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„Physiology, Metabolism and Biohydrogen Production of *Desulfurococcus fermentans*“

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Abstract

Desulfurococcus fermentans is an anaerobic, hyperthermophilic crenarchaeon, belonging to the archaeal family *Desulfurococcaceae*. *D. fermentans* was examined with respect to its physiology, metabolism and its ability to produce biohydrogen. It is able to use a variety of different carbon sources ranging from monosaccharides to polysaccharides, especially cellulose. Growth was confirmed on starch, fructose, arabinose, cellulose, sucrose, lactose and maltose with an increasing growth rate in that order. A metabolic reconstruction on the basis of the full genome of *D. fermentans* revealed a large number of broad ABC transporters for sugar uptake. *D. fermentans* possesses all necessary genes for glycolysis and contains the classical but also the modified Embden-Meyerhof pathway. The reductive citric acid cycle is incomplete. The potential of biohydrogen production was analyzed in bioreactor vessels on fructose and cellulose. Yields of biohydrogen were below 0.01 mmol L^{-1} h^{-1}, not even roughly close to the "Thauer Limit", although genes for two membrane bound [NiFe] hydrogenases and other H_2 promoting genes like pyruvate fomate lyase and formate dehydrogenase have been detected. *D. fermentans* does not seem to be the best performer for biohydrogen production but it has an interesting metabolism worth being investigated.

1. Introduction

1.1 Role of Biohydrogen

Biohydrogen, the biologically generated molecular hydrogen (H_2) is a renewable energy source and an environmentally friendly biofuel. Its use avoids greenhouse gases associated with global warming, as there is no direct CO_2 emission (Chou, *et al.*, 2008, Hallenbeck, 2009, Willquist, *et al.*, 2010, Rittmann & Herwig, 2012). The final combustion product of biohydrogen is water (H_2O) with no further emission (van Niel, *et al.*, 2003). Great cost reductions can be achieved, when the focus lies on second generation of biofuels, produced from waste products of biomass (Willquist, et al., 2010). Microorganisms which can use a broad range of substrates, in particular cellulosic or lignocellulosic material, are therefore very attractive as producers of biofuels (VanFossen, *et al.*, 2009). Unfortunately there is a major drawback, as down to the present day storage of biohydrogen as well as delivery and end user application remain a problem (Hallenbeck, 2009, Pawar & Niel, 2013).

There are three basic biological mechanisms for biohydrogen production: direct or indirect photolysis, photo-fermentation and dark fermentation. Dark fermentation, the focus of this study, is used by facultative and obligate anaerobic microorganisms. It does not require light as energy source and has a higher hydrogen evolution rate (HER) [mmol L^{-1} h^{-1}] compared to the other methods (Rittmann & Herwig, 2012). Especially hyperthermophilic and extremely thermophilic bacteria and archaea are promising in this respect, as thermodynamic reactions are more favourable at elevated temperatures (Verhaart, *et al.*, 2010). However dark fermentation is only able to reach a maximum H_2 yield of four molecules of H_2 per glucose molecule, which is called the “Thauer Limit” (Thauer, *et al.*, 1977). To reach this number, all electron carriers like NADH and reduced ferredoxin ($Fd_{(red)}$) have to transfer their electrons to protons to form H_2. Otherwise other intracellular reactions and catabolic pathways, like the formation of lactate and ethanol, can limit the biohydrogen yield by joint use of these electron donors (Chou, *et al.*, 2008, Verhaart, *et al.*, 2010, Zeidan & van Niel, 2010, Martinez-Porqueras, *et al.*, 2013). In order to exceed this limit a close evaluation of physiology and biochemistry has to be done (Chou, *et* *al.*, 2008) and the production of carbon rich metabolites as well as CO_2 and H_2 must be studied in closer detail for individual microorganisms (Rittmann & Herwig, 2012).

1.2 Hydrogenases

The two most important oxidation steps to generate reducing equivalents from glucose degradation are: the conversion from glyceraldehyde-3-phosphate to glycerate-3-phosphate via the modified Emden-Meyerhof pathway and conversion from pyruvate to acetyl-CoA, where either NADH or $Fd_{(red)}$ are generated (Verhaart*, et al.*, 2010). To reduce the protons to H_2 by disposal of excess electrons generated during metabolism, hydrogenases are needed (Hallenbeck, 2009).

Hydrogenases are oxygen-sensitive enzymes and key enzymes for biohydrogen production or consumption. Biohydrogen can be gained by oxidation of reducing equivalents like NADH or $Fd_{(red)}$ (Chou*, et al.*, 2008):

$$2H^+ + 2e^- \leftrightarrow H_2$$

The majority of anaerobic microorganisms encode one or more hydrogenases. They are divided into three groups: two classes containing iron-sulfur clusters differentiated by their active metal sites [NiFe] or [FeFe] and one class of [Fe]-only hydrogenases. All three groups need other accessory proteins with different functional roles for maturation.
[FeFe] hydrogenases are often found in anaerobic heterotrophic organisms which use ferredoxins as electron carriers. The catalytic center has a [4Fe-4S] cluster connected to a binuclear [FeFe] site, which forms an H-cluster. [FeFe] hydrogenases exist in different variations using various other accessory subunits (Schut*, et al.*, 2013).

[NiFe] hydrogenases are common in all three domains of life and widely distributed. They always have a large subunit with an active site and a small subunit. Some [NiFe] hydrogenases also possess enzymes to oxidize formate like the formate hydrogen lyase or hydrogenase 3 (Hedderich, 2004, Schut*, et al.*, 2013). They can also contain subunits homologous to the NADH: quinone oxidoreductase complex I of the respiratory system in bacteria (Yano, 2002).

Membrane bound hydrogenases (MBH) with the ability to generate H_2 from ferredoxin and NADH are found in many hyperthermophilic archaea like *Pyrococcus furiosus*, *Thermoanaerobacter tengocongensis* (Hallenbeck, 2009*)* and as claimed by genome annotation also in *D. fermentans* (Susanti*, et al.*, 2012).

1.3 Characteristics of *Desulfurococcus fermentans*

Desulfurococcus fermentans, isolated from a freshwater hot spring in the Uzon caldera (Kamchatka Penninsula, Russia), is an anaerobic and hyperthermophilic crenarchaeon*,* belonging to the archaeal family *Desulfurococcaceae*. The coccoid cells range in size from 1-4 µm in diameter with one flagellum. The optimum growth temperature was found to be between 80 to 82 °C with an optimum pH at 6.0. *D. fermentans* is able to grow on a broad range of carbohydrates including agarose, amygdalin, arabinose, arbutin, casein hydrolysate, dextran, dulcitol, fructose, lactose, laminarin, lichenan, maltose, pectin, peptone, ribose, starch and sucrose. Exceptional is the ability to grow on cellulose since it is the first known hyperthermopilic archaeaon that does so. As *D. fermentans* is able to grow on these momomeric and polymeric carbohydrates, it differs significantly from other *Desulfurococcus* species, which prefer proteinaceous substrates. No growth was detected on glucose, xylose, mannitol or sorbitol (Perevalova*, et al.*, 2005).

Hyperthermophilic strains like *D. fermentans,* compared to mesophilic strains, are more favourable for biohydrogen production than mesophilic strains, as yields of H_2 are higher with higher temperature. They are energetically better adjusted (Hallenbeck, 2009, Rittmann & Herwig, 2012, Pawar & Niel, 2013) and have a lower risk of contamination (Verhaart*, et al.*, 2010). It was already shown that *D. fermentans* is able to produce biohydrogen on media with starch or sucrose. Moreover, it seems to be able to tolerate 100% H_2 in the gas phase and elemental sulphur is not required for growth (Perevalova*, et al.*, 2005).

The genome was sequenced by Susanti, et al., 2012, and consists of 1,384,116 bp with a GC content of 44.8%. 1,075 out of 1,475 protein-coding genes have predicted functions. With respect to the production of biohydrogen, membrane-bound hydrogenases have been found (Susanti*, et al.*, 2012).

1.4 Aims of this Study

This study is focused on *D. fermentans* and its role in the production of biohydrogen with a major emphasis on its physiology and the reconstruction of its metabolism. Growth on selected carbohydrates: arabinose, cellulose, fructose, lactose, maltose, starch and sucrose should be examined quantitatively and qualitatively and evaluated, hydrogen production should be tested and a preliminary prediction of its major metabolic pathways from the known genome sequence should be achieved.

This work is based on the following hypothesis and questions:

(I) *D. fermentans* is an excellent biohydrogen producer and is able to produce H_2 not only on starch and sucrose (Perevalova*, et al.*, 2005) but also on all other test substrates, in particular other low cost carbohydrates such as cellulose.

(II) *D. fermentans* will grow on selected substrates according to its catabolic burden and energy gain.

(III) *D. fermentans* will encompass a higher cell specific H_2 evolution rate on substrates with a fast growth rate and high cell density.

(IV) Will the physiology of *D. fermentans* be altered by omitting yeast extract in the media composition? As the use of yeast extract in large scale fermentors is “very expensive” (Willquist*, et al.*, 2010, van Niel*, et al.*, 2012), high cost reduction can be achieved by working on chemically defined media.

(V) Is *D. fermentans* able to grow autotrophically by a CO_2-fixation pathway? This could be possible if it possesses genes like pyruvate formate lyase (PFL) and formate hydrogen lyase (FHL) (Ramírez-Morales*, et al.*, 2015).

2. Material and Methods

2.1 Chemicals

The gases H_2, CO_2, N_2, H_2/CO_2 (ratio 4:1), CO_2/N_2 (ratio 4:1), gas-mix 1 (95.5% N_2; 4.5% H_2), gas-mix 2 (28.3% CO; 22.4% H_2; 19.7% CO_2; 15.5% N_2; 14.1% CH_4) and gas-mix 3 (28.3% CO; 22.4% H_2; 19.7% CO_2; 12.2% N_2; 14.1% CH_4; 3.3% C_2H_4) (Air Liquide) and the standard-gas (Messer) for gas chromatography (99.92% N_2; 0.01% CH_4; 0.08% CO_2) were used for sparging or generating a standard curve. All gases had a measurement inaccuracy of approximately ±2. All other chemicals were of highest grade available.

2.2 Microorganism and Medium Composition

The strain *Desulfurococcus fermentans* DSM 16532 was purchased from the "Deutsche Sammlung von Mikroorganismen und Zellkulturen" (DSMZ). A slightly modified medium of DSMZ No. 395 was used for all cultivations containing (L^{-1}): NH_4Cl 0.33 g; KH_2PO_4 0.33 g; KCl 0.33 g; $CaCl_2$ x 2 H_2O 0.44 g; $MgCl_2$ x 6 H_2O 0.70 g; NaCl 0.50 g; $NaHCO_3$ 0.80 g; 0.20 g yeast extract (YE); Na_2S x 9 H_2O 0.50 g; trace elements SL-10 1 ml; vitamin solution 10 ml. For cultures to grow on a chemically defined medium YE was omitted. Carbon source varied in type (arabinose, agarose, cellulose, fructose, lactose, maltose, starch and sucrose) and concentration (between 2-5 g L^{-1}). Trace elements solution consisted of (L^{-1}): HCl (25%; 7.7 M) 10 ml; $FeCl_2$ x 4 H_2O 1.50 g, $ZnCl_2$ 0.07 g; $MgCl_2$ x 4 H_2O 0.1 g; H_3BO_3 0.006 g; $CoCl_2$ x 6 H_2O 0.19 g; $CuCl_2$ x 2 H_2O 0.002 g; $NiCl_2$ x 6 H_2O 0.024 g; Na_2MgO_4 x 2 H_2O 0.036 g. Vitamin solution contained (L^{-1}): biotin 0.002 g; folic acid 0.002 g; pyridoxine-HCl 0.01 g; thiamine-HCl 0.005 g; riboflavin 0.005 g; nicotinic acid 0.005 g; d-Ca-pantothenate 0.005 g; vitamin B_{12} 0.0001 g; p-aminobenzoic acid 0.005 g; lipoic acid 0.005 g.

The medium was prepared anaerobically under 80% N_2 and 20% CO_2 or 100% N_2 and distributed into 120 ml serum bottles with rubber stoppers (Butyl Ruber 20 mm, Chemglas LifeScience), containing 50 ml media and 70 ml headspace. Medium pH was adjusted to 6.2-6.4 with NaOH and sterilized at 121 °C for 20 min. After sterilization the vitamin solution and $NaHCO_3$ were added separately in the sterile laminar-air-flow-chamber FASTER BH-EN 2005 (Szabo-Scandic).

Shortly before inoculation the medium was reduced by adding sterile $Na_2S \times 9 H_2O$, prepared under N_2.

2.3 Growth Conditions & Fermentation

2.3.1 Closed Batch – Serum Bottles

Cultures of *D. fermentans* were grown anaerobically in a closed batch system (Rittmann & Herwig, 2012) in 120 ml serum bottles containing 50 ml medium with different carbon sources (arabinose, agarose, cellulose, fructose, lactose, maltose, starch and sucrose), yeast extract from 0-2 g L^{-1} and 70 ml headspace under 80% N_2 and 20% CO_2 or 100% N_2 at 0.5 bar. For inoculation 5% (v/v) of pre-culture was added anaerobically in the anaerobic glove box (Coy Laboratory Products) by using a gas-tight syringe (Soft-Ject, Henke Sass Wolf). After inoculation the bottles were flushed again with 80% N_2 and 20% CO_2 or 100% N_2 and kept on 80 °C (Labwit-Zwyr-2102c, Lab Xperts Laboratory Solutions Austria) with a stirring rate of 100 rpm. Cultures were grown in duplicates together with a negative control. The negative control was inoculated with 5 ml of the anaerobic atmosphere inside the anaerobe chamber. Every three to seven days samples of 1 ml were taken and afterwards flushed again with the respective gas.

Table 1 gives an overview of the varying parameters: substrate, atmosphere and yeast extract of the experimental setup. Media composition was already described in section 2.2.

Table 1 - Overview of serum bottle experiments: different sparging gases (N_2 and CO_2/N_2) yeast extract concentrations (with or without YE) and number of duplicates.

Substrate	N_2	N_2/CO_2	with YE	w/o YE	Replicates
Starch		x	x		3x2
Fructose		x	x		2x2
Fructose	x			x	2x2
Fructose		x		x	3x2
Arabinose		x	x		2x2
Maltose		x	x		2x2
Cellulose	x		x		3x2
Cellulose		x	x		3x2
Lactose		x	x		2x2
Sucrose		x	x		2x2

2.3.2 Batch cultivation in bioreactor

Cultures of *D. fermentans* were grown anaerobically in a 2 L bioreactor (Eppendorf) with the same media composition as in the closed batch experiments, again with different carbon sources (fructose or cellulose) and yeast extract from 0-2 g L^{-1}. To ensure anaerobic conditions inside the bioreactor vessels, prior to inoculation, the system was flushed with N_2 for approximately 5 min. For inoculation 5% (v/v) of a stock solution (prepared in closed batch) was transferred into the reactor using a gas-tight syringe. Cultivation was performed at 80-85 ± 0.5 °C with a stirring rate of 100 rpm and a gas flow of 1-6 sL h^{-1}. The pH was measured by a pH probe (Mettler Toledo GmbH) and kept constant at pH 5.8, pH 6.0 or pH 6.4 by addition of 0.5 M NaOH or 0.5 M HCl. The oxidation reduction potential (ORP) was measured by a redox probe (Mettler Toledo GmbH). The ORP was altered to a value below -300mV by adding a 0.5 M $Na_2S \times 9 H_2O$ solution. To ensure anaerobic conditions all solutions were flushed with N_2 and kept under pressure by being plugged to a gas bag filled with N_2. The reaction volume increased during cultivation by keeping the pH at a constant level and decreased when samples were taken. Setup with an overview of the varying parameters is shown in Table 3 in section 3.2.

For off-gas analysis, H_2 and CO_2, were taken from the bioreactor as follows (Figure 1): First the 120 ml flasks were flushed with the off-gas for a minimum of 30 minutes. Secondly a gas bag (SKC Quality Sampling Bag) was connected to the off-gas tube and filled. With a 50 ml gas-tight syringe, gas was taken from the bag and pressed into the flushed flasks. Inside pressure reached about 1700 mbar.

2.4 Microscopy & Cell Counting

Samples were taken directly from serum bottles or from the bioreactor. Cells were counted by applying 10 µl of the sample onto the Neubauer improved cell counting chamber (Superior Marienfeld Germany) with a depth of 0.1 mm. Cultures were scanned under the microscope (Nikon Eclipse 50i).

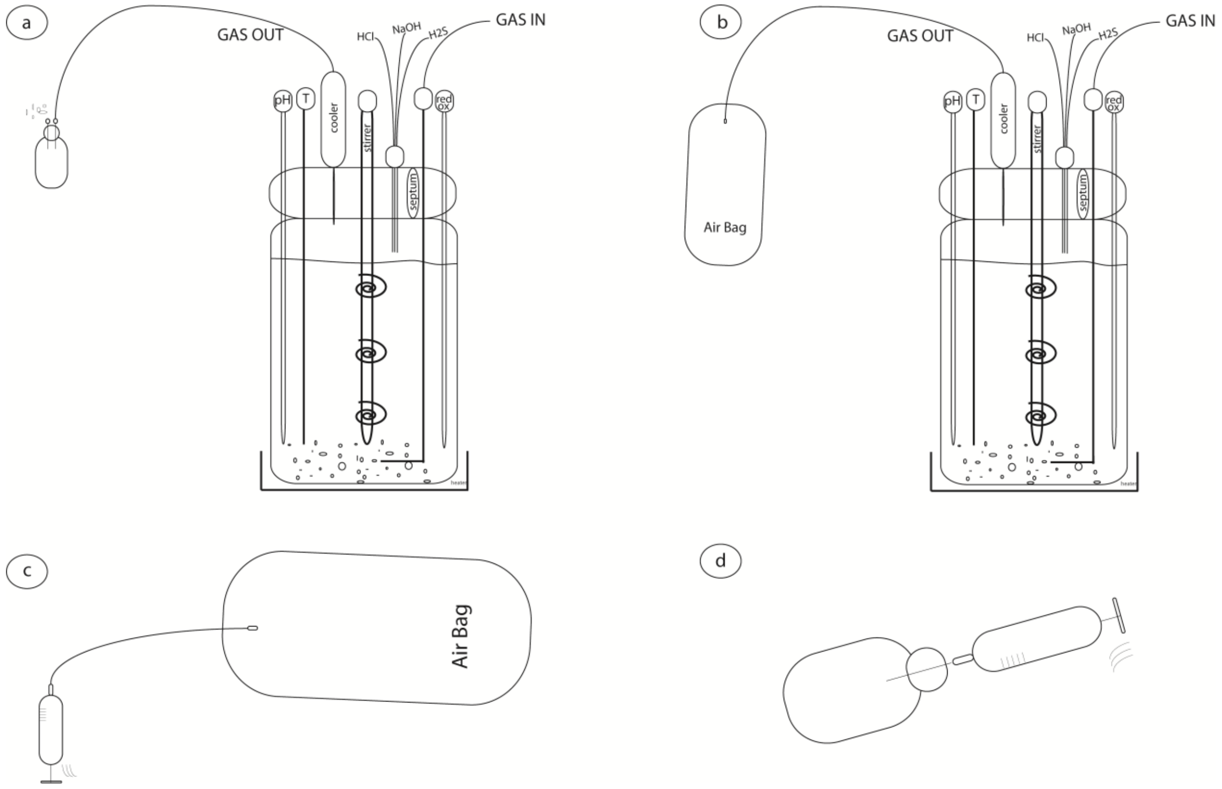

Figure 1 – Off-gas collection from bioreactor: flushing of serum bottles (a) followed by the collection in a gas bag (b), taking gas from the air bag (c) and pressing it into the flushed serum bottles (d).

Further a test for coenzyme F_{420} dependent autofluorescence was performed. Autofluorescence was visible in living cells by exciting with ultraviolet light and use of the Filter BV2A.

Measurement of the optical density (OD) (Eppendorf Bio Photometer) for estimation of cell density was not successful, as there were too many disturbing particles inside the media.

2.5 Gas Chromatography

Off-gas inside the flasks was measured (Figure 2) by using the Agilent Gas Chromatograph (Agilent 78790A GC) with a thermal conductivity detector (TCD). Gases were separated at 170 °C (Heater) with helium as carrier gas. Setting for the reference flow was 10 ml per minute and for the makeup flow 1 ml per minute.

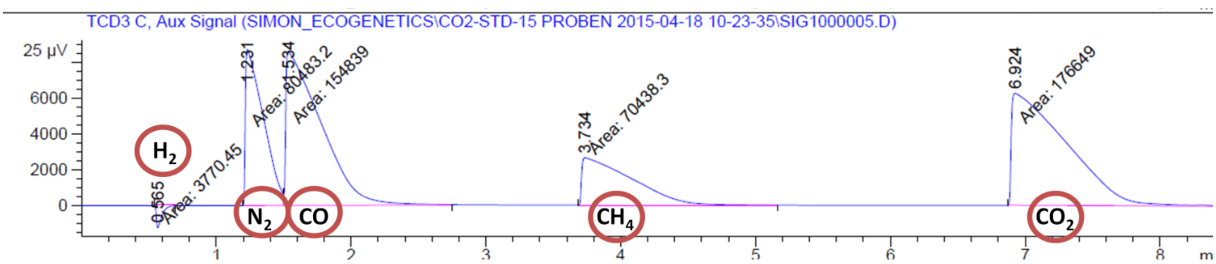

Figure 2 – Excerpt from gas chromatography analysis. Analyzed gases H_2, N_2, CO, CH_4 and CO_2 are highlighted.

To generate a standard curve for H_2, N_2 and CO_2 the following gases were used: 100% H_2, 100% CO_2, 100% N_2, H_2/CO_2 (ratio 4:1), CO_2/N_2 (ratio 4:1), gas-mix 1 (95.5% N_2; 4.5% H_2), gas-mix 2 (28.3% CO; 22.4% H_2; 19.7% CO_2; 15.5% N_2; 14.1% CH_4) and gas-mix 3 (28.3% CO; 22.4% H_2; 19.7% CO_2; 12.2% N_2; 14.1% CH_4; 3.3% C_2H_4) as well as the standard-gas for gas chromatography (99.92% N_2; 0.01% CH_4; 0.08% CO_2). Standard curve results always gave an R^2 of 0.99 or higher (Figure 3).

