Central carbon metabolism

Although *P. desulfatans* F1^T encodes and highly expresses a phosphoenolpyruvate (PEP)-dependent phosphotransferase (PTS) system, it is unclear if this is used for the transport of monosaccharides. It includes two phosphoenolpyruvate-protein phosphotransferases (*ptsI*), three phosphocarrier proteins, a HPr kinase/ phosphorylase (*hprK*) and ten predicted proteins with PTS enzyme II_A type-2 domains, of which most were highly expressed, but lacks the conventional II_B and II_C proteins constituting the actual sugar transporter. The II_A phosphorylation enzymes are adjacent to or fused with various transporter proteins that are supposedly activated by the PTS system, but none of these transporters is homologous with known monosaccharide transporters. Alternatively, sodium:glucose cotransporters could import D-glucose, some of which were expressed (PDESU_00573, PDESU_04471, Table S3e). Glycolysis would then start with the phosphorylation of D-glucose by the expressed hexokinase (PDESU_02759).

Genes with functions further downstream the glycolysis/gluconeogenesis pathway were expressed in all conditions, with no substantial differential expression between test conditions and D-glucose (Figure S3a, Table S3e). There was a high level of functional redundancy. Three ATP-dependent 6-phosphofructokinase (*pfk*) genes and one pyrophosphate (PP_i)-fructose 6-phosphate-1-kinase (*pfp*, PDESU_05674) gene were expressed. Two different phosphoglycerate mutase genes were expressed (*apgM*, PDESU_03290 and *gpmI*, PDESU_02723). Both pyruvate dikinase (*ppdk*, PDESU_00626) and pyruvate kinase (*pyk*, PDESU_05944) were expressed, but *ppdk* with tenfold higher expression. Four enzymes catalyzing interconversion of pyruvate and acetyl-CoA were expressed: pyruvate formate-lyase (*pfl*, PDESU_03399), the pyruvate dehydrogenase complex (*pdhBCD*; PDESU_01462, 01461, 00986) and two pyruvate:ferredoxin oxidoreductase (*pfor*; PDESU_01790, 05178) genes, with one of the latter having the highest expression values.

The PP_i-dependent glycolysis enzymes Pfp and PPdK are less common among bacteria, yet were highly expressed by *P. desulfatans* F1^T. They catalyze a PP_i-dependent glycolysis variant in anaerobic unicellular eukaryotes (Reeves, 1968) and *Firmicutes* bacteria (Bielen et al., 2010; Olson et al., 2017; Koendjbiharie et al., 2018), and presumably also in the close relative *Kiritimatiella glycovorans* based on genome analysis (Spring et al., 2016). Although this pathway could yield more ATP than normal glycolysis, the nature and energetic cost of the required PP_i synthesis pathway are unclear (Zhou et al., 2013). PP_i may be generated through glycogen cycling or a H^+/Na^+-translocating pyrophosphatase (Koendjbiharie et al., 2019), both of which were expressed in *P. desulfatans* F1^T. The advantage of the PP_i-dependent enzymes over their standard counterparts may alternatively lie in the reversibility of the catalyzed reactions, enabling flexibility

between glycolysis and gluconeogenesis (Chastain et al., 2011). This would be especially advantageous if the L-fucose degradation pathway links to glycolysis only at the pyruvate level, and not further upstream.

D-lactate dehydrogenase for producing lactate from pyruvate was medium expressed (Table S3f). Other fermentation genes for the conversion of acetyl-CoA to acetate (phosphate acetyltransferase, *pta*, PDESU_04375; acetate kinase, *ackA*, PDESU_04376) and ethanol (aldehyde-alcohol dehydrogenase, *adhE*, PDESU_03407) were highly expressed. Various hydrogenase complexes for generating the fermentation product H_2 were expressed. A [FeFe] Group A hydrogenase (PDESU_02256-02260) was highly expressed across conditions. Another such hydrogenase (PDESU_05553-05555) was highly expressed only on fucoidan and chondroitin sulfate. A [NiFe] Group 3d hydrogenase (PDESU_03105-03108) was upregulated and highly expressed on fucoidan. Two closely located [FeFe] hydrogenases of Group B (PDESU_03402) and Group C3 (PDESU_03405) were expressed constitutively at medium level.

