) Enzymatic reactions of the TCA cycle. (B) 2DE gel image of succinate-adapted cells. Identified proteins of the TCA cycle are marked. (C) Protein ID, predicted function, fold change in abundance of marked proteins and percentage share of each protein spot from the cumulative spot volume of a single protein. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose.

4. Gluconeogenesis (FIG S12)

A



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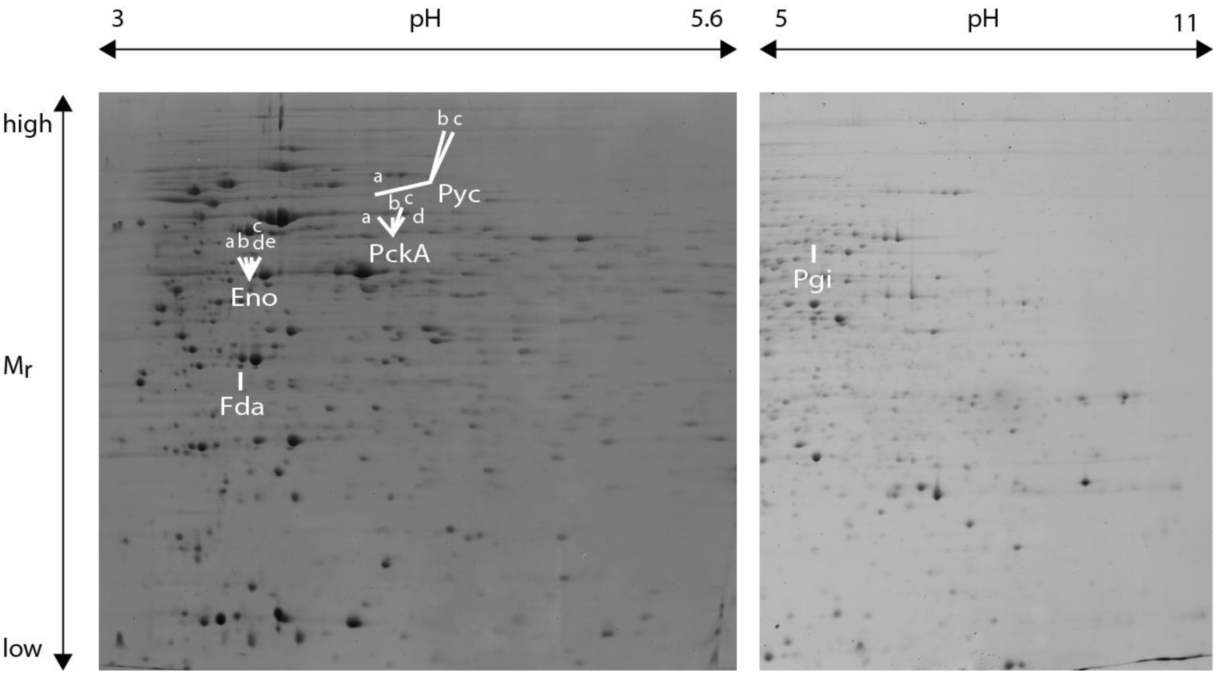

C

Acc. No. (PGA_)	Name	Predicted function	Spot	% of cumulative spot volume of single protein	Fold change in abundance as compared to succinate-adapted cells (2D DIGE)				
					NAG	MTL	SUCR	GLC	XYL
CARBOHYDRATE METABOLISM									
Gluconeogenesis									
c09420	Pyc	Pyruvate carboxylase	a	73.6	1.1	-1.3	-1.3	-1.5	-1.2
c09420	Pyc	Pyruvate carboxylase	b	6.7	-1.1	1.1	1.4	1.3	1.6
c09420	Pyc	Pyruvate carboxylase	c	19.6	-1.0	1.3	1.3	1.5	1.5
c01140	PckA	Phosphoenolpyruvate carboxykinase	a	15.4	-1.1	-2.4	-1.6	-2.1	-2.0
c01140	PckA	Phosphoenolpyruvate carboxykinase	b	14.2	-1.5	-1.8	-1.4	-1.5	-1.5
c01140	PckA	Phosphoenolpyruvate carboxykinase	c	22.2	-1.6	-1.7	-1.8	-1.7	-1.7
c01140	PckA	Phosphoenolpyruvate carboxykinase	d	48.1	-4.0	-3.4	-3.0	-2.9	-3.6
c11420	Eno	Enolase	a	3.8	1.5	2.5	3.4	2.2	4.1
c11420	Eno	Enolase	b	13.5	2.1	2.6	3.4	2.5	3.9
c11420	Eno	Enolase	c	59.0	2.0	2.4	2.6	2.3	3.1
c11420	Eno	Enolase	d	9.8	2.0	2.3	2.6	2.2	3.1
c11420	Eno	Enolase	e	13.8	1.9	2.3	2.6	2.0	3.1
c23910	Fda	Fructose-bisphosphate aldolase class 1			-2.7	-3.3	-3.6	-3.4	-2.6
c27980	Pgi	Glucose-6-phosphate isomerase			5.0	3.5	6.4	6.2	3.4

Fold change < -1.5 | > - 1.5 | > 1.5 | > 5.0 | > 10.0

FIG S12 Proteomic data and genomic predictions for the gluconeogenesis of *P. inhibens* DSM 17395. (A) Gluconeogenesis. (B) 2DE gel image of succinate-adapted cells. Identified proteins of gluconeogenesis are marked. (C) Protein ID, predicted function, fold change in abundance of marked proteins and percentage share of each protein spot from the cumulative spot volume of a single protein. Abbreviations of adaptation substrates: NAG, *N*-acetylglucosamine; MTL, mannitol; SUCR, sucrose; GLC, glucose; XYL, xylose.

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Thole S, Kalhöfer D, Voget S, Berger M, Engelhardt T, Liesegang H, Wollherr A, Kjelleberg S, Daniel R, Simon M, Thomas T, Brinkhoff T. 2012. *Phaeobacter gallaeciensis* genomes from globally opposite locations reveal high similarity of adaptation to surface life. ISME J. **6:**2229–2244.