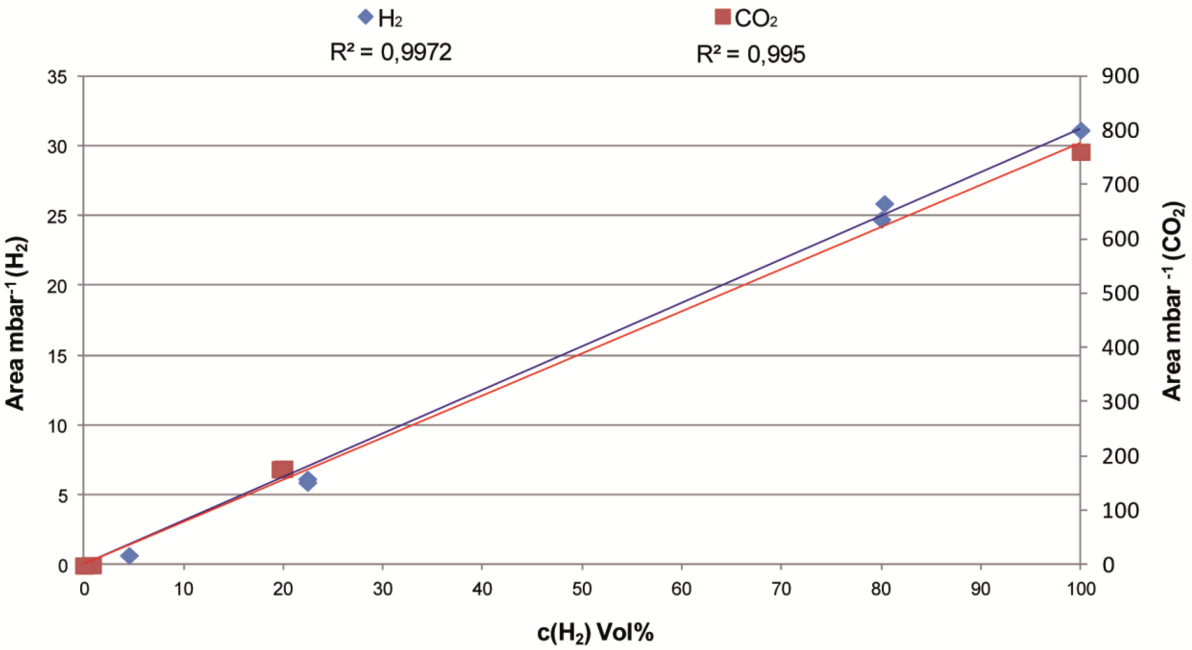

Figure 3 – Standard Curve measurements of H_2 (R^2=0.9972) in blue and CO_2 (R^2=0.995) in red.

2.6 Calculation

To determine fastest growth (μ_{max}) and doubling time, μ was calculated by

$$N = N_0 \cdot e^{\mu t}$$

with N, final cell number [cells ml^{-1}], N_0, initial cell number [cells ml^{-1}], t, time [h] and μ, specific growth rate [h^{-1}].

Hydrogen and carbon dioxide evolution rates (HER and CER) [mmol L^{-1} h^{-1}] and specific hydrogen/carbon dioxide productivity (qH_2/qCO_2) [mmol g^{-1} h^{-1}] were calculated according to the total gas flow [sL h^{-1}], the gas flow correction (the measured H_2 or CO_2 content in the offgas) and the concentrations of H_2 and CO_2 using the ideal gas law. Product yield of $Y_{(H_2/CO_2)}$ were calculated by dividing the amount of HER by the amount of CER.

2.7 Genome Analysis

Metabolic reconstruction from the genome of *D. fermentans* was done manually using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Comprehensive Enzyme Information System (BRENDA). KEGG pathway maps gave the enzyme information for the Carbon Metabolism, Carbon Fixation Pathway in Prokaryotes, Glycolysis and Glyconeogenesis, Pentose Phosphate Pathway, Citrate Cycle, Pyruvate Metabolism, Starch and Sucrose Metabolism, Fructose and Mannose Metabolism, Galactose Metabolism, Glyoxylate and Dicarboxylate Metabolism, ABC Transporter Systems and Bacterial Secretion System.

NCBI database entries of Gene-ID or Protein-ID for each enzyme obtained from KEGG and BRENDA were further analyzed with the Basic Local Alignment Search (BLAST). BLAST was performed with the following settings: tblastn, tblastx and blastn within the database reference genomic sequences (refseq_genomic) and blastp within the database reference proteins (refseq_protein). For a shorter calculation time, search was limited to the taxa *Desulfurococcales* (taxid: 114380). Blastn calculation was extended to the BLAST algorithm “somewhat similar sequences”. Results for Query coverage, E Value and Identity can be found in Supplementary Table 1 and 2.

3. Results

3.1 Growth Experiments in Serum Flasks

To determine the carbon source that resulted in fastest growth and high cell densities *Desulfurococcus fermentans* was cultivated in a closed batch system (Rittmann & Herwig, 2012) on arabinose, cellulose, fructose, lactose, maltose, starch and sucrose. To examine if growth is affected in a defined medium without YE, a very expensive nutrient source for biotechnology (Willquist & van Niel, 2012), the YE was subsequently eliminated by serial dilution from the medium with fructose in selected experiments. All other media components stayed the same.

Serum flask experiments started with a long lag phase (Supplementary Figure 1) of about 200 hours before growth started. Only with starch as substrate growth started earlier. Starch also seems to result in the highest growth rate for *D. fermentans*, although sugar particles in the background made it difficult to count the cells. As presented in Table 2 the best growth conditions, apart from starch, could be found by cultivation with fructose and arabinose, both with YE and N_2/CO_2 atmosphere reaching a doubling time of 14 h. They were followed by cellulose with YE, grown by gassing with N_2 with a doubling time of 33 h. Growth of *D. fermentans* on fructose in a defined medium resulted in a doubling time of 74 h. Weaker growth could be observed when the strain was grown on maltose, lactose and sucrose. No growth could be detected when *D. fermentans* was cultivated on cellulose supplied with an atmosphere consisting of N_2/CO_2.

Highest final cell density and fastest growth (Figure 4) was detected in starch and fructose, grown with YE and sparging of $CO_2/N_{2,}$ with a final cell density of about 2.0-4.0 x 10^7 cells ml^{-1}. Followed by arabinose and cellulose with a final cell density of around 1.0-2.0 x 10^7 cells ml^{-1}. Lower cell densities were observed with fructose as sole carbon source. Lactose and sucrose showed a very low number of cells.

Table 2 - Overview of growth results showing the hours needed to reach the max. cell density, μ_{max}, doubling time and cells ml^{-1}; Abbreviations: wY, with yeast extract; woY, without yeast extract

Substrate	**Growth [h]**	**μ_{max}**	**Doubling Time [h]**	**Mean Cells [ml^{-1}]**
Starch_wY_N_2/CO_2	228	0,056	12	3,00E+07
Fructose wY_N_2/CO_2	672	0,051	14	2,98E+07
Fructose_woY_N_2	972	0,009	74	2,59E+07
Fructose_woY_N_2/CO_2	1032	0,007	91	2,23E+07
Arabinose_wY_N_2/CO_2	672	0,049	14	1,58E+07
Maltose_wY_N_2/CO_2	936	0,004	155	1,49E+07
Cellulose_wY_N_2	512	0,021	33	1,40E+07
Cellulose_wY_N_2/CO_2	0	0,000	0	0,00E+00
Lactose_wY_N_2/CO_2	864	0,009	73	4,81E+06
Sucrose_wY_N_2/CO_2	864	0,006	110	5,25E+06

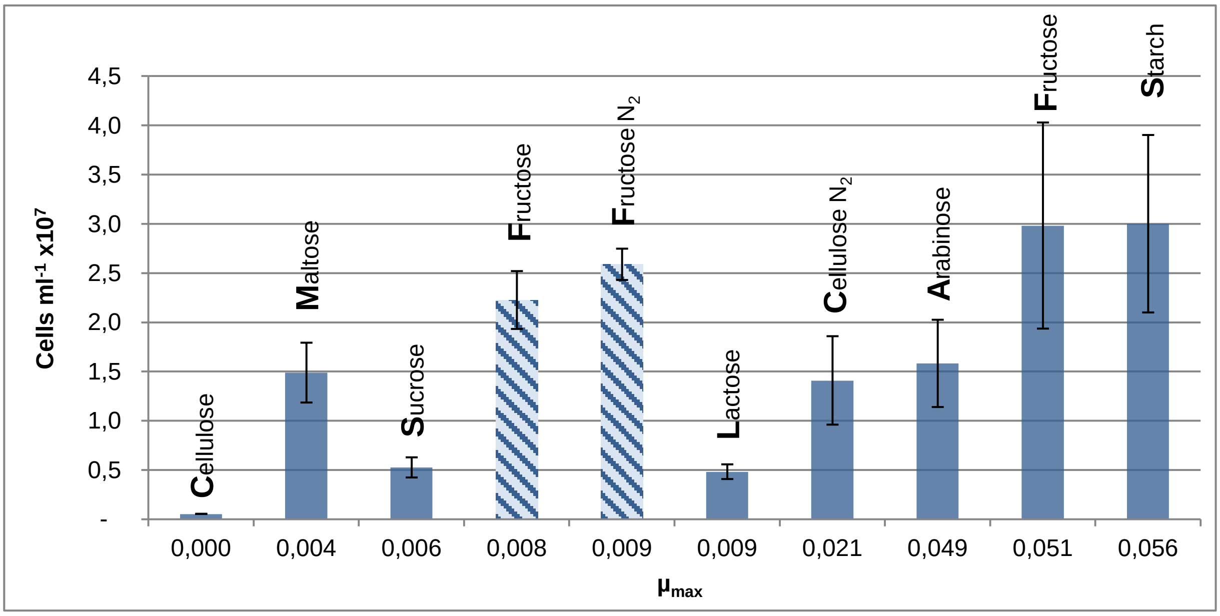

Figure 4 – Growth of *D. fermentans* on different media compositions in relation to maximum growth rate (μ_{max}) and cell densities (cells [ml^{-1}]). Dashed lines indicate growth without yeast extract.

3.2 Growth Experiments in Bioreactors

According to growth results from serum flasks experiments, cellulose and fructose, which compared to other substrates resulted in faster growth rate and higher cell densitiy, seemed promising for cultivation. Furthermore cellulose is an interesting substrate, since it is an industrial waste product of biomass processing industry and *D. fermentans* is unique among the archaea in its ability to grow on it. Based on these facts, bioreactor experiments were performed with these substrates as carbon sources. All experiments in the bioreactor were executed by sparging with N_2.

Again there was a long lag phase of about 180 hours, which is slightly shorter than it was in serum bottles, until growth of *D. fermentans* started. Only when *D. fermentans* was cultivated on cellulose, grown in a medium containing yeast extract, growth started after 120h, whereas in fructose_1 (see Table 3) it took 312 h before the cells shifted to exponential growth phase (Supplementary Figure 2). Results and an overview of the varying parameters of the experimental setup can be found in Table 3. Cellulose_1 with YE reached the fastest growth with a doubling time of 9.1 h, followed by cellulose_3 without YE with a doubling time of 11.8 h and the highest cell density. A lower pH of 5.8 and higher temperature of 85 °C resulted in a doubling time of 42.8 h and lower growth. Fructose grew to a cell density of 1.89×10^7 cells ml^{-1} and doubling time of 53.6 h and 9.44×10^6 cells ml^{-1} with doubling time of 57.0 h.

Table 3 - Overview of growth results showing the varying parameters: yeast extract concentrations (with or without YE), number simultaneous bioreactor experiments, pH, Temp [°C], hours needed to reach the max. cell density, μ_{max}, doubling time and cells ml^{-1}; Abbreviations: wYE, with yeast extract; woYE, without yeast extract. [a] pH-probe got damaged during measurements, after 120 h pH was not detected anymore. [b] run was cancelled due to measurement problems.

Substrate	**with YE**	**w/o YE**	**Repli-cate**	**pH**	**Temp [°C]**	**growth [h]**	**μ_{max}**	**Doubling Time**	**Mean Cells [ml^{-1}]**
Fructose_1		x	1	6.0	80	746	0,013	53,6	1,89E+07
Fructose_2		x	2	6.0	80	449	0,012	57,0	9,44E+06
Cellulose_1	x		2	~6.4[a]	80	168	0,076	9,1	3,38E+06
Cellulose_2[b]		x	2	6.0	80	96	0,008	83,7	3,60E+05
Cellulose_3		x	1	6.4	80	215	0,059	11,8	2,15E+07
Cellulose_4		x	1	5.8	85	314	0,016	42,8	7,00E+06

The high variations in cell densities can be explained by the formation of biofilm (Figure 5a), which was discovered at the end of growth in bioreactor experiments. This will be explained in greater detail in section 4.1.

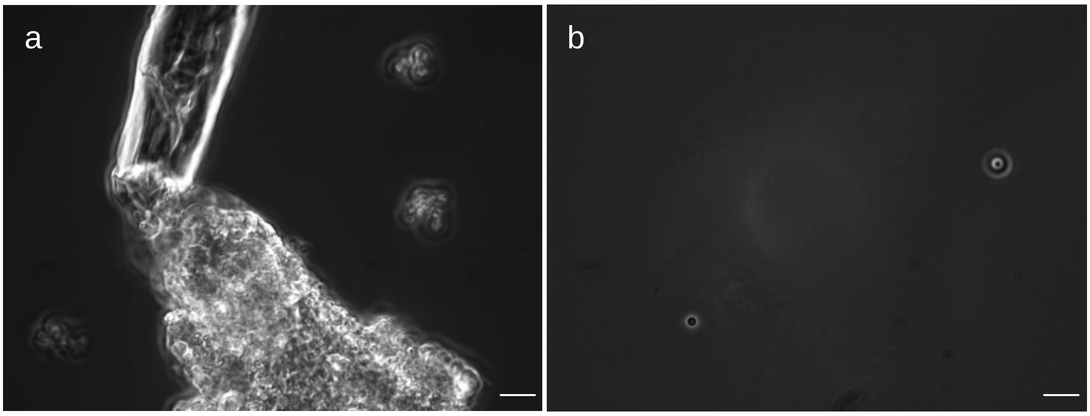

Figure 5 – Biofilm formation in bioreactor experiments (a) in comparison to single cells (b). Bars 5 µm.

3.3 Biohydrogen production rates

Volumetric hydrogen evolution rate (HER), specific hydrogen evolution rate (qH_2), volumetric carbon dioxide evolution rate (CER), specific carbon dioxide evolution rate (qCO_2) and product to product yield of H_2 and CO_2 ($Y_{(H_2/CO_2)}$) has been determined for fermentation of fructose and cellulose of *D. fermentans* (Supplementary Table 3). The aim was to detect H_2 and CO_2 in relation to growth. All gas chromatographic results showed an extremely low HER (Figure 6) and CER from zero to max. 0.02 or 0.07 mmol L^{-1} h^{-1}, meaning a very low gas development. Furthermore there was no visible regularity in H_2 or CO_2 production, as the detection limit of the gas chromatography was reached (Figure 7).

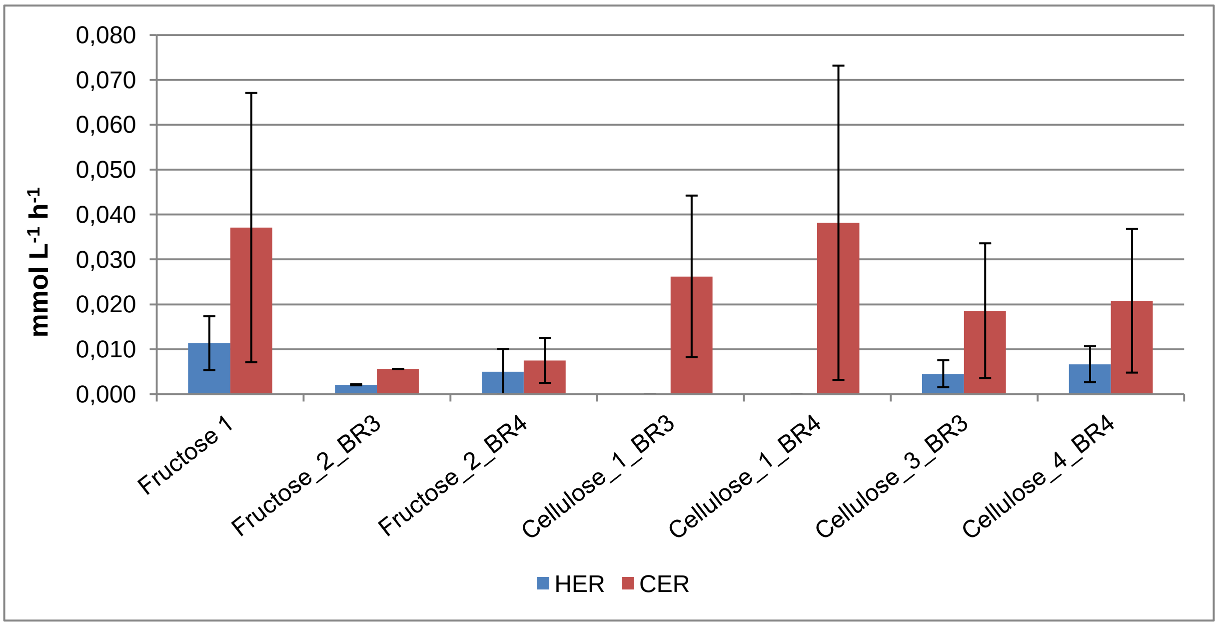

Figure 6 – Hydrogen evolution rate (HER) in blue and carbon dioxide evolution rate (CER) in red of all bioreactor experiments. Low gas production and high irregularity is visible.

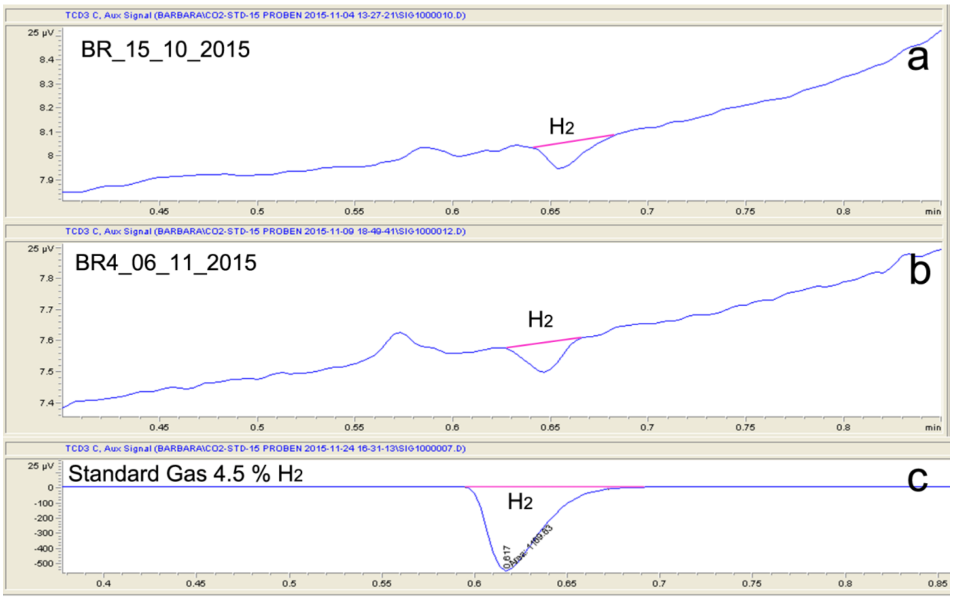

Figure 7 – Excerpt from gas chromatographic determination of H_2. Gas samples from 15th of October 2015 (a) and 06th of November 2015 (b) showed a very low and noisy H_2 signal compared to a standard gas measurement filled with 4.5 % H_2 (c) with a clear signal.

3.4 Evaluation of the Metabolism

Whole genome sequencing was previously performed by Susanti, et al., 2012. *D. fermentans* is able to metabolise a variety of carbohydrates (Perevalova*, et al.*, 2005), including the tested monosaccharides fructose (hexose) and arabinose (pentose), disaccharides maltose (formed by two glucose molecules), lactose (formed by glucose and galactose) and sucrose (formed by glucose and fructose) and polysaccharides starch and cellulose by using a variety of encoded sugar transporters (Supplementary Figure 3 and in greater detail attached on the last page in ratio 1:1). A complete list can be found in the Supplementary Table 1 and 2.

3.4.1 Central Carbon Metabolism

The central metabolism is divided into two sections: the conversion of sugars to pyruvate and the further utilization to organic end products or CO_2. Analysis of the genome revealed a complete pathway for glycolysis using the Embden-Meyerhof pathway (EMP), starting from glucose to glyceraldehyde-3-phosphate with a ROK family glucokinase (Desfe_0578), bifunctional phosphoglucose/phosphomannose isomerase (Desfe_1128), phosphofructokinase (Desfe_0717 and Desfe_0968), fructose-bisphosphate aldolase, class I (Desfe_0718) and fructose 1,6-bisphosphate aldolase/phosphatase (Desfe_1349).

D. fermentans is able to use two pathways to convert glyceraldehyde-3-phosphate to glycerate-3-phosphate (Figure 8): via the classical EMP pathway by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Desfe_0262) and phosphoglycerate kinase (Desfe_0261), resulting in the production of NADPH and ATP and via the modified EMP pathway by an aldehyde ferredoxin oxidoreductase (GAPOR) (Desfe_0557), resulting in 2 mole of $Fd_{(red)}$ or by a non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Desfe_0067) resulting in NADH. This modified pathway was also described in *P. furiosus* (Verhaart*, et al.*, 2010, Pawar & Niel, 2013).

From glycerate-3-phosphate the glycolysis pathway continues with phosphonopyruvate decarboxylase-related protein (Desfe_0416), enolase (Desfe_0063), pyruvate kinase (Desfe_1347) or pyruvate, water dikinase (Desfe_0879) to pyruvate.

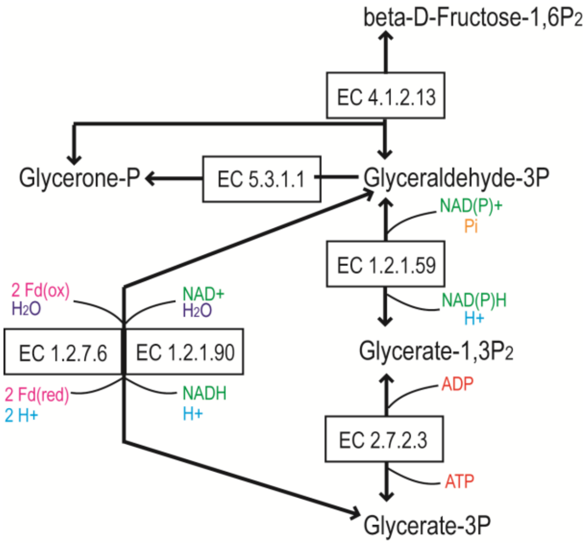

Figure 8 – Classic EMP pathway with EC 1.2.1.59 (GAPDH) and EC 2.7.2.3 resulting in NAD(P)H and ATP in comparision to the modified EMP pathway with EC 1.2.7.6 (GAPOR) resulting in 2 $Fd_{(red)}$.