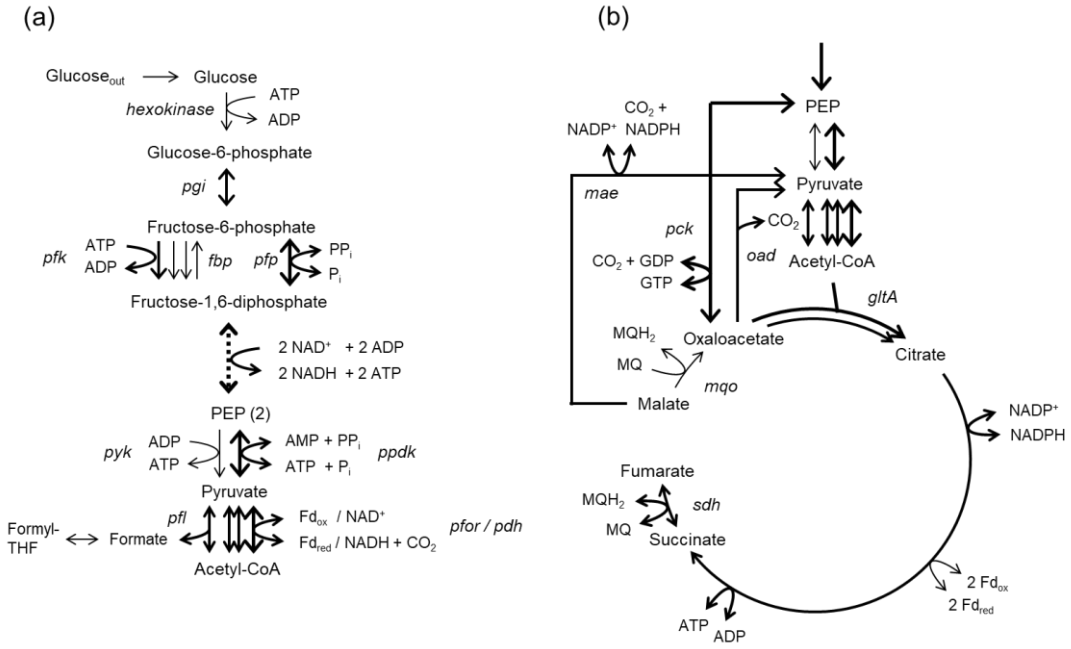

Figure S3. Expression map of **(a)** glycolysis and **(b)** the incomplete tricarboxylic acid cycle during growth on D-glucose. The three arrow thicknesses indicate TPM values of 100-200, 200-500 and >500. Abbreviations: AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; P_i, phosphate; PP_i, pyrophosphate; NAD(P)$^+$, oxidized nicotinamide adenine dinucleotide (phosphate); NAD(P)H, reduced nicotinamide adenine dinucleotide (phosphate); Fd_{ox}, oxidized ferredoxin; Fd_{red}, reduced ferredoxin PEP, phosphoenolpyruvate; NDP, nucleotide diphosphate; NTP, nucleotide triphosphate; MQ, menaquinone; MQH_2, menaquinol. Genes: *pgi*, glucose 6-phosphate isomerase; *pfk*, 6-phosphofructokinase; *fbp*, fructose-1,6-bisphosphatase; *pfp*, PPi—fructose 6-phosphate-1-kinase; *pyk*, pyruvate kinase; *ppdk*, pyruvate dikinase; *pfl*, pyruvate formate-lyase; *pfor*, pyruvate:ferredoxin oxidoreductase; *pdh*, pyruvate dehydrogenase; *pck*,

phosphoenolpyruvate carboxykinase; *oad,* oxaloacetate decarboxylase; *mae*, malic enzyme; *gltA*, citrate synthase; *sdh*, succinate dehydrogenase; *mqo*, malate:quinone oxidoreductase.

An incomplete oxidative tricarboxylic acid (TCA) cycle was expressed (Figure S3b, Table S3g), producing the amino acid precursors oxaloacetate and 2-oxoglutarate and the fermentation product succinate. Functionally redundant enzymes were again expressed, here in the form of two citrate synthase (*gltA*; PDESU_01399, 05439) genes, two NADP-dependent isocitrate dehydrogenase (*icd*, PDESU_00163, 02878) genes and two 2-oxoglutarate:ferredoxin oxidoreductase (*korAB*) gene sets. The succinate dehydrogenase (*sdhABC*, PDESU_05445-05447) complex probably functions as fumarate reductase as sink for fumarate produced by purine metabolism and arginine biosynthesis, which were highly expressed. Both aerobic and anaerobic fumarase (*fumAB*; PDESU_00109, 00106) genes were present, but were below the threshold of expression on D-glucose, consistent with an incomplete TCA cycle. The high expression level of malic enzyme (*mae*, PDESU_05521), which catalyzes malate conversion to pyruvate, indicates production of malate. However, aforementioned fumarases and a putative malate dehydrogenase are the only identifiable routes towards malate, but both are poor candidates due their low expression. On fucoidan, several genes of the TCA cycle were upregulated, including *fumAB* genes, consistent with a higher succinate production.

The oxidative branch of the pentose phosphate pathway (PPP), which converts D-glucose 6-phosphate into the five-carbon D-ribose 5-phosphate and $NADP^+$ into NADPH, was constitutively expressed at a low to medium level. The non-oxidative branch of the PPP is not canonical, lacking a transaldolase of which the functionality is most likely replaced by one of the four aforementioned 6-phosphofructokinases in combination with fructose-1,6-bisphosphatase aldolase as recently demonstrated for *Pseudoclostridium thermosuccinogenes* (Koendjbiharie et al., 2020). The non-oxidative PPP branch was constitutively highly expressed.

The high expression levels of GDP-dependent PEP carboxykinase (*pck*) and Na^+-transporting oxaloacetate decarboxylase (*oadABC,* Table S3e) are also unusual. Pck probably has a role in both anaplerosis and catabolism, since oxaloacetate is required for amino acid synthesis (Sauer and Eikmanns, 2005) as well as succinate production (Schöcke and Weimer, 1997). OadABC is normally involved in dicarboxylic acid fermentations (Sauer and Eikmanns, 2005), so its role here is unclear. We speculate that together these enzymes could form a route for conversion of PEP to pyruvate conserving energy in the form of GTP and sodium-motive force. This would be analogous to the 'malate shunt' in *Clostridium thermocellum*, which coincidentally utilizes PPdK in parallel for this conversion (Olson et al., 2017). The malate shunt is probably not active in *P. desulfatans* $F1^T$, as putative malate dehydrogenase was lowly expressed (Table S3g).

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