It proceeds with pyruvate ferredoxin oxidoreductase (PFOR) which decarboxylates pyruvate (Figure 9) as end product of the EMP to acetyl coenzyme A (acetyl-CoA). *D. fermentans* possesses 8 PFOR, two alpha subunits (Desfe_0503 and Desfe_1298), 2 beta subunits (Desfe_0502 and Desfe_1299), two gamma subunits (Desfe_0505 and Desfe_1296) and two delta subunits (Desfe_0504 and Desfe_1297). *D. fermentans* is further able to use dihydrolipoamide dehydrogenase (Desfe_0667) for the same reaction. Decarboxylation with PFOR results in 2 $Fd_{(red)}$, with dihydrolipoamide dehydrogenase in NADH. Further the citrate cycle (TCA cycle) can be entered by pyruvate carboxylase subunit B while converting pyruvate, ATP and HCO_3^- to oxaloacetate. Acetate and ATP is generated from acetyl-CoA by acetyl-CoA synthetase (Desfe_0782 and Desfe_1050) (Figure 9).

D. fermentans also possesses a pyruvate formate-lyase (PFL) (Desfe_0583). PFL provides acetyl-CoA and formate (Figure 9) and has a key role in the anaerobic metabolism (Crain & Broderick, 2014). PFL is activated by the pyruvate formate lyase-activating protein (PFL-AE) (Desfe_1164) and involves the H-atom abstraction by a radical SAM protein (Desfe_0007, Desfe_0149, Desfe_0201, Desfe_0288, Desfe_0298, Desfe_0313, Desfe_0363, Desfe_0369, Desfe_0376, Desfe_0576, Desfe_0583, Desfe_0693, Desfe_0860, Desfe_1164) (Broderick*, et al.*, 2014).

CO_2 is produced by oxidation of formate using formate dehydrogenase (FDH) (Desfe_1134) while generating NADH. CO_2 can be further reduced to CO by carbon-monoxide dehydrogenase, whereof *D. fermentans* possesses three medium (Desfe_0376, Desfe_0379 and Desfe_0769) and three small (Desfe_0767, Desfe_0770 and Desfe_0380) subunits (Figure 9). With a decarboxylase enzyme (Desfe_0759) the resulting CO is combined with a methyl-group to receive acetyl-CoA which enters glycolysis (Supplementary Figure 3).

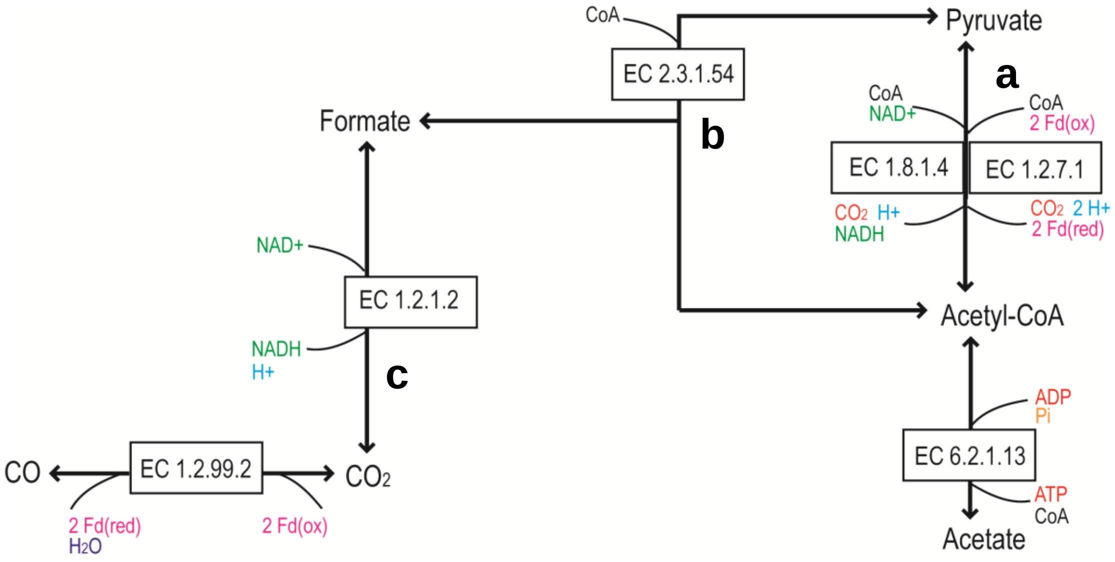

Figure 9 – Conversion of pyruvate: to acetyl-CoA and acetate via PFOR and acetyl-CoA synthase generating 2 Fd(red) and ATP (a) or PFL and FDH to formate and CO_2 generating NADH and acetyl-CoA (b) and conversion of CO_2 to CO by CMO-DH generating 2 $Fd_{(red)}$ and H_2O. Abbreviations: PFOR, pyruvate ferredoxin oxidoreductase; PFL, pyruvate formate-lyase; FDH, formate dehydrogenase; CMO-DH, carbon-monoxide dehydrogenase.

3.4.2 Incomplete Citrate Cycle

Genome analysis revealed that the reductive citrate cycle in *D. fermentans* is incomplete because of the absence of enzymes for some reactions. TCA can be entered either from phosphoenolpyruvate or from pyruvate resulting in oxaloacetate, which is reduced to (S)-malate by FAD-dependent oxidoreductase (Desfe_0284). Decarboxylation of malate to fumarate is not known, which is also the case in *Thermococcus kodakarensis* and *Pyrococcus furiosus*. Both are able to decarboxylate malate to pyruvate by the malic enzyme (Fukuda*, et al.*, 2005). BLAST of the malic enzyme gave no results for *D. fermentans* homologues, so this step remains to be discovered. Fumarate and succinate are coupled by fumarate reductase/succinate dehydrogenase flavoprotein domain protein (Desfe_0481). Succinate is further converted to succinyl-CoA by succinyl-CoA synthetase alpha subunit (Desfe_1155) and succinyl-CoA synthetase beta subunit (Desfe_1156). The reaction from succinyl-CoA to 2-oxoglutarate is catalysed by a 2-oxoglutarate ferredoxin oxidoreductase complex containing two alpha subunits (Desfe_0475 and Desfe_0499), two beta subunits (Desfe_0474 and Desfe_0498), one gamma subunit (Desfe_0497) and one delta subunit (Desfe_0500). No homolog to the enzyme isocitrate dehydrogenase linking isocitrate to 2-

oxoglutarate was found. Aconitate (Desfe_0217 and Desfe_0218) catalyzes the isomerisation of citrate and isocitrate. To overcome the lack of isocitrate dehydrogenase *D. fermentans* can use the aspartate aminotransferase (Desfe_0590) to go from oxaloacetate together with glutamate directly to 2-oxoglutarate and aspartate. With glutamate dehydrogenase (Desfe_0075) *D. fermentans* is able to convert glutamate to 2-oxoglutarate with the production of NADPH. Further aspartate and fumarate are coupled via adenylosuccinate lyase (Desfe_0494) and adenylosuccinate synthetase (Desfe_0482).

3.4.3 Fermentation End-Products

Acetate as end product could be generated from acetyl-CoA by acetyl-CoA synthetase (Desfe_0782 and Desfe_1050). Theoretically each synthesis to acetate results in formation of 1 ATP.

For synthesis of lactate and ethanol, reducing equivalents like NAD(P)H and $Fd_{(red)}$ can be used. For the formation of ethanol an aldehyde dehydrogenase (Desfe_0067) catalyzes the reaction from acetate to acetaldehyde followed by an alcohol dehydrogenase (Desfe_0019 and Desfe_1240) where the formation of ethanol is completed. In the end 2 NAD(P)H are needed for this two reactions. The formation of lactate only needs one NADH and is catalysed by a lactate dehydrogenase (Desfe_1212).

3.4.4 Hydrogenases

Two gene cluster of membrane bound [NiFe] hydrogenases (Desfe_0337-Desfe_0348 and Desfe_1135-Desfe_1141) have been found (Table 4). Both consist of large and small subunits, a [4Fe-4S] cluster and various other subunits and proton antiporters. The second cluster possesses a hydrogenase 3 large subunit hycE (Desfe_1139) a homologue to the large hydrogenase subunit (PF1332) in *P. furiosus (Jenney & Adams, 2008)*. This subunit transcribes a formate hydrogen lyase subunit 5 which is part of the formate hydrogen lyase system.

For maturation of these hydrogenases a complex process with many genes of different function is required. *D. fermentans* possesses hycE associated with the large subunit, the assembly proteins HupF for Ni incorporation and maturation, HypC used as chaperone for metal insertion and HypE for possible purine binding and a maturation protein hypF for delivery of the ligands CN and CO (Casalot & Rousset, 2001). A comparison of bacterial and archaeal hydrogenase maturation proteins can be found in Table 5.

Apart from these clusters it contains the expression proteins HupK, resembling a large subunit, for binding of Fe and the ligands CN and CO and HypD again for Ni incorporation and maturation (Casalot & Rousset, 2001). Additionally it holds a hydrogenase maturation protease (Desfe_0501) including a transcript for coenzyme F_{420}-reducing hydrogenase delta subunit.

Table 4 – Hydrogenase and maturation proteins of *D. fermentans* according to NCBI search.

Desfe locus	Desfe NCBI Name	Subunit	Special features
Desfe_0087	AIR Synthase	HypE	HypE hydrogenase maturation factor
Desfe_0168	NAD-ubiquinone oxidoreductase subunit 4L	Subunit C 4L	Na^+/H^+ antiporter subunit C Multisubunit Na^+/H^+ antiporter MnhC subunit
Desfe_0175	NAD-ubiquinone oxidoreductase subunit B	Subunit B	
Desfe_0189	NAD-ubiquinone oxidoreductase subunit 4L	Suunit 4L	
Desfe_0337	Hydrogenase assembly protein HupF	HupF, HypE	hydrogenase expression formation protein HypE
Desfe_0338	Hydrogenase assembly protein HypC	HypC, HupF	HupF/HypC family
Desfe_0339	Hydrogen maturation protein HypF	HypF, NuoD	carbamoyltransferase
Desfe_0340	NADH-quinone oxidoreductase subunit B	Subunit B	[NiFe]-hydrogenase III small subunit
Desfe_0341	NADH dehydrogenase		
Desfe_0342	NADH-ubiquinone oxidoreductase	Subunit D HycE2	NADH-quinone oxidoreductase subunit D HycE2 [NiFe]-hydrogenase III large subunit NuoD NADH:ubiquinone oxidoreductase 49 kD subunit (chain D)
Desfe_0343	NADH-ubiquinone oxidoreductase	Subunit I	[4Fe-4S] double cluster binding domain
Desfe_0345	cation:proton antiporter	MnhG	Multisubunit Na^+/H^+ antiporter, MnhG subunit
Desfe_0346	sodium:proton antiporter		unknown
Desfe_0347	sodium:proton antiporter		unknown
Desfe_0348	NAD-ubiquinone oxidoreductase subunit 4L	Subunit 4L MnhC	Na^+/H^+ antiporter subunit C Multisubunit Na^+/H^+ antiporter, MnhC subunit

Desfe_0501	Hydrogen maturation protease		coupled with a region for frhD (coenzyme F_{420}-reducing hydrogenase delta subunit)
Desfe_0788	Hydrogenase exprression protein HupK	HupK hypA	Hydrogenase expression/synthesis hypA
Desfe_0861	Hydrogenase exprression protein HypD	HypD	
Desfe_1135	NAD ubiquinone oxidoreductase	Subunit D	Na^+/H^+ antiporter subunit D Formate hydrogenlyase subunit 3/Multisubunit Na^+/H^+ antiporter, MnhD subunit
Desfe_1136	NAD ubiquinone oxidoreductase		
Desfe_1137	hypothetical protein		
Desfe_1138	Hydrogenase		NAD dehydrogenase
Desfe_1139	Hydrogenase 3 large subunit hycE	HycE	formate hydrogenlyase subunit 5; HycBCDEFG is part of the formate hydrogenlyase system which is involved in the cleaving of formate to dihydrogen and carbon dioxide [NiFe]-hydrogenase III large subunit NADH:ubiquinone oxidoreductase 49 kD subunit (chain D) Respiratory-chain NADH dehydrogenase
Desfe_1140	[4Fe-4S] ferredoxin	Subunit H	Hydrogenase 4 subunit H
Desfe_1141	NAD ubiquinone oxidoreductase		

Table 5 – Comparison of bacterial (Excerpt from Casalot & Rousset, 2001) and archaeal hydrogenase maturation proteins. [a] according to Jenney & Adams, 2008; [b] according to van de Werken, et al., 2008.

Function	**Species**							
	Escherichia	***Desulfovibrio***		***Methanosarcina***		**Desulfuro-coccus**	**Pyrococcus**	**Caldicellulo-siruptor**
	coli	***gigas***	***fructo-sovorans***	***barkeri***	***mazei***	***fermentans***	***furiosus*** [a]	***saccaro-lyticus*** [b]
membrane bound hydrogenase						*MBH*	*MBH*	*MBH*
Histidine kinase								
Response regulator (NtrC)	hydG							
[2Fe-2S], NAD and FMN binding								
[4Fe-4S] binding								
Small subunit (SSU)	*hyaA*	*hynA*	*hynA*	*echC*	*vhoG*	*SU-C*		*hydA*
Large subunit (LSU)	*hyaB*	hynB	hynB	echE	vhoA	*hycE2*		hydB
Cytochrome b	*hyaC*				vhoC			hydC
C-terminal peptidase	*hyaD*	hynC	hynC		vhoD		*hycI*	
Ni incorporation/ maturation						*hupF*		
SSU maturation?	*hyaE*							
SSU maturation	*hyaF*							
Fe(CN)$_2$CO binding?						*HupK*		
Ni incorporation/ maturation	*hybF*						*hypA*	*hypA*
Nickelin/Ni insertion	*hypB*						*hypB*	*hypB*
Chaperone/maturation	*hypC/hybG*	hynD				hypC	hypC	hypC
Ni incorporation/ maturation	*hypD*					*hypD*	*hypD*	*hypD*
Purine derivative binding?	*hypE*					*hypE*	*hypE*	*hypE*
CN/CO delivery	*hypF*					hypF	hypF	hypF
Membrane protein				*echB*				
Membrane protein				*echA*				
Iron sulfur protein				*echF*				
								hydD?

3.4.5 Transfer of Carbon Sources

D. fermentans has a broad spectrum of general sugar transporters. According to NCBI 97 ABC transporters (i.e. Desfe_0184, Desfe_0187, Desfe_0620, Desfe_0639, Desfe_0721 and Desfe_0754) were found. Additionally is possesses various other enzymes with important functions in relation to sugar transport like the multiple sugar transport system ATP-binding protein (Desfe_1188) which channels, the sugar ABC transporter permease (Desfe_0355 and Desfe_0366), the ABC transporter substrate-binding protein (Desfe_0354) and the ABC transporter permease (Supplementary Table 1 and 2).

Starch is phosphorylated by starch phosphorylase (Desfe_0264) to glycogen and alpha-D-glucose-1-phosphate. From glycogen it is possible to go further to ADP-glucose by starch synthase (glycosyl-transferring) (Desfe_0403). From there it enters the glycolysis at alpha-D-glucose-1-phosphate via nucleotidyltransferase (Desfe_0189) or sugar-phosphate nucleotidyltransferase (Desfe_ 0962) gaining one ATP.

Cellulose is degraded by endoglucanase (Desfe_0691) to cellobiose and further to beta-D-glucose by a beta-glucosidase (Desfe_0624) followed by an ATP consuming step to beta-d-glucose-6-phosphate by a ROK family protein (Desfe_0578). With the bifunctional phosphoglucose/phosphomannose isomerase (Desfe_1128) it enters the glycolysis at beta-D-fructose-6-phosphate.

Fructose enters the pathway via phosphofructokinase (Defe_0719 and Desfe_0968) to beta-D-fructose 6-phosphate and consumes one ATP.

Sucrose is split into D-fructose and alpha-D-glucose by a hypothetical protein annotated as sucrose alpha-glucosidase (Desfe_0611) and from the latter further to alpha-D-glucose-phosphate by a ROK family protein (Desfe_0578) using one ATP.

Lactose is split into D-galactose and D-glucose by beta-glucosidase (Desfe_0624) and followed again generating alpha-D-glucose-6-phosphate by a ROK family protein (Desfe_0578).

Maltose is degraded by the alpha-amylase (Desfe_0406) using one ATP, followed by the pullulanase (Desfe_0644) to amylase and starch synthase (glycosyl-transferring) (Desfe_0403) to ADP-glucose. From there it enters the glycolysis at alpha-D-glucose-1-phosphate via nucleotidyltransferase (Desfe_0189) or sugar-phosphate nucleotidyltransferase (Desfe_ 0962).

Degradation of arabinose in archaea is not fully resolved yet and needs further evaluation. *D. fermentans* possesses an alcohol dehydrogenase Desfe_1240 containing a D-arabino-1 dehydrogenase transcript, homologue to *Sulfolobus* *solfataricus* SSO1300. This would form D-arabino-1,4-lactate, generating NADH. Additional genes for arabinose degredation like SSO3124, SSO3117 and SSO3118 were not found (Peng*, et al.*, 2011). The situation was similar with genes of *Haloferax volcanii*. The homologue NAD-dependend epimerase (Desfe_0989) was found for HVO_B0032 epimerase/dehydrogenase which would result in L-arabinoate and NADH. Unfortunately no similar genes to HVO_B0038A, HVO_B0027 or HVO_B0039 were found for further degredation and incorporation to glycolysis (Johnsen*, et al.*, 2013).

In theory *D. fermentans* could be able to use formate or CO_2 as carbon source. Formate could be degraded to CO_2 using formate dehydrogenase while generating NADH, coupled with a reduction to CO by carbon-monoxide dehydrogenase (Figure 9). It would then enter the end of glycolysis by forming acetyl-CoA with the methyl-group from 5-methyl-tetrahydrofuran.

4. Discussion

4.1 Metabolism of *D. fermentans*

D. fermentans was able to grow on all test substrates, although with differences in growth rate, cell density and doubling time. It is clearly visible from Table 2 and Table 3 that growth in the bioreactor was faster than in the serum bottle experiments, but reached lower cell densities. Compared to Perevalova*, et al.*, 2005 the experiments resulted in general in a lower growth rate and in the majority reached lower final cell densities (Table 6).

Table 6 – Comparison of growth rate and maximum cell number of *D. fermentans* between our experiments (Exp.) and the literature (Lit.) (Perevalova*, et al.*, 2005).

Substrate	**Doubling Time [h]**		**max. number of cells [ml^{-1}]**	
	Exp.	**Lit.**	**Exp.**	**Lit.**
Starch_wY_N_2/CO_2	12	6	2.0-4.0E+07	5.0E+07
Fructose wY_N_2/CO_2	14		2.0-4.0E+07	2.0E+07
Fructose_woY_N_2	74		2.5E+07	
Fructose_woY_N_2/CO_2	91		2.3E+07	
Arabinose_wY_N_2/CO_2	14		1.3-2.0E+07	2.5 - 5.0E+07
Maltose_wY_N_2/CO_2	155		1.3-1.8E+07	2.0E+07
Cellulose_wY_N_2	33	11	1.3-2.0E+07	2.5E+07
Lactose_wY_N_2/CO_2	73		0.5E+07	2.0E+07
Sucrose_wY_N_2/CO_2	110		0.5E+07	2.5 - 5.0E+07

One reason could be the formation of biofilm in our experiments with the result that proper counting was not possible anymore. Evaluation of pictures taken from counting with the Neubauer chamber showed that biofilm formation took place in the bioreactor after approximately 150 h of growth. In general one can note that hyperthermophiles, compared to mesophilic organisms, show a tendency to grow in lower cell densities. Up to today there are still many characteristics unknown about the factors that limit growth for hyperthermophilic organisms (Chou*, et al.*, 2008, Pawar & Niel, 2013).

A different way to determine growth could be the measurement of the optical density (OD). Unfortunately the results of OD measurements were irregular and as already described sugar particles disturbed the measurement.

Comparison of the sparging gas (20% CO_2 in 80% N_2 and 100% N_2) on fructose showed that sparging with the gas mix including CO_2 resulted in a six time higher growth rate. According to van Niel*, et al.*, 2012 and Willquist*, et al.*, 2009 CO_2 can act as growth initiation and as additional carbon source for CO_2-fixation by reduction of CO_2 to CO (Figure 9). Growth was limited by the omission of Yeast extract. This confirms an earlier study in which growth of *D. fermentans* with only YE as carbon source was demonstrated (Perevalova*, et al.*, 2005).

By only considering experiments in which *D. fermentans* was grown on YE and sparging gas CO_2/N_2, the highest growth rate in this study was found on the following substrates and in the following order: starch (μ_{max} 0.056), fructose (μ_{max} 0.051), arabinose (μ_{max} 0.049), lactose (μ_{max} 0.009), sucrose (μ_{max} 0.006) and maltose (μ_{max} 0.004). Although no growth was visible with cellulose on N_2/CO_2, based on the growth rate on sparging with N_2 (μ_{max} 0.021), I would assume, that if growth would have been detected, it could line up before fructose and arabinose (Table 2).

According to the metabolic burden, from transport to the production of acetate starch would be the best substrate resulting in ATP gain. For the other substrates it would be energy neutral, with no ATP gain or loss. That leads to the question on how the energy metabolism of *D. fermentans* is functioning and if it safes energy during the metabolism. For that we have to take a closer look to the whole energy metabolism in relation to the carbon metabolism. ABC transporters for example have different affinities to specific sugars (Willquist*, et al.*, 2010, Bräsen*, et al.*, 2014). Utilization of the substrate with the lowest energy cost could save ATP in the beginning. Knowing which transporters are coupled to which sugars could be advantageous for further studies.

Generating more ATP could also be possible by the use of triosephosphate isomerase (Desfe_1348) on glycerine-phosphate, synthetized by the conversion from beta-D-fructose-1,6-bisphosphate resulting in more glyceraldehyde-3-phosphate ready for use in glycolysis.

The ability of *D. fermentans* to grow on formate and CO_2 has to be examined in further studies. This would be an energy neutral step and even result in electron carriers for H_2 production (Figure 9). Nevertheless formate is also a potent cell toxin and is often detoxified via FHL (Rittmann*, et al.*, 2015).

A closer look should be taken to the whole metabolism like the transport and utilization of arabinose (Sato & Atomi, 2011) or other carbohydrates and the use of the modified EMP pathway. Not to forget the incomplete citrate cycle or the possibility of *D. fermentans* to use different carbon assimilation pathways. *Desulfurococcales* for example are apparently able to use the dicarboxylate/4-hydroxybutyrate cycle, which partly resembles the reductive citrate cycle, with succinyl-CoA as key enzyme (Huber*, et al.*, 2008, Berg*, et al.*, 2010, Bar-Even*, et al.*, 2011).

Regarding the ecosystem of its origin site, the hot spring in the Uzon caldera *D. fermentans* probably forms complex food webs with other hyperthermophiles where it consumes available organic material or CO_2 as carbon and energy source. The formation of possible fermentative end products like acetate, formate, CO_2 and H_2 could then be utilized by other hyperthermophilic bacteria and archaea producing e.g. methane (Lowe*, et al.*, 1993, Huber*, et al.*, 2000).

4.2 Production of biohydrogen

In theory *D. fermentans* has the potential to be an excellent biohydrogen producer from cellulosic substrates, as the strain has a lot of features that can promote H_2 production.

It possesses the glyceraldehyde ferredoxin oxidoreductase (GAPOR), a unique enzyme ot the archaeal EM pathway (Verhees*, et al.*, 2003), which supplies 2 $Fd_{(red)}$. The generation of $Fd_{(red)}$ with -398 mV reduction potential is more favourable than NADH (resulting from GAPDH) with -320 mV (Pawar & Niel, 2013). Together with acetate as end product this could result in 4 $Fd_{(red)}$ to function as electron carrier and be advantageous in relation to H_2 production (Willquist*, et al.*, 2009). In case of *D. fermentans* although omission of the ATP-generating way via GAPDH seems disadvantageous, as most test substrates will need this energy from acetate production. Therefore acetate will most likely be the only end product of the fermentative metabolism.

This was confirmed according to Perevalova, et al., 2005 where only acetate could be detected from growth on starch and sucrose. This again, would not support the hypothesis of biohydrogen production. If *D. fermentans* is focused on generating other end products like ethanol or lactate which consume NADH, production of H_2 is avoided, as important electron carriers would be used up. This can occur if the organism is under stress (Pawar & Niel, 2013). Additionally ATP generated from acetate would be missing. There is also a possibility that acetate production might inhibit growth and the fermentation ability, which was shown in *C. saccharolyticus* (van Niel*, et al.*, 2003). Metabolic pathways and products still need to be evaluated in greater detail.

In relation to acetate assimilation, the possibility for use of the dicarboxylate/4-hydroxybutyrate cycle arises. Genome evaluation of *D. fermentans* showed the presence of homologous genes of this cycle, in particular the degradation of the key enzyme succinyl-CoA (Berg*, et al.*, 2007, Huber*, et al.*, 2008).

D. fermentans also possesses genes for the pyruvate formate-lyase and formate dehydrogenase ($HCOO^- \rightarrow CO_2 + 2e^-$) generating NADH, in other words, more electrons to use. *T. onnurineus* for example has a coupled mechanism to generate ATP from formate lyase by a hydrogenase, where the reduction to H_2 takes place ($2H^+ + 2e^- \rightarrow H_2$), linked to a proton antiporter involved in the formation of a sodium gradient which is used for ATP generation (Chou*, et al.*, 2008, Hallenbeck, 2009, Kim*, et al.*, 2010). PFL and FDH in *D. fermentans* are coupled to a membrane bound [NiFe] hydrogenase with a proton antiporter. If there is such an ATP synthesis system in *D. fermentans* has to be evaluated. Although, the function of PFL coupled with PFOR is not always related to biohydrogen production as some organisms have multiple homologues encoded in their genomes but use them in different pathways and for different functions (Hallenbeck, 2012).

Despite all these possibilities to produce biohydrogen but due to its slow growth and putative poor energy metabolism, HER and qH_2 were very low to nonexistent (Supplementary Table 3). Constant sparging with N_2 with 1-6 sL h^{-1} in the bioreactor could have been too high with the result, that H_2 got diluted below gas chromatographic detection limit. As HER was that low it was not possible to evaluate if chemical or physical parameters like pH, substrate solubility, temperature, agitation or partial gas pressure had an effect on biohydrogen production.

5. Conclusion

This study focused on the analysis of *D. fermentans* as possible biohydrogen producer and on a reconstruction of its physiology from the genome sequence. *D. fermentans* is able to grow on different carbohydrates with highest growth rate on starch followed by cellulose, fructose and arabinose. The growth rate is elevated by the use of yeast extract and the sparging gas CO_2/N_2 both potentially acting as additional carbon sources or by the use of more favourable metabolic energy routes coupled with the generation of ATP.

Metabolic reconstruction revealed a large number of broad ABC transporters, all necessary genes for glycolysis containing the classical but also the modified Embden-Meyerhof pathway. The reductive citrate cycle is incomplete, lacking the step from malate to fumarate. The possession of biohydrogen promoting genes like pyruvate formate-lyase and formate dehydrogenase has been confirmed as well as the possibility for CO_2-fixation by reducing CO_2 to CO with a carbon-monoxide dehydrogenase. *D. fermentans* also contains genes for 2 membrane bound [NiFe] hydrogenases indicating the potential to produce hydrogen. Nevertheless the experimentally determined hydrogen production of *D. fermentans* was minor, as HER was below 0.02 mmol L^{-1} h^{-1} under all conditions tested.

6. Acknowlegements

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7. References

1. **Bar-Even A**, Noor E & Milo R (2011) A survey of carbon fixation pathways through a quantitative lens. *Journal of experimental botany*.
2. **Berg IA**, Kockelkorn D, Buckel W & Fuchs G (2007) A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in Archaea. *Science* 318: 1782-1786.
3. **Berg IA**, Kockelkorn D, Ramos-Vera WH*, et al.* (2010) Autotrophic carbon fixation in archaea. *Nat Rev Micro* 8: 447-460.
4. **Bräsen C**, Esser D, Rauch B & Siebers B (2014) Carbohydrate Metabolism in Archaea: Current Insights into Unusual Enzymes and Pathways and Their Regulation. *Microbiology and Molecular Biology Reviews: MMBR* **78**: 89.
5. **Broderick JB**, Duffus BR, Duschene KS & Shepard EM (2014) Radical S-Adenosylmethionine Enzymes. *Chemical Reviews* 114: 4229-4317.
6. **Casalot L & Rousset M** (2001) Maturation of the [NiFe] hydrogenases. *Trends in Microbiology* 9: 228-237.
7. **Chou CJ**, Jenney Jr FE, Adams MWW & Kelly RM (2008) Hydrogenesis in hyperthermophilic microorganisms: Implications for biofuels. *Metabolic Engineering* 10: 394-404.
8. **Crain AV & Broderick JB** (2014) Pyruvate Formate-lyase and Its Activation by Pyruvate Formate-lyase Activating Enzyme. *The Journal of Biological Chemistry* 289: 5723-5729.
9. **Fukuda W**, Ismail YS, Fukui T, Atomi H & Imanaka T (2005) Characterization of an archaeal malic enzyme from the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. *Archaea (Vancouver, B.C.)* 1: 293-301.
10. **Hallenbeck PC** (2009) Fermentative hydrogen production: Principles, progress, and prognosis. *International Journal of Hydrogen Energy* 34: 7379-7389.
11. H**allenbeck PC** (2012) Fundamentals of dark hydrogen fermentations: multiple pathways and enzymes.
12. **Hedderich R (**2004) Energy-Converting [NiFe] Hydrogenases from Archaea and Extremophiles: Ancestors of Complex I. *Journal of Bioenergetics and Biomembranes* 36: 65-75.
13. **Huber H**, Gallenberger M, Jahn U*, et al.* (2008) A dicarboxylate/4-hydroxybutyrate autotrophic carbon assimilation cycle in the hyperthermophilic

Archaeum Ignicoccus hospitalis. *Proceedings of the National Academy of Sciences* 105: 7851-7856.

14. **Huber R**, Huber H & Stetter KO (2000) Towards the ecology of hyperthermophiles: biotopes, new isolation strategies and novel metabolic properties. *FEMS Microbiology Reviews* 24: 615-623.
15. **Jenney FE & Adams MWW** (2008) Hydrogenases of the Model Hyperthermophiles. *Annals of the New York Academy of Sciences* 1125: 252-266.
16. **Johnsen U**, Sutter J-M, Zaiß H & Schönheit P (2013) l-Arabinose degradation pathway in the haloarchaeon *Haloferax volcanii* involves a novel type of l-arabinose dehydrogenase. *Extremophiles* 17: 897-909.
17. **Kim JYH**, Jo BH & Cha HJ (2010) Production of biohydrogen by recombinant expression of [NiFe]-hydrogenase 1 in *Escherichia coli*. *Microb Cell Fact* 9.
18. **Lowe SE**, Jain MK & Zeikus JG (1993) Biology, ecology, and biotechnological applications of anaerobic bacteria adapted to environmental stresses in temperature, pH, salinity, or substrates. *Microbiological reviews* 57: 451-509.
19. **Martinez-Porqueras E**, Rittmann S & Herwig C (2013) Analysis of H2 to CO2 yield and physiological key parameters of *Enterobacter aerogenes* and *Caldicellulosiruptor saccharolyticus*. *International Journal of Hydrogen Energy* 38: 10245-10251.
20. **Pawar SS & Niel EWJ** (2013) Thermophilic biohydrogen production: how far are we? *Applied Microbiology and Biotechnology* 97: 7999-8009.
21. **Peng N**, Ao X, Liang Yun X & She Q (2011) Archaeal promoter architecture and mechanism of gene activation. *Biochemical Society Transactions* 39: 99-103.
22. **Perevalova AA**, Svetlichny VA, Kublanov IV*, et al.* (2005) *Desulfurococcus fermentans sp. nov.*, a novel hyperthermophilic archaeon from a Kamchatka hot spring, and emended description of the genus *Desulfurococcus*. *International Journal of Systematic and Evolutionary Microbiology* 55: 995-999.
23. **Ramírez-Morales JE**, Tapia-Venegas E, Toledo-Alarcón J & Ruiz-Filippi G (2015) Simultaneous production and separation of biohydrogen in mixed culture systems by continuous dark fermentation. *Water Science and Technology* 71: 1271-1285.
24. **Rittmann S & Herwig C** (2012) A comprehensive and quantitative review of dark fermentative biohydrogen production. *Microbial Cell Factories* 11: 1-18.

25. **Rittmann SKMR**, Lee HS, Lim JK, Kim TW, Lee J-H & Kang SG (2015) One-carbon substrate-based biohydrogen production: Microbes, mechanism, and productivity. *Biotechnology Advances* 33: 165-177.
26. **Sato T & Atomi H** (2011) Novel metabolic pathways in Archaea. *Current Opinion in Microbiology* 14: 307-314.
27. **Schut GJ,** Boyd ES, Peters JW & Adams MWW (2013) The modular respiratory complexes involved in hydrogen and sulfur metabolism by heterotrophic hyperthermophilic archaea and their evolutionary implications. *FEMS Microbiology Reviews* 37: 182-203.
28. **Susanti D**, Johnson EF, Rodriguez JR*, et al.* (2012) Complete genome sequence of *Desulfurococcus fermentans*, a hyperthermophilic cellulolytic crenarchaeon isolated from a freshwater hot spring in Kamchatka, Russia. *Journal of bacteriology* 194: 5703-5704.
29. **Thauer RK**, Jungermann K & Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological Reviews* 41: 100-180.
30. v**an de Werken HJ**, Verhaart MR, VanFossen AL*, et al.* (2008) Hydrogenomics of the extremely thermophilic bacterium *Caldicellulosiruptor saccharolyticus*. *Applied and environmental microbiology* 74: 6720-6729.
31. **van Niel EW**, Willquist K, Zeidan AA, de Vrije T, Mars AE & Claassen PA (2012) Hydrogen production by thermophilic fermentation. *State of the Art and Progress in Production of Biohydrogen*), pp. 137-159. Bentham Science Publishers.
32. **van Niel EWJ**, Claassen PAM & Stams AJM (2003) Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*. *Biotechnology and Bioengineering* 81: 255-262.
33. **VanFossen AL**, Verhaart MR, Kengen SM & Kelly RM (2009) Carbohydrate utilization patterns for the extremely thermophilic bacterium *Caldicellulosiruptor saccharolyticus* reveal broad growth substrate preferences. *Applied and environmental microbiology* 75: 7718-7724.
34. **Verhaart MRA**, Bielen AAM, Oost Jvd, Stams AJM & Kengen SWM (2010) Hydrogen production by hyperthermophilic and extremely thermophilic bacteria and archaea: mechanisms for reductant disposal. *Environmental Technology* 31: 993-1003.

35. **Verhees CH**, Kengen SWM, Tuininga JE, Schut GJ, Adams MWW, de Vos WM & Oost Jvd (2003) The unique features of glycolytic pathways in Archaea. *Biochemical Journal* 375: 231-246.
36. **Willquist K & van Niel EWJ** (2012) Growth and hydrogen production characteristics of *Caldicellulosiruptor saccharolyticus* on chemically defined minimal media. *International Journal of Hydrogen Energy* 37: 4925-4929.
37. **Willquist K**, Claassen PA & Van Niel EW (2009) Evaluation of the influence of CO 2 on hydrogen production by *Caldicellulosiruptor saccharolyticus*. *International Journal of Hydrogen Energy* 34: 4718-4726.
38. **Willquist K**, Zeidan AA & van Niel EWJ (2010) Physiological characteristics of the extreme thermophile *Caldicellulosiruptor saccharolyticus*: an efficient hydrogen cell factory. *Microb Cell Fact* 9.
39. **Yano T** (2002) The energy-transducing NADH: quinone oxidoreductase, complex I. *Molecular Aspects of Medicine* 23: 345-368.
40. **Zeidan AA & van Niel EWJ** (2010) A quantitative analysis of hydrogen production efficiency of the extreme thermophile *Caldicellulosiruptor owensensis* OL. *Int J Hydrogen Energ* 35.

8. Zusammenfassung - Summary

Biowasserstoff ist eine erneuerbare Energie-Quelle und ein umweltfreundlicher Kraftstoff, bei dem keine Treibhausgase gebildet werden. Verbrennung von Wasserstoff resultiert in Wasser. Durch den Nutzen von Abfallprodukten aus der industriellen Verwertung kann eine kosteneffiziente Bildung von Biowasserstoff erreicht werden. Ein potentieller Kandidat für die Produktion von Biowasserstoff ist *Desulfuroccus fermentans.* Er gehört zu den hyperthermophilen Archaea und der Familie *Desulfurococcacaea*.

In dieser Studie wurde das Wachstum, der Metabolismus und das Potential zur Biowasserstoff Produktion von *D. fermentans* untersucht.

Die Wachstumsrate wurde in geschlossenen Systemen in Serumflaschen und im Bioreaktor getestet mit dem Resultat, dass er auf verschiedenen Zuckern wie Arabinose, Stärke, Fructose, Maltose, Saccharose, Laktose und Zellulose mit unterschiedlicher Wachstumsrate und Zelldichte wachsen kann. Das ist abhängig von der Medium Zusammenstellung, ob Hefe Extrakt enthalten ist und dem verwendeten Atmosphären-Gas. Hefe und das Gas Gemisch H_2/CO_2 waren dabei wachstumsfördernd.

Eine genom-basierte Rekonstruktion des Metabolismus von *D. fermentans* zeigte, dass dieser Organismus viele Gene besitzt, die die Biowasserstoffproduktion fördern oder möglich machen. Es kann einige Wege nutzen, die zum Erhalt von Reduktionsäquivalenten führen, wie NAD(P)H oder $Fd_{(red)}$. Diese gelten als Elektronen-Spender und werden von verschiedenen Mechanismen zum Biowasserstoff Produktion genutzt. Als Beispiel die Existenz der Glyzerinaldehyde-Ferredoxin-Oxidoreduktase (GAPOR), ein modifizierter Weg des Embden-Meyerhof-Stoffwechselweges, den vor allem Archaea benutzen. Weiters enthält das Genom Gene für eine Umwandlung von Pyruvate zu Formate, welches wiederum oxidiert werden kann um Elektronen zu generieren. Zur Biowasserstof Produktion werden Hydrogenasen eingesetzt. *D. fermentans* besitzt zwei Gen-Cluster mit [NiFe] Hydrogenasen und den dazugehörigen Aufbau-Proteinen.

Einige Schritte im Bezug auf den metabolischen Weg weisen noch einige experimentell zu adressierende Lücken auf, wie der reduktive Zitrat-Zyklus und der Transport einiger Zucker, zum Beispiel Arabinose.

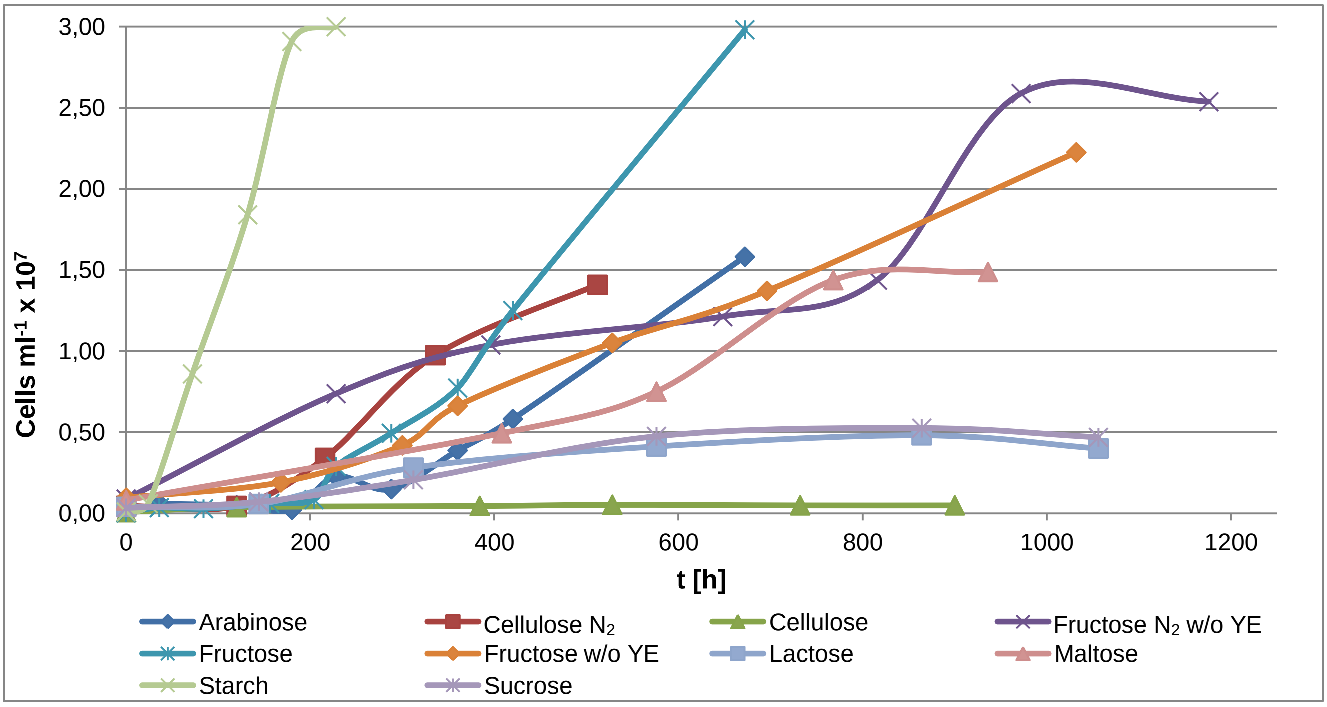
Trotz der vielen Möglichkeiten Wasserstoff zu generieren, war die Ausbeute der „Wasserstoff-Entstehungs-Rate“ (HER) sehr gering oder nicht existent. Der Gebrauch der richtigen Wege zur effizienten Biowasserstoff Produktion muss noch verdeutlicht werden.

9. List of Figures

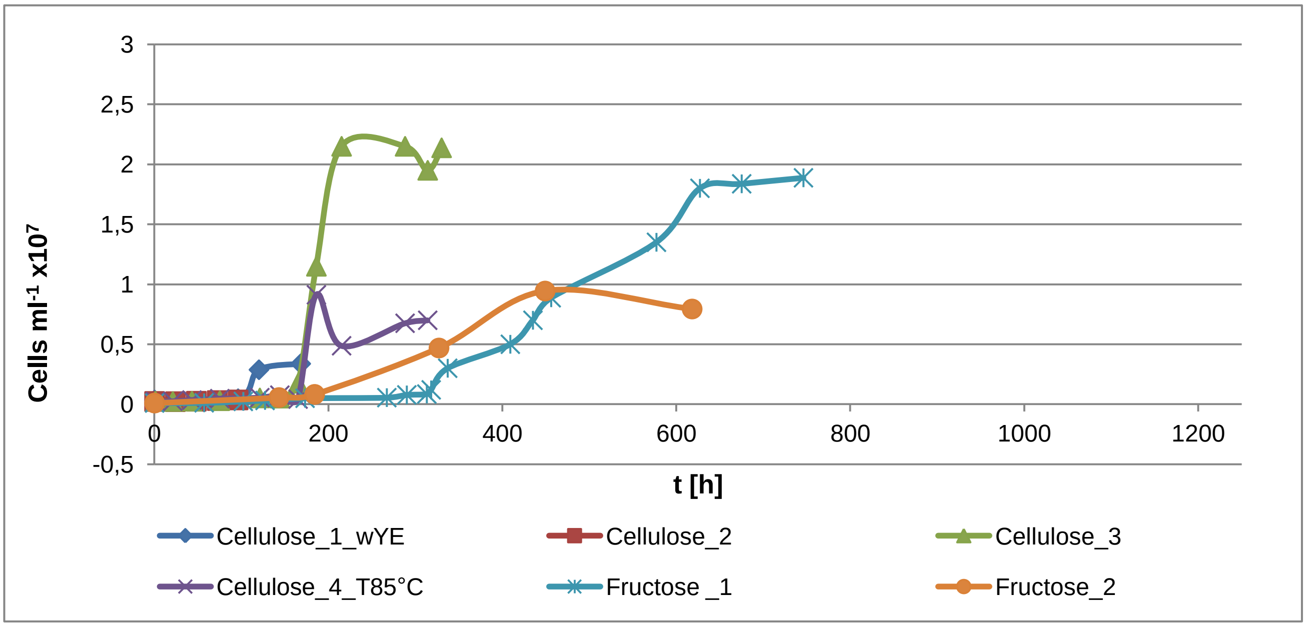
10. List of Tables

11. Appendix

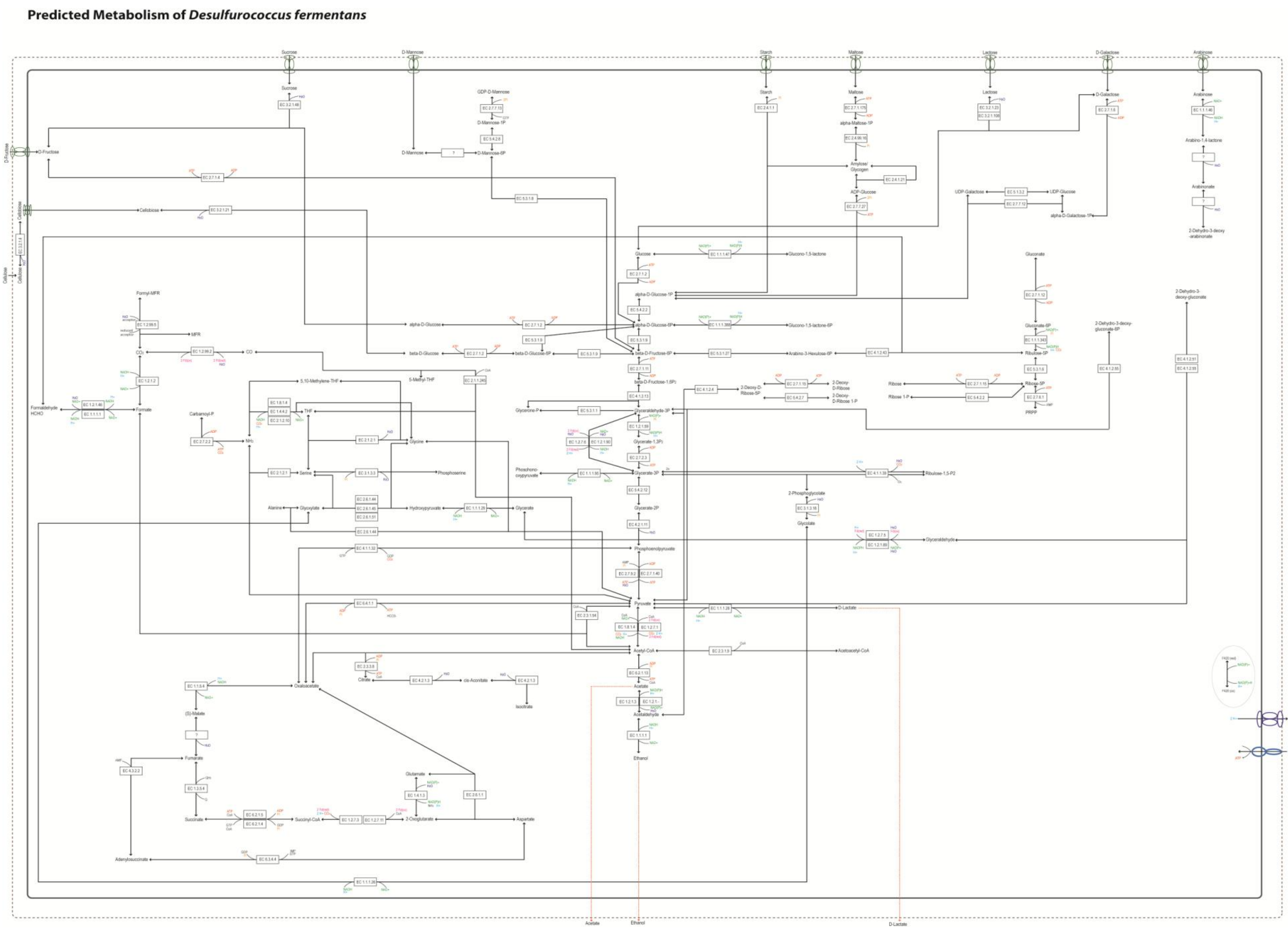
Supplementary Figure 1– Growth Curve of *D. fermentans* in serum bottles with varying parameters like carbon source, sparging gas and yeast extract. If not indicated otherwise all experiments were performed with YE and sparging with CO_2/N_2.



Supplementary Figure 2 – Growth Curve of *D. fermentans* in bioreactor experiments of cellulose and fructose with varying parameters like pH and temperature. If not indicated otherwise all experiments were performed without YE, a temperature of 80 °C and and sparging with N_2.



Supplementary Figure 3 - Overview of metabolism and transport of D. fermentans (will be attached on the last page in ratio of 1:1)



Supplementary Table 1 – Genes of *D. fermentans* according to BLAST search (annotated from KEGG), Abbreviations: DF, D. fermentans; nt, nucleotide length; aa, amino acid length, +, genes present, -, genes not present.

EnzymeNr.		**Locus_Tag KEGG**	**Enzyme *D.fermentans* KEGG**	**DF Protein Number NCBI**	**nt (NCBI)**	**aa (NCBI)**	**Blastp**	**Q cover [%]**	**E-value**	**Identity [%]**
Glycolysis										
EC 5.4.2.2	+	Desfe_0111	phosphomannomutase phosphoglucomutase	WP_014766929.1	1377	458	phosphoglucosamine mutase [D. fermentans] phosphoglucomutase [D. fermentans]	94 99	9E-165 3E-48	56 31
EC 5.3.1.9	+	Desfe_1128	bifunctional phosphoglucose phosphomannose isomerase	WP_014767896	999	332	mannose-6-phosphate isomerase [Desulfurococcus fermentans]	96	4,00E-32	33
EC 4.1.2.13	+	Desfe_0718	fructose-bisphosphate aldolase, class I	WP_014767518.1	819	272	fructose-bisphosphate aldolase [Desulfurococcus fermentans]	98	1,00E-105	55
	+	Desfe_1349	fructose 1,6-bisphosphate aldolase/phosphatase	WP_014768106.1	1149	382	fructose 1,6-bisphosphatase [Desulfurococcus fermentans]	93	0.0	74
EC 1.2.1.59		Desfe_0262	glyceraldehyde 3-phosphate dehydrogenase (NAD(P)+)	WP_014767076.1	1074	357	glyceraldehyde-3-phosphate dehydrogenase [Desulfurococcus fermentans]	97	1,00E-111	49
EC 1.2.7.6	+	Desfe_0557	Aldehyde ferredoxin oxidoreductase	WP_014767359.1	1953	650	aldehyde ferredoxin oxidoreductase [Desulfurococcus fermentans] aldehyde ferredoxin oxidoreductase [Desulfurococcus fermentans]	98 55	2,00E-147 7,00E-16	39 24
EC 1.2.1.90	+	Desfe_0067	glyceraldehyde-3-phosphate dehydrogenase [NAD(P)+]	WP_014766885.1	1521	506	aldehyde dehydrogenase [Desulfurococcus fermentans]	98	0.0	62
EC 2.7.2.3	+	Desfe_0261	phosphoglycerate kinase	WP_014767075.1	427	1284	phosphoglycerate kinase [Desulfurococcus fermentans]	99	6,00E-146	53
EC 5.4.2.12	+	Desfe_0416	phosphonopyruvate decarboxylase-related protein	WP_048815972.1	424	1239	phosphonopyruvate decarboxylase [Desulfurococcus fermentans]	96	8,00E-112	46
							phosphonopyruvate decarboxylase [Desulfurococcus fermentans]	88	6,00E-67	37
	-	not found								

EC 4.2.1.11	+	Desfe_0063	enolase	WP_014766881.1	435	1308	enolase [Desulfurococcus fermentans]	96	1,00E-131	49
EC 2.7.1.40	+	Desfe_1347	pyruvate kinase	WP_014768104.1	473	1422	pyruvate kinase [Desulfurococcus fermentans]	99	8,00E-88	38
							pyruvate kinase [Desulfurococcus fermentans]	93	5,00E-59	31
EC 2.7.1.2		Desfe_0578	ROK family protein	AFL66479.1 WP_048815982.1	332 329	999 990	glucokinase [Desulfurococcus fermentans]	99	2,00E-61	41
EC 3.1.3.11	-	Desfe_1349	archaeal fructose 1,6-bisphosphatasefructose 1,6-bisphosphate aldolase/phosphatase	WP_014768106.1	1149	382	-	-	-	-
							fructose 1,6-bisphosphatase [Desulfurococcus fermentans]	93	0.0	74
EC 5.3.1.1	+	Desfe_1348	triosephosphate isomerase	WP_014768105.1	231	696	triose-phosphate isomerase [Desulfurococcus fermentans]	96	4,00E-72	52
EC 4.1.1.32	+	Desfe_0003	Phosphoenolpyruvate carboxykinase (GTP)	WP_014766823.1	629	1890	phosphoenolpyruvate carboxykinase [Desulfurococcus fermentans]	99	0,00E+00	47
EC 1.2.7.1	+	Desfe_1298	pyruvate ferredoxin oxidoreductase alpha subunit	WP_014768058.1	393	1182	pyruvate ferredoxin oxidoreductase [Desulfurococcus fermentans] 2-ketoisovalerate ferredoxin oxidoreductase subunit alpha [Desulfurococcus fermentans]	100 95	0 3,00E-80	71 42
	+	Desfe_0503	pyruvate ferredoxin oxidoreductase alpha subunit	WP_014767308.1						
	+	Desfe_1296	pyruvate ferredoxin oxidoreductase gamma subunit	WP_014768056.1	582	193	pyruvate/ketoisovalerate oxidoreductase subunit gamma [Desulfurococcus fermentans] pyruvate/ketoisovalerate oxidoreductase subunit gamma [Desulfurococcus fermentans]	98 95	6,00E-81 7,00E-35	62 39
	+	Desfe_0505	pyruvate ferredoxin oxidoreductase gamma subunit	WP_014767310.1	558	185	pyruvate/ketoisovalerate oxidoreductase subunit gamma [Desulfurococcus fermentans] pyruvate/ketoisovalerate oxidoreductase subunit gamma [Desulfurococcus fermentans]	98 95	6,00E-81 7,00E-35	62 39
	+	Desfe_0504	pyruvate ferredoxin oxidoreductase delta subunit	WP_014767309.1	282	93				

	+	Desfe_1297	pyruvate ferredoxin oxidoreductase delta subunit	WP_014768057.1	201	93				
	+	Desfe_0502	pyruvate ferredoxin oxidoreductase beta subunit	WP_014767307.1	1059	352				
	+	Desfe_1299	pyruvate ferredoxin oxidoreductase beta subunit	WP_048816009.1	867	288				
EC 6.2.1.13	+	Desfe_0782	acetyl-CoA synthetase (ADP-forming)	WP_014767577.1	232	699	acetyl-CoA synthetase [Desulfurococcus fermentans] acetyl-CoA synthetase [Desulfurococcus fermentans]	91 87	3,00E-60 9,00E-57	46 44
	-	Desfe_0977	acetyl-CoA synthetase (ADP-forming)				acetyl-CoA synthetase [Desulfurococcus fermentans]acetyl-CoA synthetase [Desulfurococcus fermentans]	9187	3,00E-609,00E-57	4644
	+	Desfe_1050	acetyl-CoA synthetase (ADP-forming)	WP_014767819.1	232	699	acetyl-CoA synthetase [Desulfurococcus fermentans] acetyl-CoA synthetase [Desulfurococcus fermentans]	91 87	3,00E-60 9,00E-57	46 44
EC 1.8.1.4	+	Desfe_0667	dihydrolipoamide dehydrogenase	WP_014767468.1	456	1371	dihydrolipoamide dehydrogenase [Desulfurococcus fermentans]	96	4,00E-44	28
EC 1.1.1.1	+	Desfe_1240	zinc-binding alcohol dehydrogenase family protein	BAE84834	334	1005	alcohol dehydrogenase [Desulfurococcus fermentans] alcohol dehydrogenase [Desulfurococcus fermentans]	98 55	1,00E-80 8,00E-15	45 32
Pentose Phosphate										
EC 5.3.1.9	+	Desfe_1128	bifunctional phosphoglucose phosphomannose isomerase	WP_014767896	999	332	mannose-6-phosphate isomerase [Desulfurococcus fermentans]	96	4,00E-32	33
EC 3.1.3.11	**-**	Desfe_1349	archaeal fructose 1,6-bisphosphatase fructose 1,6-bisphosphate aldolase/phosphatase	WP_014768106.1	1149	382	-	-	-	-

							fructose 1,6-bisphosphatase [Desulfurococcus fermentans]	93	0.0	74
EC 4.1.2.13	+	Desfe_0718	fructose-bisphosphate aldolase, class I	WP_014767518.1	819	272	fructose-bisphosphate aldolase [Desulfurococcus fermentans]	98	1,00E-105	55
	+	Desfe_1349	fructose 1,6-bisphosphate aldolase/phosphatase	WP_014768106.1	1149	382	fructose 1,6-bisphosphatase [Desulfurococcus fermentans]	93	0.0	74
EC 4.1.2.4	-	Desfe_0673	deoxyribose-phosphate aldolase	WP_048815987.1	657	218	type 2 isopentenyl-diphosphate Delta-isomerase [Desulfurococcus fermentans]	12	9,00E-06	50
EC 2.7.1.15	+	Desfe_0717	ribokinase / PfkB domain protein	AFL66616 WP_048815869.1	924	307	phosphofructokinase [Desulfurococcus fermentans] phosphofructokinase [Desulfurococcus fermentans]	98 90	6,00E-102 5,00E-32	47 30
	+	Desfe_0968	ribokinase / PfkB domain protein	AFL66854 WP_014767752.1	927	308	phosphofructokinase [Desulfurococcus fermentans] phosphofructokinase [Desulfurococcus fermentans]	98 90	6,00E-102 5,00E-32	47 30
EC 5.3.1.27	+	Desfe_0297	6-phospho-3-hexuloisomerase	AFL66208.1 / WP_014767110.1"	606	201	hypothetical protein [Desulfurococcus fermentans]	100	2,00E-113	77
							hypothetical protein [Desulfurococcus fermentans]	94	3,00E-49	35
							hypothetical protein [Desulfurococcus fermentans]	94	3,00E-20	34
EC 4.1.2.43	+	Desfe_0079	3-hexulose-6-phosphate synthase	WP_014766897.1	651	216	orotidine 5'-phosphate decarboxylase [Desulfurococcus fermentans]	92	6,00E-36	37
							orotidine 5'-phosphate decarboxylase [Desulfurococcus fermentans]	72	6,00E-33	42
EC 2.7.1.12	+	Desfe_1214	gluconokinase	WP_014767979.1	1512	503	carbohydrate kinase [Desulfurococcus fermentans]	77	4,00E-46	30
EC 5.3.1.6	+	Desfe_0020	ribose 5-phosphate isomerase B	WP_014766840.1	441	146	ribose-5-phosphate isomerase [Desulfurococcus fermentans]	98	5,00E-34	40
	+	Desfe_0594	ribose 5-phosphate isomerase A	AFL66495.1 / WP_048815860.1	741	246	ribose 5-phosphate isomerase B [Desulfurococcus fermentans]	88	3,00E-21	39
EC 2.7.6.1	+	Desfe_0584	ribose-phosphate pyrophosphokinase	WP_014767386.1	903	300	ribose-phosphate pyrophosphokinase [Desulfurococcus fermentans]	92	1,00E-63	39

EC 5.4.2.2		Desfe_0111	phosphomannomutase phosphoglucomutase	WP_014766929.1	1377	458	phosphoglucosamine mutase [D. fermentans] phosphoglucomutase [D. fermentans]	94 99	9E-165 3E-48	56 31
EC 1.2.7.5	+	Desfe_0695	aldehyde:ferredoxin oxidoreductase	AFL66594.1 / WP_048815866.1	1884	627	aldehyde ferredoxin oxidoreductase [D. fermentans] aldehyde ferredoxin oxidoreductase [D. fermentans] aldehyde ferredoxin oxidoreductase [D.fermentans]	98 95 49	0.0 4E-97 7E-13	56 35 24
	+	Desfe_1093	aldehyde:ferredoxin oxidoreductase	WP_014767861.1	1827	608				
EC 1.2.1.90	+	Desfe_0067	glyceraldehyde-3-phosphate dehydrogenase [NAD(P)+]	WP_014766885.1	1521	506	aldehyde dehydrogenase [Desulfurococcus fermentans]	98	0.0	62
Carbon Metabolism										
EC 2.7.1.2		Desfe_0578	ROK family protein	AFL66479.1 WP_048815982.1	332 329	999 990	glucokinase [Desulfurococcus fermentans]	99	2,00E-61	41
EC 5.3.1.9	+	Desfe_1128	bifunctional phosphoglucose phosphomannose isomerase	WP_014767896	999	332	mannose-6-phosphate isomerase [Desulfurococcus fermentans]	96	4,00E-32	33
EC 5.3.1.8	+	Desfe_1128	mannose-6-phosphateisomerase	WP_014767896.1	999	332	mannose-6-phosphate isomerase [Desulfurococcus fermentans]	96	4,00E-32	33
EC 4.1.2.13	+	Desfe_0718	fructose-bisphosphate aldolase, class I	WP_014767518.1	819	272	fructose-bisphosphate aldolase [Desulfurococcus fermentans]	98	1,00E-105	55
	+	Desfe_1349	fructose 1,6-bisphosphate aldolase/phosphatase	WP_014768106.1	1149	382	fructose 1,6-bisphosphatase [Desulfurococcus fermentans]	93	0.0	74
EC 3.1.3.11	-	Desfe_1349	archaeal fructose 1,6-bisphosphatase fructose 1,6-bisphosphate aldolase/phosphatase	WP_014768106.1	1149	382	-	-	-	-
							fructose 1,6-bisphosphatase [Desulfurococcus fermentans]	93	0.0	74
EC 1.2.1.90	+	Desfe_0067	glyceraldehyde-3-phosphate dehydrogenase	WP_014766885.1	1521	506	aldehyde dehydrogenase [Desulfurococcus fermentans]	98	0.0	62

			[NAD(P)+]							
EC 1.2.7.6	+	Desfe_0557	Aldehyde ferredoxin oxidoreductase	WP_014767359.1	1953	650	aldehyde ferredoxin oxidoreductase [Desulfurococcus fermentans] aldehyde ferredoxin oxidoreductase [Desulfurococcus fermentans]	98 55	2,00E-147 7,00E-16	39 24
EC 1.2.1.59	+	Desfe_0262	glyceraldehyde 3-phosphate dehydrogenase (NAD(P)+)	WP_014767076.1	1074	357	glyceraldehyde-3-phosphate dehydrogenase [Desulfurococcus fermentans]	97	1,00E-111	49
EC 2.7.2.3	+	Desfe_0261	phosphoglycerate kinase	WP_014767075.1	427	1284	phosphoglycerate kinase [Desulfurococcus fermentans]	99	6,00E-146	53
EC 5.4.2.12	+	Desfe_0416	phosphonopyruvate decarboxylase-related protein	WP_048815972.1	424	1239	phosphonopyruvate decarboxylase [Desulfurococcus fermentans]	96	8,00E-112	46
							phosphonopyruvate decarboxylase [Desulfurococcus fermentans]	88	6,00E-67	37
	-	not found								
EC 4.2.1.11	+	Desfe_0063	enolase	WP_014766881.1	435	1308	enolase [Desulfurococcus fermentans]	96	1,00E-131	49
EC 2.7.9.2	+	Desfe_0879	pyruvate, water dikinase	WP_014767668.1	830	2493	phosphoenolpyruvate synthase [Desulfurococcus fermentans]	99	0,00E+00	86
EC 2.7.1.40	+	Desfe_1347	pyruvate kinase	WP_014768104.1	473	1422	pyruvate kinase [Desulfurococcus fermentans]	99	8,00E-88	38
							pyruvate kinase [Desulfurococcus fermentans]	93	5,00E-59	31
EC 1.2.7.1	+	Desfe_1298	pyruvate ferredoxin oxidoreductase alpha subunit	WP_014768058.1	393	1182	Dihydrolipoamide dehydrogenase [Desulfurococcus fermentans]	100 95	0 3,00E-80	71 42
	+	Desfe_0503	pyruvate ferredoxin oxidoreductase alpha subunit	WP_014767308.1						
	+	Desfe_1296	pyruvate ferredoxin oxidoreductase gamma subunit	WP_014768056.1	582	193	pyruvate/ketoisovalerate oxidoreductase subunit gamma [Desulfurococcus fermentans] pyruvate/ketoisovalerate oxidoreductase subunit gamma [Desulfurococcus fermentans]	9895	6,00E-817,00E-35	6239

	+	Desfe_0505	pyruvate ferredoxin oxidoreductase gamma subunit	WP_014767310.1	558	185	pyruvate/ketoisovalerate oxidoreductase subunit gamma [Desulfurococcus fermentans] pyruvate/ketoisovalerate oxidoreductase subunit gamma [Desulfurococcus fermentans]	98 95	6,00E-81 7,00E-35	62 39
	+	Desfe_0504	pyruvate ferredoxin oxidoreductase delta subunit	WP_014767309.1	282	93				
	+	Desfe_1297	pyruvate ferredoxin oxidoreductase delta subunit	WP_014768057.1	201	93				
	+	Desfe_0502	pyruvate ferredoxin oxidoreductase beta subunit	WP_014767307.1	1059	352				
	+	Desfe_1299	pyruvate ferredoxin oxidoreductase beta subunit	WP_048816009.1	867	288				
EC 1.8.1.4	+	Desfe_0667	dihydrolipoamide dehydrogenase	WP_014767468.1	456	1371	dihydrolipoamide dehydrogenase [Desulfurococcus fermentans]	96	4,00E-44	28
EC 2.3.1.9	+	Desfe_0849	acetyl-CoA C-acetyltransferase	WP_014767639.1	391	1176	acetyl-CoA acetyltransferase [Desulfurococcus fermentans]	99	7,00E-115	46
EC 6.2.1.13	+	Desfe_0782	acetyl-CoA synthetase (ADP-forming)	WP_014767577.1	232	699	acetyl-CoA synthetase [Desulfurococcus fermentans] acetyl-CoA synthetase [Desulfurococcus fermentans]	91 87	3,00E-60 9,00E-57	46 44
	-	Desfe_0977	acetyl-CoA synthetase (ADP-forming)				acetyl-CoA synthetase [Desulfurococcus fermentans] acetyl-CoA synthetase [Desulfurococcus fermentans]	91 87	3,00E-60 9,00E-57	46 44
	+	Desfe_1050	acetyl-CoA synthetase (ADP-forming)	WP_014767819.1	232	699	acetyl-CoA synthetase [Desulfurococcus fermentans] acetyl-CoA synthetase [Desulfurococcus fermentans]	91 87	3,00E-60 9,00E-57	46 44
EC 4.1.1.32	+	Desfe_0003	Phosphoenolpyruvate carboxykinase (GTP)	WP_014766823.1	629	1890	phosphoenolpyruvate carboxykinase [Desulfurococcus fermentans]	99	0,00E+00	47
EC 2.7.1.12	-	Desfe_1214	gluconokinase	WP_014767979.1	503	1512				
	+	Desfe_1214					carbohydrate kinase [Desulfurococcus fermentans]	77	4,00E-46	30

EC 5.3.1.27	+	Desfe_0297	6-phospho-3-hexuloisomerase	WP_014767110.1	606	201	hypothetical protein [Desulfurococcus fermentans]	92	4,00E-79	60
EC 4.1.2.43	+	Desfe_0079	3-hexulose-6-phosphate synthase	WP_014766897.1	651	216	orotidine 5'-phosphate decarboxylase [Desulfurococcus fermentans]			
EC 5.3.1.6	+	Desfe_0020	ribose 5-phosphate isomerase B	WP_014766840.1	441	146	ribose 5-phosphate isomerase B [Desulfurococcus fermentans]	86	6,00E-20	36
	+	Desfe_0594	ribose 5-phosphate isomerase A	AFL66495.1	741	246	ribose-5-phosphate isomerase [Desulfurococcus fermentans]	88	7,00E-22	36
EC 2.7.6.1		Desf_0584	ribose-phosphate pyrophosphokinase	WP_014767386.1	903	300	ribose-phosphate pyrophosphokinase [Desulfurococcus fermentans]	97	5,00E-56	36
EC 5.3.1.1	+	Desfe_1348	triosephosphate isomerase	WP_014768105.1	696	231	triose-phosphate isomerase [Desulfurococcus fermentans] geranylgeranylglyceryl/heptaprenylglyceryl phosphate synthase [Desulfurococcus fermentans]	52 7	3,00E-16 0,14	28 45
EC 4.1.1.39	+	Desfe_1247	ribulose-bisphosphate carboxylase large chain	WP_014768010.1	1338	445	ribulose 1,5-bisphosphate carboxylase large subunit [Desulfurococcus fermentans]	99	0.0	84
EC 1.2.7.5	+	Desfe_0695	aldehyde:ferredoxin oxidoreductase	AFL66594.1 / WP_048815866.1	1884	627	aldehyde ferredoxin oxidoreductase [D. fermentans] aldehyde ferredoxin oxidoreductase [D. fermentans] aldehyde ferredoxin oxidoreductase [D.fermentans]	98 95 49	0.0 4E-97 7E-13	56 35 24
	+	Desfe_1093	aldehyde:ferredoxin oxidoreductase	WP_014767861.1	1827	608				
EC 1.2.99.5	+	Desfe_0059	formylmethanofuran dehydrogenase subunit E	WP_014766877.1	690	229	formylmethanofuran dehydrogenase subunit E [Desulfurococcus fermentans]	96	2,00E-18	28
EC 1.2.99.2	+	Desfe_0379	carbon-monoxide dehydrogenase medium subunit	AFL66289.1	882	293	(2Fe-2S)-binding protein [Desulfurococcus fermentans]	91	4,00E-42	45
	+	Desfe_0380	carbon-monoxide dehydrogenase small subunit	WP_014767191.1	534	177	aldehyde oxidase [Desulfurococcus fermentans]	98	5,00E-90	31
	+	Desfe_0766	carbon-monoxide dehydrogenase medium subunit	WP_014767563.1	903	300	aldehyde oxidase [Desulfurococcus fermentans]	99	1,00E-87	31

	-	Desfe_0767	carbon-monoxide dehydrogenase small subunit	WP_014767564.1	462	153	carbon monoxide dehydrogenase large subunit [Desulfurococcus fermentans]	98	3,00E-76	29
	+	Desfe_0769	carbon-monoxide dehydrogenase medium subunit	AFL66666.1	906	301	carbon monoxide dehydrogenase [Desulfurococcus fermentans] carbon monoxide dehydrogenase medium subunit [Desulfurococcus fermentans]	99 94	6,00E-58 2,00E-54	40 39
	+	Desfe_0770	carbon-monoxide dehydrogenase small subunit	WP_014767567.1	462	153	molybdopterin dehydrogenase [Desulfurococcus fermentans]	75	4,00E-27	30
EC 1.8.1.4	+	Desfe_0667	dihydrolipoamide dehydrogenase	WP_014767468.1	1371	456	dihydrolipoamide dehydrogenase [Desulfurococcus fermentans]	96	4,00E-44	28
EC 1.4.4.2	+	Desfe_0668	glycine dehydrogenase subunit 2	WP_014767469.1	1572	523	glycine dehydrogenase (aminomethyl-transferring) [Desulfurococcus fermentans]	84	2,00E-77	39
	+	Desfe_0669	glycine dehydrogenase subunit 1	AFL66569.1	1419	472	glycine dehydrogenase (aminomethyl-transferring) [Desulfurococcus fermentans]	97	4,00E-105	42
EC 2.1.2.10	+	Desfe_0670	aminomethyltransferase	WP_014767471.1	1110	369	glycine cleavage system protein T [Desulfurococcus fermentans]	98	2w-47	33
EC 2.1.2.1	+	Desfe_0671	glycine hydroxymethyltransferase	AFL66571.1	1347	448	serine hydroxymethyltransferase [Desulfurococcus fermentans] glycine dehydrogenase (aminomethyl-transferring) [Desulfurococcus fermentans]	92 21	0.0 0,14	64 31
EC 2.5.1.47	~	Desfe_0378	cysteine synthase A	WP_014767189.1	1008	335	pyridoxal-5'-phosphate-dependent protein subunit beta [Desulfurococcus fermentans]	98	2,00E-62	42
EC 3.1.3.3	+	Desfe_0998	phosphoserine phosphatase	WP_014767776.1	648	215	-	-	-	-
EC 1.1.1.95	+	Desfe_0945	D-3-phosphoglycerate dehydrogenase	WP_014767730.1	942	313	2-hydroxyacid dehydrogenase [Desulfurococcus fermentans] D-glycerate dehydrogenase [Desulfurococcus fermentans] lactate dehydrogenase [Desulfurococcus fermentans]	94 83 78	1,00E-63 100E-41 3,00E-33	38 30 35
EC 6.4.1.1	+	Desfe_0591	pyruvate carboxylase subunit B	WP_014767393.1	1410	469	pyruvate carboxylase [Desulfurococcus fermentans]	75	0.0	69

EC 6.2.1.5	+	Desfe_1155	succinyl-CoA synthetase alpha subunit	WP_014767922.1	876	291	succinyl-CoA synthetase subunit alpha [Desulfurococcus fermentans]	99	6,00E-130	62
	+	Desfe_1156	succinyl-CoA synthetase beta subunit	AFL67027.1	1151	383	succinate--CoA ligase subunit beta [Desulfurococcus fermentans]	100	2,00E-114	50
EC 1.2.7.3 and EC 1.2.7.11	+	Desfe_0474	2-oxoglutarate ferredoxin oxidoreductase subunit beta	WP_014767279.1	927	308	2-oxoacid ferredoxin oxidoreductase subunit beta [Desulfurococcus fermentans]2-oxoglutarate ferredoxin oxidoreductase subunit beta [Desulfurococcus fermentans]	9860	3E-1051e-31	5438
	+	Desfe_0475	2-oxoglutarate ferredoxin oxidoreductase subunit alpha	WP_014767280.1	1896	631	2-oxoacid:ferredoxin oxidoreductase subunit alpha [Desulfurococcus fermentans] 2-oxoglutarate ferredoxin oxidoreductase subunit alpha [Desulfurococcus fermentans]	55 97	5,00E-48 60,,E-135	38 37
	+	Desfe_0497	2-oxoglutarate ferredoxin oxidoreductase subunit gamma	WP_014767302.1	531	176	2-oxoglutarate ferredoxin oxidoreductase subunit delta [Desulfurococcus fermentans] 4Fe-4S ferredoxin [Desulfurococcus fermentans] more	96 68	7e-40 3E-06	71 44
	+	Desfe_0498	2-oxoglutarate ferredoxin oxidoreductase subunit beta	WP_014767303.1	831	276	2-oxoglutarate oxidoreductase [Desulfurococcus fermentans] pyruvate ferredoxin oxidoreductase [Desulfurococcus fermentans]	98 32	1,00E-71 0,039	58 36
	+	Desfe_0499	2-oxoglutarate ferredoxin oxidoreductase subunit alpha	WP_014767304.1	1125	374				
	+	Desfe_0500	2-oxoglutarate ferredoxin oxidoreductase subunit delta	WP_014767305.1	261	86				
EC 1.8.1.4	+	Desfe_0667	dihydrolipoamide dehydrogenase	WP_014767468.1	456	1371	dihydrolipoamide dehydrogenase [Desulfurococcus fermentans]	96	4,00E-44	28
EC 1.4.1.3		Desfe_0075	glutamate dehydrogenase (NAD(P)+)	WP_014766893.1	1281	426	glutamate dehydrogenase [Desulfurococcus fermentans]	97	4,00E-138	51
Fructose & Mannose										

EC 5.3.1.8	+	Desfe_1128	mannose-6-phosphate isomerase	WP_014767896.1	999	332	mannose-6-phosphate isomerase [Desulfurococcus fermentans]	96	4,00E-32	33
EC 5.4.2.8	+	Desfe_0111	phosphomannomutase	WP_014766929.1	1377	458	phosphoglucosamine mutase [Desulfurococcus fermentans] phosphoglucomutase [Desulfurococcus fermentans]	99 99	5,00E-161 2,00E-47	52 30
EC 2.7.7.13	+	Desfe_0189	mannose-1-phosphate guanylyltransferase	WP_014767004.1	1128	375	nucleotidyltransferase [Desulfurococcus fermentans] nucleotidyltransferase [Desulfurococcus fermentans]	86 88	1,00E-28 6,00E-27	30 25
	+	Desfe_0962		WP_014767746.1	738	245	nucleotidyltransferase [Desulfurococcus fermentans]sugar-phosphate nucleotidyltransferase [Desulfurococcus fermentans]	5863	1,00E-217,00E-10	3323
EC 3.1.3.11	-	Desfe_1349	archaeal fructose 1,6-bisphosphatase fructose 1,6-bisphosphate aldolase/phosphatase	WP_014768106.1	1149	382	-	-	-	-
							fructose 1,6-bisphosphatase [Desulfurococcus fermentans]	93	0.0	74
EC 4.1.2.13	+	Desfe_0718	fructose-bisphosphate aldolase, class I	WP_014767518.1	819	272	fructose-bisphosphate aldolase [Desulfurococcus fermentans]	98	1,00E-105	55
	+	Desfe_1349	fructose 1,6-bisphosphate aldolase/phosphatase	WP_014768106.1	1149	382	fructose 1,6-bisphosphatase [Desulfurococcus fermentans]	93	0.0	74
EC 5.3.1.1	+	Desfe_1348	triosephosphate isomerase	WP_014768105.1	231	696	triose-phosphate isomerase [Desulfurococcus fermentans]	96	4,00E-72	52
EC 4.1.2.17	+	Desfe_0510	L-fuculose-phosphate aldolase	WP_014767315.1	627	208	aldolase [Desulfurococcus fermentans]	79	1,00E-22	36
Galactose										
EC 2.7.1.6	+	Desfe_0628	galactokinase	WP_014767430.1	1248	415	GHMP kinase [Desulfurococcus fermentans]	97	3,00E-106	46
EC 2.7.7.12	+	Desfe_0627	UDPglucose--hexose-1-phosphate uridylyltransferase	WP_048815984.1	924	307	galactose-1-phosphate uridylyltransferase [Desulfurococcus fermentans]	75	5,00E-37	32
EC 5.1.3.2	+	Desfe_0535	UDP-glucose 4-epimerase	WP_014767340.1	909	302	UDP-glucose 4-epimerase [Desulfurococcus fermentans]	96	1,00E-122	60

	+	Desfe_0629	UDP-glucose 4-epimerase	WP_014767431.1	936	320	UDP-glucose 4-epimerase [Desulfurococcus fermentans]	94	1,00E-21	31
EC 3.2.1.23	+	Desfe_0357	beta-galactosidase	WP_014767168.1	2922	973	beta-galactosidase [Desulfurococcus fermentans]	29	2,00E-30	40
EC 5.4.2.2 (similar to EX 5.4.2.7)	+	Desfe_0111	phosphomannomutase / phosphoglucomutase	WP_014766929.1	1377	458	phosphoglucosamine mutase [D. fermentans] phosphoglucomutase [D. fermentans]	94 99	9E-165 3E-48	56 31
EC 2.7.1.2		Desfe_0578	ROK family protein	AFL66479.1 WP_048815982.1	332 329	999 990	glucokinase [Desulfurococcus fermentans]	99	2,00E-61	41
Pyruvate										
EC 2.3.1.9	+	Desfe_0849	acetyl-CoA C-acetyltransferase	WP_014767639.1	391	1176	acetyl-CoA acetyltransferase [Desulfurococcus fermentans]	99	7,00E-115	46
EC 2.7.9.2	+	Desfe_0879	pyruvate, water dikinase	WP_014767668.1	830	2493	phosphoenolpyruvate synthase [Desulfurococcus fermentans]	99	0,00E+00	86
EC 6.4.1.1	+	Desfe_0591	pyruvate carboxylase subunit B	WP_014767393.1	1410	469	pyruvate carboxylase [Desulfurococcus fermentans]	75	0.0	69
Glyoxylate and Dicarboxylate										
3.1.3.18	+	Desfe_0132	phosphoglycolate phosphatase	WP_014766950.1	675	224	phosphatase [Desulfurococcus fermentans]	95	5,00E-59	46
	+	Desfe_0131	phosphoglycolate phosphatase	WP_014767899.1	660	219	haloacid dehalogenase [Desulfurococcus fermentans]	90	5,00E-33	30
EC 4.1.1.39	+	Desfe_1247	ribulose-bisphosphate carboxylase large chain	WP_014768010.1	1338	445	ribulose 1,5-bisphosphate carboxylase large subunit [Desulfurococcus fermentans]	99	0.0	84
EC 1.1.1.26	+	Desfe_1189	glyoxylate reductase	WP_014767955	1011	336	D-glycerate dehydrogenase [Desulfurococcus fermentans] 2-hydroxyacid dehydrogenase [Desulfurococcus fermentans] lactate dehydrogenase [Desulfurococcus fermentans]	100 92 77	7,00E-173 1,00E-81 3,00E-40	67 44 38
Starch and Sucrose										

EC 3.2.1.4	+	Desfe_0691	endoglucanase	WP_014767491.1	1101	366	peptidase M42 [Desulfurococcus fermentans]	97	2,00E-143	55
EC 3.2.1.21	+	Desfe_0624	beta-glucosidase	WP_014767426.1	1263	420	beta-glucosidase [Desulfurococcus fermentans]	98	1,00E-167	54
EC 2.4.1.1	+	Desfe_0264	starch phosphorylase	WP_014767078.1	1533	510	glycosyl transferase family 1 [Desulfurococcus fermentans]	99	2,00E-110	39
EC 2.4.1.21	+	Desfe_0403	starch synthase	WP_014767214.1	1539	512	glycosyl transferase family 1 [Desulfurococcus fermentans]	86	2,00E-49	29
Citrat										
EC 6.2.1.5	+	Desfe_1155	succinyl-CoA synthetase alpha subunit	WP_014767922.1	876	291	succinyl-CoA synthetase subunit alpha [Desulfurococcus fermentans]	99	6,00E-130	62
	+	Desfe_1156	succinyl-CoA synthetase beta subunit	AFL67027.1	1151	383	succinate--CoA ligase subunit beta [Desulfurococcus fermentans]	100	2,00E-114	50
EC 1.8.1.4	+	Desfe_0667	dihydrolipoamide dehydrogenase	WP_014767468.1	456	1371	dihydrolipoamide dehydrogenase [Desulfurococcus fermentans]	96	4,00E-44	28
EC 1.2.7.3	+	Desfe_0474	2-oxoglutarate ferredoxin oxidoreductase subunit beta	WP_014767279.1	927	308	2-oxoacid ferredoxin oxidoreductase subunit beta [Desulfurococcus fermentans]2-oxoglutarate ferredoxin oxidoreductase subunit beta [Desulfurococcus fermentans]	9860	3E-1051e-31	5438
	+	Desfe_0475	2-oxoglutarate ferredoxin oxidoreductase subunit alpha	WP_014767280.1	1896	631	2-oxoacid:ferredoxin oxidoreductase subunit alpha [Desulfurococcus fermentans] 2-oxoglutarate ferredoxin oxidoreductase subunit alpha [Desulfurococcus fermentans]	55 97	5,00E-48 60,,E-135	38 37
	+	Desfe_0497	2-oxoglutarate ferredoxin oxidoreductase subunit gamma	WP_014767302.1	531	176	2-oxoglutarate ferredoxin oxidoreductase subunit delta [Desulfurococcus fermentans] 4Fe-4S ferredoxin [Desulfurococcus fermentans] more	96 68	7e-40 3E-06	71 44
	+	Desfe_0498	2-oxoglutarate ferredoxin oxidoreductase subunit beta	WP_014767303.1	831	276	2-oxoglutarate oxidoreductase [Desulfurococcus fermentans] pyruvate ferredoxin oxidoreductase [Desulfurococcus fermentans]	98 32	1,00E-71 0,039	58 36
	+	Desfe_0499	2-oxoglutarate ferredoxin oxidoreductase subunit	WP_014767304.1	1125	374				

			alpha							
	+	Desfe_0500	2-oxoglutarate ferredoxin oxidoreductase subunit delta	WP_014767305.1	261	86				
Bacterial Secretion System										
ffh	+	Desfe_0034	signal recognition particle subunit SRP54	AFL65947.1	1356	451	signal recognition particle protein [Desulfurococcus fermentans] signal recognition particle-docking protein FtsY [Desulfurococcus fermentans]	99 65	7,00E-173 1,00E-36	55 31
SecY	+	Desfe_1263	preprotein translocase subunit SecY	WP_014768024.1	1425	474	preprotein translocase subunit SecY [Desulfurococcus fermentans]	99	1,00E-149	49
FtsY	+	Desfe_1222	fused signal recognition particle receptor	WP_014767987.1	891	296	signal recognition particle-docking protein FtsY [Desulfurococcus fermentans] signal recognition particle protein [Desulfurococcus fermentans]	99 89	6,00E-95 7,00E-39	49 33
ABC Transporter										
MalK	+	Desfe_1188	multiple sugar transport system ATP-binding protein	AFL67058.1	1203	400	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans] 6 more	100	2,00E-153	60
GanO	+	Desfe_0354	arabinogalactan oligomer / maltooligosaccharide transport system substrate-binding protein	WP_014767165.1	1545	514	ABC transporter substrate-binding protein [Desulfurococcus fermentans]	89	2,00E-08	24
GanP	+	Desfe_0355	arabinogalactan oligomer / maltooligosaccharide transport system permease protein	WP_014767166.1	1434	477	sugar ABC transporter permease [Desulfurococcus fermentans] sugar ABC transporter permease [Desulfurococcus fermentans]	82 22	1,00E-22 0,034	26 34

GanQ		Desfe_0356	arabinogalactan oligomer / maltooligosaccharide transport system permease protein	WP_014767167.1	855	284	sugar ABC transporter permease [Desulfurococcus fermentans]	93	3,00E-14	25
MsmX	+	Desfe_1188	multiple sugar transport system ATP-binding protein	AFL67058.1	1203	400	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans] 6 more	100	2,00E-153	60
MsmK	+	Desfe_1188	multiple sugar transport system ATP-binding protein	AFL67058.1	1203	400	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans] 6 more	100	2,00E-153	60
SmoK	+	Desfe_1188	multiple sugar transport system ATP-binding protein	AFL67058.1	1203	400	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans] 6 more	100	2,00E-153	60
AglK	+	Desfe_1188	multiple sugar transport system ATP-binding protein	AFL67058.1	1203	400	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans] 6 more	100	2,00E-153	60
MsiK	+	Desfe_1188	multiple sugar transport system ATP-binding protein	AFL67058.1	1203	400	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans] 6 more	100	2,00E-153	60
MsmX	+	Desfe_1188	multiple sugar transport system ATP-binding protein	AFL67058.1	1203	400	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans]6 more	100	2,00E-153	60
MalK	+	Desfe_1188	multiple sugar transport system ATP-binding protein	AFL67058.1	1203	400	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans] 6 more	100	2,00E-153	60

Supplementary Table 2 – Genes of *D. fermentans* according to BLAST search (annotated from KEGG), only results of plastp is shown, Abbreviations: nt, nucleotide length; aa, amino acid length.

EnzymeNr.	**Locus_Tag**	**Protein Nr. *D. fermentans* NCBI**	**Enzyme Name *D. fermentans* KEGG**	**Name Synonym NCBI**	**Protein Nr. Synonym NCBI**	**Blastp**	**Q cover [%]**	**E value**	**Identity [%]**
Glycolysis									
EC 2.7.1.69	not found		PTS system, glucose-specific IIA component	PTS glucose transporter subunit IIA [Desulfotomaculum reducens]	WP_011876718.1	-	-	-	-
EC 2.7.1.11	Desfe_0717 Desfe_0968	WP_048815869.1 WP_014767752.1	6-phosphofructokinase 1 (also 2)	ATP-dependent 6-phosphofructokinase [Aeropyrum pernix K1]	BAA78921.1	phosphofructokinase [Desulfurococcus fermentans] phosphofructokinase [Desulfurococcus fermentans]	97 90	6,00E-97 5,00E-28	46 29
EC 2.7.1.146	not found		ADP-dependent phosphofructokinase/glucokinase	phosphofructokinase [Thermococcus litoralis DSM 5473]	WP_020953560.1	-	-	-	-
EC 1.2.1.12	not found		glyceraldehyde 3-phosphate dehydrogenase	NAglyceraldehyde-3-phosphate dehydrogenase [Haloferax]	WP_004044440.1	-	-	-	-
EC 5.4.2.4	not found		bisphosphoglycerate mutase	NA	NA	-	-	-	-
EC 1.2.1.9	Desfe_0067	WP_014766885.1	glyceraldehyde-3-phosphate dehydrogenase (NADP+)	Glyceraldehyde-3-phosphate dehydrogenase, NADP dependent (gapN-1) [Sulfolobus solfataricus P2][Sulfolobus solfataricus P2]	AAK41843.1		-	-	-
EC 3.1.3.13	Desfe_0416	AFL66326	bisphosphoglycerate mutase		NA	-	-	-	-
EC 5.4.2.11	not found		2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase 1 [Acidilobus	ADL19899.1	-	-	-	-

				saccharovorans 345-15]					
EC 3.1.3.80	not found		multiple inositol-polyphosphate phosphatase / 2,3-bisphosphoglycerate 3-phosphatase		NA	-	-	-	-
EC 2.3.1.12	not found		pyruvate dehydrogenase E2 component (dihydrolipoamide acetyltransferase)	ATP-NAD kinase [Sulfolobus solfataricus]	WP_010923453.1	-	-	-	-
EC 1.2.4.1	not found		pyruvate dehydrogenase E1 component alpha subunit , etc	pyruvate dehydrogenase E1 component, beta subunit [Aeropyrum pernix K1]	BAA80675.1				
EC 4.1.1.1	Desfe_1298 Desfe_0503 Desfe_0499 Desfe_0475	WP_014768058.1 WP_014767308.1 WP_014767304.1 WP_014767280.1	pyruvate decarboxylase	UniProt: Pyruvate ferredoxin oxidoreductase subunit alpha	UniProt: W8CQR1	pyruvate ferredoxin oxidoreductase [Desulfurococcus fermentans]2-ketoisovalerate ferredoxin oxidoreductase subunit alpha [Desulfurococcus fermentans]2-oxoglutarate ferredoxin oxidoreductase subunit alpha [Desulfurococcus fermentans]	999886	1,00E-1042,00E-962,00E-20	414428
EC 1.1.1.27	not found	WP_014767018.1	L-lactate dehydrogenase	L-lactate dehydrogenase [Haloferax volcanii DS2]	WP_004045328.1	-	-	-	-
EC 6.2.1.1	not found		acetyl-CoA synthetase	acetyl-coenzyme A synthetase [Aeropyrum pernix K1]	WP_004044044.1				

EC 1.2.1.3	Desfe_0067	WP_014766885.1	aldehyde dehydrogenase (NAD+), aldehyde dehydrogenase family 7 member A1, etc	aldehyde dehydrogenase [Sulfolobus acidocaldarius]	WP_011278508.1	aldehyde dehydrogenase [Desulfurococcus fermentans]	83	3,00E-48	29
EC 1.2.1.5	not found		aldehyde dehydrogenase (NAD(P)+)	aldehyde dehydrogenase [Anaeromyxobacter dehalogenans]	WP_011420200.1	-	-	-	
EC 1.2.1.-	Desfe_0067	WP_014766885.1	aldehyde dehydrogenase	aldehyde dehydrogenase [Deinococcus radiodurans R1]	NP_285671.1	aldehyde dehydrogenase [Desulfurococcus fermentans]	93	3,00E-47	27
EC 1.1.2.7	not found		methanol dehydrogenase (cytochrome c) subunit 1	methanol dehydrogenase [Paracoccus]	WP_049792378.1	-	-	-	
EC 1.1.2.8	not found		alcohol dehydrogenase (cytochrome c)	dehydrogenase [Natrinema pellirubrum]	WP_006179367.1	-	-	-	
EC 1.1.1.2	Desfe_0889	WP_014767677.1	alcohol dehydrogenase (NADP+)	alcohol dehydrogenase [Haloarcula marismortui]	WP_049938537.1	oxidoreductase [Desulfurococcus fermentans]	41	4,00E-19	27
Pentose Phosphate									
EC 1.1.1.47	Desfe_0025 Desfe_1209	WP_014766844.1 WP_014767974.1	glucose 1-dehydrogenase	glucose dehydrogenase [Haloarcula marismortui ATCC 43049]	AAV47505	short-chain dehydrogenase [D. fermentans]	98	9,00E+62	43
EC 1.1.3.4	not found	WP_014767363.1	glucose oxidase (* more names in KEGG)	Glucose oxidase Aspergillus niger	P13006 UniProt	-	-	-	-
EC 1.1.3.5	not found		hexose oxidase			-	-	-	-

EC 1.1.1.360	not found		glucose/galactose 1-dehydrogenase (NADP+)	bifunctional 5,10-methylene-tetrahydrofolate dehydrogenase/5,10-methylene-tetrahydrofolate cyclohydrolase [Thermoplasma acidophilum]	WP_010901308	-	-	-	-
EC 1.1.1.359	Desfe_1240	WP_014768004.1	aldose 1-dehydrogenase [NAD(P)+]	Glucose 1-dehydrogenase (dhg-1) [Sulfolobus solfataricus P2]	WP_009992653.1	alcohol dehydrogenase [D. fermentans]	39	3,00E-11	32
EC 1.1.5.9	not found		glucose dehydrogenase (acceptor)	uncharacterized protein Dmel_CG12398 [Drosophila melanogaster]	NP_572985.1	-	-	-	-
EC 1.1.5.2	not found	WP_014767867.1	quinoprotein glucose dehydrogenase	glucose dehydrogenase [Haloarcula marismortui]	WP_011222555.1	CRISPR-associated protein Cas4 [Desulfurococcus fermentans]	6	1	36
EC 3.1.1.17	not found		gluconolactonase	gluconolactonase [Haloferax volcanii]	WP_004041072.1	-	-	-	-
EC 4.2.1.39	not found		gluconate dehydratase, D-gluconate hydro-lyase	Thermoproteus tenax Kra 1 gluconate dehydratase	G4RJQ1 UniProt	-	-	-	-
EC 4.2.1.140	not found		gluconate/galactonate dehydratase	D-gluconate/D-galactonate dehydratase OS=Sulfolobus solfataricus	Q97U27 UniProt	-	-	-	-
EC 4.3.1.9	Desfe_1363	WP_014768119	glucosaminate ammonia-lyase	GlcNA-DH alpha subunit, partial [Pseudomonas fluorescens]	BAB64360	thioredoxin reductase [Desulfurococcus fermentans]	88	2,00E+43	37
EC 4.1.2.55	Desfe_1213	WP_014767978.1	2-dehydro-3-deoxy-phosphogluconate/2-dehydro-3-deoxy-6-phosphogalactonate aldolase	2-keto-3-deoxy gluconate aldolase [Metallosphaera sedula]	WP_012021241.1	-	-	-	-
EC 4.1.2.51	Desfe_1213	WP_014767978.2	2-dehydro-3-deoxy-D-gluconate aldolase	dihydrodipicolinate synthase family protein [Thermoplasma	WP_010917486.1	dihydrodipicolinate synthase family protein	82	8,00E-24	28

				volcanium]		[Desulfurococcus fermentans]			
EC 1.2.99.8	Desfe_0765 Desfe_0766 Desfe_0769 Desfe_0770	WP_014767562.1 WP_014767563.1 WP_048815992.1 WP_014767576.1	glyceraldehyde dehydrogenase large subunit (also medium and small SU)	Carbon monoxide dehydrogenase, medium chain. (cutB-2) [Sulfolobus solfataricus P2]	WP_009988542.1	carbon monoxide dehydrogenase medium subunit [Desulfurococcus fermentans] carbon monoxide dehydrogenase [Desulfurococcus fermentans]	97 96	4e-44 4e-44	35 33
	Desfe_0381 Desfe_0765 Desfe_0768	WP_014767192.1 WP_014767562.1 WP_014767565.1	glyceraldehyde dehydrogenase large subunit (also medium and small SU)	aldehyde dehydrogenase, large subunit [Aeropyrum pernix K1]	BAA79684.2	aldehyde oxidase [Desulfurococcus fermentans] carbon monoxide dehydrogenase large subunit [Desulfurococcus fermentans]	98 98	3E-104 2E-70	32 29
	Desfe_0766 Desfe_0767 Desfe_0769	WP_014767563.1 WP_014767564.1 WP_048815992.1	glyceraldehyde dehydrogenase large subunit (also medium and small SU)	glyceraldehyde oxidoreductase medium chain [Metallosphaera cuprina Ar-4]	AEB95870	carbon monoxide dehydrogenase medium subunit [Desulfurococcus fermentans] carbon monoxide dehydrogenase [Desulfurococcus fermentans]	95 94	3e-38 2e-32	36 33
EC 1.2.1.89	Desfe_0067	WP_014766885.1	D-glyceraldehyde dehydrogenase (NADP+)	aldehyde dehydrogenase [Thermoplasma acidophilum]	WP_010901221.1	aldehyde dehydrogenase [Desulfurococcus fermentans]	96	5,00E-69	31
EC 2.7.1.165	not found		glycerate 2-kinase	glycerate kinase [Aeropyrum pernix K1]	BAA79980.2	-	-	-	-

EC 1.1.99.3	Desfe_0795 Desfe_0538	WP_014767588.1 WP_014767342.1	gluconate 2-dehydrogenase alpha chain (also gamma chain)	Gluconate 2-dehydrogenase (acceptor) [Haloterrigena turkmenica DSM 5511]	ADB60204	ribulose-1,5-biphosphate synthetase [Desulfurococcus fermentans]	5	0,29	46
EC 2.7.1.13	Desfe_0717	WP_048815869.1	dehydrogluconokinase	2-ketogluconate kinase [Pseudomonas aeruginosa PAO1]	NP_250951	phosphofructokinase [Desulfurococcus fermentans]	86	2,00E-22	27
EC 1.1.1.215	Desfe_1189 Desfe_0945	WP_014767955.1 WP_014767730.1	gluconate 2-dehydrogenase	gluconate 2-dehydrogenase [Picrophilus torridus DSM 9790]	AAT44023	D-glycerate dehydrogenase [Desulfurococcus fermentans] 2-hydroxyacid dehydrogenase [Desulfurococcus fermentans]	90 91	2E-61 1,00E-47	38 35
EC 1.1.1.43	Desfe_1189 Desfe_0945	WP_014767955.1 WP_014767730.1	phosphogluconate 2-dehydrogenase	2-hydroxyacid dehydrogenase [Pseudomonas aeruginosa PAO1	NP_250953.1	D-glycerate dehydrogenase [Desulfurococcus fermentans] 2-hydroxyacid dehydrogenase [Desulfurococcus fermentans]	83 89	1,00E-73 5,00E-54	47 39
EC 2.7.1.45	Desfe_0717 Desfe_0968	WP_048815869.1 WP_014767752.1	2-dehydro-3-deoxygluconokinase	2-dehydro-3-deoxygluconate kinase [Thermococcus barophilus MP]	ADT82998	phosphofructokinase [Desulfurococcus fermentans]	88	2,00E-22	28
EC 2.7.1.178	Desfe_0968 Desfe_0717	WP_014767752.1 WP_048815869.1	2-dehydro-3-deoxygluconokinase / 2-dehydro-3-deoxygalactonokinase	sugar kinase [Sulfolobus solfataricus]	WP_009991690.1	phosphofructokinase [Desulfurococcus fermentans]	88	2,00E-21	28
EC 4.2.1.12	not found		phosphogluconate dehydratase	phosphogluconate dehydratase [Pseudomonas aeruginosa PAO1]	NP_251884	-	-	-	-

EC 4.1.2.14	not found		2-dehydro-3-deoxyphosphogluconate aldolase / (4S)-4-hydroxy-2-oxoglutarate aldolase	2-dehydro-3-deoxyphosphogluconate aldolase/4-hydroxy-2-oxoglutarate aldolase [Haloarcula marismortui ATCC 43049]	AAV47830	-	-	-	-
EC 4.1.2.55	Desfe_0262	WP_014767076.1	2-dehydro-3-deoxy-phosphogluconate/2-dehydro-3-deoxy-6-phosphogalactonate aldolase	2-keto-3-deoxy gluconate aldolase (eda) [Sulfolobus solfataricus P2]	AAK43294	-	-	-	-
EC 1.2.1.9	Desfe_0067	WP_014766885.1	glyceraldehyde-3-phosphate dehydrogenase (NADP+)	Glyceraldehyde-3-phosphate dehydrogenase, NADP dependent (gapN-1) [Sulfolobus solfataricus P2][Sulfolobus solfataricus P2]	AAK41843.1	aldehyde dehydrogenase [Desulfurococcus fermentans]	98	4,00E-68	31
EC 4.3.1.29	not found		D-glucosaminate-6-phosphate ammonia-lyase	D-glucosaminate-6-phosphate ammonia lyase [Salmonella enterica]	WP_000188463	-	-	-	-
EC 2.7.1.69	not found		PTS system, D-glucosaminate-specific IIA component (also IIB-IID)	PTS fructose transporter subunit IID [Serratia fonticola]	AKG71692	-	-	-	-
EC 1.1.1.44	not found		6-phosphogluconate dehydrogenase	Putative 6-phosphogluconate dehydrogenase [Methanosaeta harundinacea 6Ac]	AET64740	-	-	-	-
EC 1.1.1.343	Desfe_1189	WP_014767955.1	6-phosphogluconate dehydrogenase	6-phosphogluconate dehydrogenase, decarboxylating [Candidatus Nitrososphaera gargensis Ga9.2]	AFU57575	D-glycerate dehydrogenase [Desulfurococcus fermentans]	24	0,89	28
EC 5.1.3.1	not found		ribulose-phosphate 3-epimerase	ribulose-phosphate 3-epimerase [Aciduliprofundum boonei T469]	ADD08292	-	-	-	-

EC 5.4.2.7	Desfe_0195 Desfe_0111	WP_014767010.1 WP_014766929.1	phosphoglucomutase / phosphopentomutase	phosphopentomutase [Thermococcus kodakarensis]	WP_011250728.1	phosphoglucomutase [Desulfurococcus fermentans] phosphoglucosamine mutase [Desulfurococcus fermentans]	98 95	5,00E-90 3,00E71	39 36
EC 2.7.4.23	not found		ribose 1,5-bisphosphokinase	phosphonate metabolism protein/1,5-bisphosphokinase (PRPP-forming) PhnN [Desulfovibrio alaskensis]	WP_011369062	NAD-dependent epimerase [Desulfurococcus fermentans]	97	1,00E-11	24
EC 1.1.1.49	not found		glucose-6-phosphate 1-dehydrogenase	glucose-6-phosphate 1-dehydrogenase [Pseudomonas aeruginosa PAO1]	NP_251873	-	-	-	-
EC 1.1.1.363	not found		glucose-6-phosphate 1-dehydrogenase	glucose-6-phosphate 1-dehydrogenase [Pseudomonas putida KT2440]	NP_743183	-	-	-	-
EC 1.1.1.388	Desfe_0989	WP_014767768.1	NAD+ dependent glucose-6-phosphate dehydrogenase	NAD-dependent epimerase [Haloferax volcanii]	WP_004044412.1	NAD-dependent epimerase [Desulfurococcus fermentans]	97	1,00E-11	24
EC 3.1.1.31	not found		6-phosphogluconolactonase	6-phosphogluconolactonase [Pseudomonas aeruginosa PAO1]	NP_251872	-	-	-	-
EC 2.7.1.11	Desfe_0717 Desfe_0968	WP_048815869.1 WP_014767752.1	6-phosphofructokinase 1 (also 2)	ATP-dependent 6-phosphofructokinase [Aeropyrum pernix K1]	BAA78921.1	phosphofructokinase [Desulfurococcus fermentans]phosphofructokinase [Desulfurococcus fermentans]	9790	6,00E-975,00E-28	4629
EC 2.7.1.146	not found		ADP-dependent phosphofructokinase/glucokinase	phosphofructokinase [Thermococcus litoralis DSM 5473]	WP_020953560.1	-	-	-	-

EC 2.2.1.1	not found		transketolase	Transketolase, N-terminal section (tkt-1) [Sulfolobus solfataricus P2]	AAK40638	-	-	-	-
EC 2.2.1.2	not found		transaldolase	Transaldolase [Methanococcus maripaludis S2]	WP_011171252	-	-	-	-
EC 4.1.2.9	not found		xylulose-5-phosphate/fructose-6-phosphate phosphoketolase	phosphoketolase [Streptococcus agalactiae 2603V/R]	NP_688789	-	-	-	-
Carbon Metabolism									
EC 1.1.1.47	Desfe_0025 Desfe_1209	WP_014766844.1 WP_014767974.1	glucose 1-dehydrogenase	glucose dehydrogenase [Haloarcula marismortui ATCC 43049]	AAV47505	short-chain dehydrogenase [D. fermentans]	98	9,00E+62	43
EC 1.1.1.359	Desfe_1240	WP_014768004.1	aldose 1-dehydrogenase [NAD(P)+]	Glucose 1-dehydrogenase (dhg-1) [Sulfolobus solfataricus P2]	WP_009992653.1	alcohol dehydrogenase [D. fermentans]	39	3,00E-11	32
EC 3.1.1.17	not found		gluconolactonase	gluconolactonase [Haloferax volcanii]	WP_004041072.1	-	-	-	-
EC 4.2.1.140	not found		gluconate/galactonate dehydratase	D-gluconate/D-galactonate dehydratase OS=Sulfolobus solfataricus	Q97U27 UniProt	-	-	-	-
EC 4.1.2.55	Desfe_1213	WP_014767978.1	2-dehydro-3-deoxy-phosphogluconate/2-dehydro-3-deoxy-6-phosphogalactonate aldolase	2-keto-3-deoxy gluconate aldolase [Metallosphaera sedula]	WP_012021241.1	-	-	-	-
EC 4.1.2.51	Desfe_1213	WP_014767978.2	2-dehydro-3-deoxy-D-gluconate aldolase	dihydrodipicolinate synthase family protein [Thermoplasma volcanium]	WP_010917486.1	dihydrodipicolinate synthase family protein [Desulfurococcus fermentans]	82	8,00E-24	28

EC 1.1.1.388	Desfe_0989	WP_014767768.1	NAD+ dependent glucose-6-phosphate dehydrogenase	NAD-dependent epimerase [Haloferax volcanii]	WP_004044412.1	-	-	-	-
EC 3.1.1.31	not found		6-phosphogluconolactonase	6-phosphogluconolactonase [Pseudomonas aeruginosa PAO1]	NP_251872	-	-	-	-
EC 4.2.1.12	not found		phosphogluconate dehydratase	phosphogluconate dehydratase [Pseudomonas aeruginosa PAO1]	NP_251884	-	-	-	-
EC 4.1.2.14	not found		2-dehydro-3-deoxyphosphogluconate aldolase / (4S)-4-hydroxy-2-oxoglutarate aldolase	2-dehydro-3-deoxyphosphogluconate aldolase/4-hydroxy-2-oxoglutarate aldolase [Haloarcula marismortui ATCC 43049]	AAV47830	-	-	-	-
EC 4.1.2.55	Desfe_0262	WP_014767076.1	2-dehydro-3-deoxy-phosphogluconate/2-dehydro-3-deoxy-6-phosphogalactonate aldolase	2-keto-3-deoxy gluconate aldolase (eda) [Sulfolobus solfataricus P2]	AAK43294	-	-	-	-
EC 1.1.1.44	not found		6-phosphogluconate dehydrogenase	Putative 6-phosphogluconate dehydrogenase [Methanosaeta harundinacea 6Ac]	AET64740	-	-	-	-
EC 1.1.1.343	Desfe_1189	WP_014767955.1	6-phosphogluconate dehydrogenase	6-phosphogluconate dehydrogenase, decarboxylating [Candidatus Nitrososphaera gargensis Ga9.2]	AFU57575	D-glycerate dehydrogenase [Desulfurococcus fermentans]	24	0,89	28
EC 2.7.1.19	not found		phosphoribulokinase	phosphoribulokinase/uridine kinase [Archaeoglobus profundus	WP_012940929	dipeptide/oligopeptide/nickel ABC transporter ATP-binding protein [Desulfurococcus fermentans]	6	3,5	52

EC 5.1.3.1	not found		ribulose-phosphate 3-epimerase	ribulose-phosphate 3-epimerase [Aciduliprofundum boonei T469]	ADD08292	-	-	-	-
EC 2.2.1.1	not found		transketolase	Transketolase, N-terminal section (tkt-1) [Sulfolobus solfataricus P2]	AAK40638	-	-	-	-
EC 2.2.1.2	not found		transaldolase	Transaldolase [Methanococcus maripaludis S2]	WP_011171252	-	-	-	-
EC 2.2.1.3	not found		dihydroxyacetone synthase	dihydroxyacetone synthase [Candida boidinii]	O93884 (UniProt) / AAC83349	-	-	-	-
EC 3.1.3.11	Desfe_1349	WP_014768106.1	fructose-1,6-bisphosphatase II	fructose 1,6-bisphosphatase [Sulfolobus tokodaii]	WP_052846263	fructose 1,6-bisphosphatase [Desulfurococcus fermentans]	96	0.0	70
EC 3.1.3.37	not found		sedoheptulose-1,7-bisphosphatase / sedoheptulose-bisphosphatase	sedoheptulose-1,7-bisphosphatase [Trypanosoma cruzi strain CL Brener]	XP_807376				
EC 4.1.2.13	not found		fructose-bisphosphate aldolase, class I/II	fructose-bisphosphate aldolase [Haloferax]	WP_004043441				
EC 2.7.1.29	not found		dihydroxyacetone kinase / glycerone kinase	Glycerone kinase [Thermobaculum terrenum ATCC BAA-798]	ACZ43374.1				
EC 2.7.1.11	Desfe_0717 Desfe_0968	WP_048815869.1 WP_014767752.1	6-phosphofructokinase 1 (also 2)	ATP-dependent 6-phosphofructokinase [Aeropyrum pernix K1]	BAA78921.1	phosphofructokinase [Desulfurococcus fermentans] phosphofructokinase [Desulfurococcus fermentans]	97 90	6,00E-97 5,00E-28	46 29
EC 2.7.1.146	not found		ADP-dependent phosphofructokinase/glucokinase	phosphofructokinase [Thermococcus litoralis DSM 5473]	WP_020953560.1	-	-	-	-

EC 2.7.1.147	not found		ADP-dependent phosphofructokinase/glucokinase	phosphofructokinase [Thermococcus litoralis DSM 5473]	WP_020953560.1	-	-	-	-
EC 3.1.3.18	Desfe_1132	WP_048815912.1	phosphoglycolate phosphatase	phosphoglycolate phosphatase [Thermoplasma acidophilum]	WP_010900602.1	phosphoglycolate phosphatase [Desulfurococcus fermentans]	96	7,00E-22	28
EC 3.1.3.48	not found		protein-tyrosine-phosphatase	phosphotyrosine protein phosphatase [Thermus thermophilus]	WP_011173248.1				
EC 1.1.3.15	not found		(S)-2-hydroxy-acid oxidase	L-lactate oxidase [Lactococcus lactis subsp. lactis Il1403]	NP_267408.1	type 2 isopentenyl-diphosphate Delta-isomerase [Desulfurococcus fermentans]	19	0,019	30
EC 1.11.1.6	not found		catalase	catalase [Methanosarcina barkeri str. Fusaro]	WP_011305841.1				
EC 2.6.1.2	Desfe_0370 Desfe_0590	WP_014767181 WP_014767392	alanine transaminase	aminotransferase class I/II [Anaeromyxobacter sp. Fw109-5]	WP_012096788.1	aminotransferase [Desulfurococcus fermentans] aspartate aminotransferase [Desulfurococcus fermentans]	36 92	3,00E-09 4,00E-15	28 24
EC 2.6.1.4	Desfe_0739 Desfe_1253	WP_014767537 WP_014768016	glycine transaminase	class V aminotransferase [Hydrogenobacter thermophilus]	WP_012962754	class V aminotransferase [Desulfurococcus fermentans] aspartate aminotransferase [Desulfurococcus fermentans]	99 91	1,00E-46 1,00E-43	32 33
EC 2.6.1.44	Desfe_0841 Desfe_0590	WP_014767632.1 WP_014767392	alanine-glyoxylate aminotransferase	aspartate aminotransferase [Hydrogenobacter thermophilus]	WP_012964464	aspartate aminotransferase [Desulfurococcus fermentans]	91 83	7,00E-34 2,00E-	26 25

						alanine--glyoxylate aminotransferase [Desulfurococcus fermentans]		15	
EC 2.7.2.1	not found		acetate kinase	Acetate kinase [Methanosarcina thermophila CHTI-55]	AKB16585.1				
EC 6.2.1.1	not found		acetyl-CoA synthetase	acetyl-coenzyme A synthetase [Aeropyrum pernix K1]	BAA80340.2				
EC 6.2.1.13	Desfe_0977 Desfe_1051	WP_014767761.1 WP_014767820.1	acetyl-CoA synthetase (ADP-forming)	acetyl-CoA synthetase [Pyrococcus furiosus]	WP_011012687	acetyl-CoA synthetase [Desulfurococcus fermentans] CoA-binding protein [Desulfurococcus fermentans]	98 99	4,00E-166 4,00E-104	56 43
EC 2.3.1.8	not found		phosphate acetyltransferase, putative phosphotransacetylase	phosphotransacetylase [Methanosarcina acetivorans]	WP_011023515.1	-	-	-	-
EC 4.1.3.24	not found		malyl-CoA lyase	Chloroflexus aurantiacus malyl-CoA/beta-methylmalyl-CoA/citramalyl-CoA lyase	S5N020 (uniProt)	-	-	-	-
EC 4.1.3.25	not found		(S)-citramalyl-CoA lyase	Chloroflexus aurantiacus malyl-CoA/beta-methylmalyl-CoA/citramalyl-CoA lyase	S5N020 (uniProt)				
EC 6.4.1.2	Desfe_0880	WP_014767669.1	acetyl-CoA carboxylase	carbamoyl-phosphate synthase subunit L	WP_013907587	carbamoyl-phosphate synthase subunit L [Desulfurococcus fermentans]	94	4,00E-73	33

EC 6.3.4.14	Desfe_0880 Desfe_0782 Desfe_1050	WP_014767669.1 WP_014767577.1 WP_014767819.1	biotin carboxylase	biotin carboxylase [Metallosphaera sedula]	WP_011921292.1	carbamoyl-phosphate synthase subunit L [Desulfurococcus fermentans] acetyl-CoA synthetase [Desulfurococcus fermentans] acetyl-CoA synthetase [Desulfurococcus fermentans]	91 6 4	6,00E-65 0,61 3,5	32 47 58
EC 6.4.1.3	not found			Biotin carboxyl carrier protein of acetyl-CoA carboxylase OS=Acidianus brierleyi	Q877I4 (UniProt)	-	-	-	-
EC 1.2.1.18	not found		malonate-semialdehyde dehydrogenase (acetylating)		not for this Enzyme, only for orthologue EC 1.2.1.18)	-	-	-	-
EC 1.2.1.27	Desfe_0067	WP_014766885.1	methylmalonate-semialdehyde dehydrogenase	methylmalonate-semialdehyde dehydrogenase [Sulfolobus solfataricus]	WP_010923256.1	aldehyde dehydrogenase [Desulfurococcus fermentans]	96	2,00E-60	29
EC 1.2.1.75	not found		malonyl-CoA reductase	Sulfolobus tokodaii str. 7 malonyl-CoA/succinyl-CoA reductase	Q96YK1 (UniProt)	-	-	-	-
EC 1.2.1.76	not found		succinyl-CoA reductase (NADPH)	aspartate-semialdehyde dehydrogenase [Sulfolobus solfataricus]	WP_009992122.1	-	-	-	-
EC 1.1.1.298	not found		3-hydroxypropionate dehydrogenase (NADP+)	3-hydroxyacyl-CoA dehydrogenase [Sulfolobus solfataricus]	WP_009991183.1	-	-	-	-
EC 3.1.2.4	not found		3-hydroxyisobutyryl-CoA hydrolase	3-hydroxyisobutyryl-CoA hydrolase [Bacillus cereus]	WP_000139775	-	-	-	-

EC 1.3.1.84	Desfe_1240 Desfe_0019	WP_014768004.1 WP_048815804.1	acrylyl-CoA reductase (NADPH)	ulfolobus tokodaii str. 7 acrylyl-CoA reductase	Q975C8 (UniProt)	alcohol dehydrogenase [Desulfurococcus fermentans]	99	4,00E-34	30
EC 4.2.1.116	not found		3-hydroxypropionyl-CoA dehydratase	3-hydroxypropionyl-coenzyme A dehydratase OS=Metallosphaera sedula	A4YI89 (UniProt)	-	-	-	-
EC 6.2.1.36	not found		3-hydroxypropionyl-coenzyme A synthetas	3-hydroxypropionyl-CoA synthetase [Sulfolobus solfataricus]	WP_009991677.1	-	-	-	-
EC 5.3.3.8	not found		dodecenoyl-CoA isomerase			-	-	-	-
EC 4.2.1.17	not found		enoyl-CoA hydratase			-	-	-	-
EC 1.1.1.35	not found		3-hydroxyacyl-CoA dehydrogenase	3-hydroxyacyl-CoA dehydrogenase [Metallosphaera sedula]	WP_048059965.1	-	-	-	-
EC 1.1.1.211	not found		long-chain-3-hydroxyacyl-CoA dehydrogenase	3-hydroxyacyl-CoA dehydrogenase [Pseudomonas putida KT2440]	NP_744197	-	-	-	-
EC 1.3.8.7	not found		acyl-CoA dehydrogenase	acyl-CoA dehydrogenase [Aeropyrum pernix K1]	BAA79340.2	-	-	-	-
EC 4.1.3.24	not found		malyl-CoA lyase	Chloroflexus aurantiacus malyl-CoA/beta-methylmalyl-CoA/citramalyl-CoA lyase	S5N020 (uniProt)	-	-	-	-
EC 6.4.1.3	not found			Biotin carboxyl carrier protein of acetyl-CoA carboxylase OS=Acidianus brierleyi	Q877I4 (UniProt)	-	-	-	-
EC 2.6.1.44	Desfe_0739 Desfe_1253	WP_014767537 WP_014768016	alanine-glyoxylate transaminase	alanine--glyoxylate aminotransferase [Haloferax volcanii]	WP_013035552.1	class V aminotransferase [Desulfurococcus fermentans] aspartate aminotransferase	86 92	9,00E-51 6,00E-47	30 32

						[Desulfurococcus fermentans]			
EC 2.6.1.45	Desfe_0739 Desfe_1253	WP_014767537 WP_014768016	serine-glyoxylate transaminase	alanine--glyoxylate aminotransferase [Haloarcula marismortui]	WP_049938957.1	class V aminotransferase [Desulfurococcus fermentans] aspartate aminotransferase [Desulfurococcus fermentans]	94 87	2,00E-48 2,00E-49	31 30
EC 2.6.1.51	Desfe_0739 Desfe_1253	WP_014767537WP_014768016	serine-pyruvate transaminase	Serine--glyoxylate transaminase [Pyrobaculum calidifontis JCM 11548]	ABO09029	class V aminotransferase [Desulfurococcus fermentans]aspartate aminotransferase [Desulfurococcus fermentans]	9687	4,00E-434,00E-44	3332
EC 1.1.1.29	Desfe_1189	WP_014767955.1	glycerate dehydrogenase, n hydroxypyruvate reductase 1 + 2	glycerate dehydrogenase [Thermoplasma acidophilum]	WP_010901268.1	D-glycerate dehydrogenase [Desulfurococcus fermentans] glutamate dehydrogenase [Desulfurococcus fermentans]	88 11	2E-29 3,9	31 36
EC 4.1.3.1	not found		isocitrate lyase	isocitrate lyase [Sulfolobus solfataricus] NCBI Reference Sequence: WP_010923328.1	WP_010923328.1	-	-	-	-
EC 4.3.1.17	not found		L-serine dehydratase, L-serine ammonia-lyase	serine dehydratase [Methanococcus voltae]	WP_013180165.1	-	-	-	-
EC 4.3.1.19	Desfe_1398 Desfe_0716	WP_014768151.1 WP_048815868.1	threonine dehydratase, threonine ammonia-lyase	threonine dehydratase [Aeropyrum pernix K1]	BAA80497	L-threonine synthase [Desulfurococcus fermentans] mannose-6-	66 13	5,00E-21 0,31	33 27

						phosphate isomerase [Desulfurococcus fermentans]			
EC 3.1.2.30	not found		(3S)-malyl-CoA thioesterase	malate synthase [Haloferax volcanii]	WP_004041864.1	peptide transporter [Desulfurococcus fermentans]	24	4,7	26
EC 2.3.3.9	not found		malate synthase	malate synthase [Sulfolobus solfataricus]	WP_010923329.1	-	-	-	-
EC 4.1.1.31	not found		phosphoenolpyruvate carboxylase	phosphoenolpyruvate carboxylase [Ignicoccus hospitalis]	WP_011998376.1	-	-	-	-
EC 4.1.1.49	not found		phosphoenolpyruvate carboxykinase (ATP)	phosphoenolpyruvate carboxykinase [ATP] [Aeropyrum pernix K1]	BAA78942.2	-	-	-	-
EC 1.1.1.38	not found		malate dehydrogenase (oxaloacetate-decarboxylating)	malate dehydrogenase [Aeropyrum pernix K1]	BAA79347.1	-	-	-	-
EC 1.1.1.39	not found		malate dehydrogenase (decarboxylating)	malate dehydrogenase [Lactobacillus paracasei ATCC 334]	YP_806019.1	-	-	-	-
EC 1.1.1.40	not found		malate dehydrogenase (oxaloacetate-decarboxylating)(NADP+)	malate dehydrogenase [Metallosphaera sedula]	WP_048060215.1	-	-	-	-
EC 4.1.3.34	not found		citryl-CoA lyase	citryl-CoA lyase [Sulfurihydrogenibium sp. YO3AOP1]	WP_012459727.1	-	-	-	-
EC 2.3.3.1	not found		citrate synthase	citrate synthase [Aeropyrum pernix K1]	BAA80714.1	-	-	-	-
EC 2.3.3.8	Desfe_1155	WP_014767922.1	ATP-citrate lyase alpha-subunit + beta-subunit	ATP-citrate lyase subunit A [Methanosaeta harundinacea]	WP_014587072.1	succinyl-CoA synthetase subunit alpha [Desulfurococcus	47	9,00E-21	27

						fermentans]			
EC 6.2.1.18	Desfe_1156	WP_014767922.2	citryl-CoA synthetase large subunit + small subunit	succinyl-CoA synthetase subunit alpha [Thermocrinis albus]	WP_012991242.1	succinyl-CoA synthetase subunit alpha [Desulfurococcus fermentans]	82	1,00E-49	37
EC 4.2.1.3	Desfe_2749	WP_014767032.1	aconitate hydratase, aconitate hydratase 2	acetyltransferase, aconitate hydratase [Thermosphaera aggregans]	BAA80618	acetyltransferase [Desulfurococcus fermentans]	92	5,00E-45	59
EC 4.2.1.99	not found		2-methylisocitrate dehydratase	aconitate hydratase B [Methanococcoides methylutens]	WP_048205444.1	-	-	-	-
EC 1.1.1.41	not found		isocitrate dehydrogenase (NAD+)	isocitrate dehydrogenase [Thermogladius cellulolyticus]	WP_014737451.1	-	-	-	-
EC 1.1.1.42	not found		isocitrate dehydrogenase	isocitrate dehydrogenase [NADP] [Aeropyrum pernix K1]	BAA79665	-	-	-	-
EC 2.6.1.1	Desfe_0370 Desfe_0590	WP_014767181 WP_014767392	aspartate aminotransferase, cytoplasmic	aspartate aminotransferase [Sulfolobus solfataricus]	WP_009992318.1	aspartate aminotransferase [Desulfurococcus fermentans]	92	9,00E-43	29
EC 1.3.5.1.			succinate dehydrogenase, succinate dehydrogenase (ubiquinone) flavoprotein subunit, etc	succinate dehydrogenase subunit B [Aeropyrum pernix K1]	BAA79930.1	-	-	-	-
EC 1.3.5.4	Desfe_0380 Desfe_0770 Desfe_0500	WP_014767191.1 WP_014767576.1 WP_014767305.1	fumarate reductase flavoprotein subunit	fumarate reductase iron-sulfur subunit [Helicobacter pylori 26695]	NP_206990.1	(2Fe-2S)-binding protein [Desulfurococcus fermentans] (2Fe-2S)-binding protein [Desulfurococcus fermentans] 2-oxoglutarate	13 11 7	2,00E-04 0,18 0,75	50 44 58

						ferredoxin oxidoreductase subunit delta [Desulfurococcus fermentans]			
EC 1.3.1.6	not found		NADH-dependent fumarate reductase subunit A/B	fumarate reductase iron-sulfur subunit [Aquifex aeolicus VF5]	NP_213452.1	-	-	-	-
EC 1.3.4.1	not found		fumarate reductase (CoM/CoB) subunit A	succinate dehydrogenase [Methanothermobacter thermautotrophicus]	WP_048061157.1	-	-	-	-
EC 4.2.1.2	Desfe_0494	WP_014767299.1	fumarate hydratase, class I + II, fumarate hydratase subunit alpha + beta	fumarate hydratase class II [Aeropyrum pernix K1]	BAA80819.2	adenylosuccinate lyase [Desulfurococcus fermentans]	77	4,00E-11	28
EC 1.1.1.37	not found		malate dehydrogenase	malate dehydrogenase [Aeropyrum pernix K1]	BAA79645.2	-	-	-	-
EC 1.1.1.82	not found		malate dehydrogenase (NADP+)	Aeropyrum pernix K1 malate dehydrogenase	Q9YEA1 (Uniprot)	-	-	-	-
EC 1.1.5.4	Desfe_0284	WP_014767098.1	malate dehydrogenase (quinone)	FAD-dependent oxidoreductase [Natronomonas moolapensis]	WP_015409055.1	FAD-dependent oxidoreductase [Desulfurococcus fermentans]	47	0,065	22
EC 6.2.1.9	Desfe_1155 Desfe_1156	WP_014767922.1 WP_048815914.1	malate-CoA ligase subunit alpha/beta	MULTISPECIES: malate--CoA ligase subunit beta [Methylobacterium]v	WP_003597633.1	succinate--CoA ligase subunit beta [Desulfurococcus fermentans]	99	5,00E-90	39
EC 2.8.3.22	Desfe_0278	WP_014767092.1	succinyl-CoA:(S)-malate CoA-transferase subunit A/B	L-carnitine dehydratase/bile acid-inducible protein F [Chloroflexus aurantiacus J-10-fl]	YP_001633821.1	formyl-CoA transferase [Desulfurococcus fermentans]	97	8,00E-58	31

EC 2.6.1.52	not found		phosphoserine aminotransferase	phosphoserine aminotransferase [Methanosarcina acetivorans]	WP_011022283.1	-	-	-	-
EC 2.3.1.30	not found		serine O-acetyltransferase	serine acetyltransferase [Haloferax volcanii]	WP_004041887.1	-	-	-	-
EC 1.14.13.25	not found		methane monooxygenase component A alpha / beta / gamma chain			-	-	-	-
EC 1.14.18.3	not found		methane monooxygenase	hypothetical protein Nmar_1500 [Nitrosopumilus maritimus SCM1]	ABX13396.1	-	-	-	-
EC 1.14.99.39	not found		ammonia monooxygenase	hypothetical protein Nmar_1500 [Nitrosopumilus maritimus SCM1]	ABX13396.2	-	-	-	-
EC 1.1.2.7	not found		methanol dehydrogenase (cytochrome c) subunit 1	alcohol oxidase [Fusarium graminearum PH-1]	XP_011327306.1	-	-	-	-
EC 1.1.3.13	not found		alcohol oxidase			-	-	-	-
EC 1.2.1.46	Desfe_1240 Desfe_0019	WP_014768004.1 WP_048815804.1	glutathione-independent formaldehyde dehydrogenase	formaldehyde dehydrogenase [Haloferax gibbonsii]	WP_004971697.1	alcohol dehydrogenase [Desulfurococcus fermentans] alcohol dehydrogenase [Desulfurococcus fermentans]	72 27	9,00E-16 8,00E-10	28 32
EC 1.2.1.43	not found		formate dehydrogenase alpha/beta subunit	formate dehydrogenase subunit alpha [Desulfomicrobium baculatum]	WP_012805379.1	NADH dehydrogenase subunit I [Desulfurococcus fermentans]	9	6,00E-04	35
EC 1.2.98.1	not found		methanol:N,N-dimethyl-4-nitrosoaniline oxidoreductase	iron-containing alcohol dehydrogenase [Desulfotomaculum	ACV64937.1	-	-	-	-

				acetoxidans DSM 771]					
EC 4.4.1.22	not found		S-(hydroxymethyl)glutathione synthase	glutathione-dependent formaldehyde-activating, GFA [Burkholderia ambifaria AMMD]	ABI91344.1	-	-	-	-
EC 1.1.1.284	Desfe_1240 Desfe_0019	WP_014768004.1 WP_048815804.1	S-(hydroxymethyl)glutathione dehydrogenase	hypothetical protein [Desulfitobacterium hafniense Y51]	BAE84833	alcohol dehydrogenase [Desulfurococcus fermentans] alcohol dehydrogenase [Desulfurococcus fermentans]	93 33	8,00E-25 6,00E-15	26 30
EC 1.1.1.1	Desfe_1240 Desfe_0019	WP_014768004.1 WP_048815804.1	alcohol dehydrogenase	hypothetical protein [Desulfitobacterium hafniense Y51]	BAE84834	alcohol dehydrogenase [Desulfurococcus fermentans] alcohol dehydrogenase [Desulfurococcus fermentans]	93 34	8,00E-25 6,00E-16	26 31
EC 3.1.2.12	not found		S-formylglutathione hydrolase	S-formylglutathione hydrolase [Myxococcus xanthus]	WP_011557012	-	-	-	-
EC 2.8.4.1	not found		methyl-coenzyme M reductase alpha/beta/gamma subunit	methyl coenzyme M reductase I, subunit gamma (mcrG) [Methanocaldococcus jannaschii DSM 2661]	NP_247839.1	-	-	-	-
EC 1.8.98.1	not found		heterodisulfide reductase subunit A-E	heterodisulfide reductase [Sulfolobus solfataricus]	WP_009989757.1	-	-	-	-
EC 2.1.1.90	not found		methanol---5-hydroxybenzimidazolylcobamide Co-methyltransferase	methanol-5-hydroxybenzimidazolylcobamide methyltransferase [Methanosarcina acetivorans]	WP_011020506.1	-	-	-	-

EC 2.1.1.246	not found	[methyl-Co(III) methanol-specific corrinoid protein]:coenzyme M methyltransferase	methylcobamide--CoM methyltransferase [Methanosarcina acetivorans]	WP_011020823.1	-	-	-	-
EC 2.1.1.247	not found	[methyl-Co(III) methylamine-specific corrinoid protein]:coenzyme M methyltransferase	methylcobamide--CoM methyltransferase [Methanosarcina acetivorans]	WP_011020204.1	-	-	-	-
EC 2.1.1.248	not found	methylamine---corrinoid protein Co-methyltransferase	monomethylamine methyltransferase [Thermofilum pendens]	WP_011752874.1	-	-	-	-
EC 2.1.1.249	not found	dimethylamine---corrinoid protein Co-methyltransferase	dimethylamine [Methanohalobium evestigatum Z-7303]	ADI74293	-	-	-	-
EC 2.1.1.250	not found	trimethylamine---corrinoid protein Co-methyltransferase	trimethylamine--corrinoid methyltransferase [Methanosarcina acetivorans]	WP_048064903.1	-	-	-	-
EC 1.5.8.1	not found	dimethylamine dehydrogenase	dimethylamine dehydrogenase [Methylotenera versatilis]	WP_013148095	-	-	-	-
EC 1.5.8.2	not found	trimethylamine dehydrogenase	dimethylamine dehydrogenase [Methylotenera versatilis]	WP_013148096	-	-	-	-
EC 2.1.1.86	not found	tetrahydromethanopterin S-methyltransferase subunit A-H	tetrahydromethanopterin S-methyltransferase subunit H [Methanosarcina acetivorans]	WP_011020327.1	-	-	-	-
EC 2.3.1.101	not found	formylmethanofuran--tetrahydromethanopterin N-formyltransferase	formylmethanofuran--tetrahydromethanopterin formyltransferase [Archaeoglobus fulgidus]	WP_010879565.1	-	-	-	-
EC 3.5.4.27	not found	methenyltetrahydromethanopterin cyclohydrolase	methenyltetrahydromethanopterin cyclohydrolase [Haloterrigena turkmenica]	WP_012943379.1	-	-	-	-

EC 1.5.1.-	not found		methylene-tetrahydromethanopterin dehydrogenase	Methylene-tetrahydromethanopterin dehydrogenase [Methylobacterium extorquens PA1]	ABY30228.1	-	-	-	-
EC 1.5.98.1	not found		methylenetetrahydromethanopterin dehydrogenase	methylenetetrahydromethanopterin dehydrogenase [Archaeoglobus fulgidus]	WP_010878217.1	-	-	-	-
EC 1.5.98.2	not found		5,10-methenyltetrahydromethanopterin hydrogenase	5,10-methylenetetrahydromethanopterin reductase [Methanothermobacter thermautotrophicus]	WP_010877358.1	-	-	-	-
EC 2.3.1.-	not found		acetyl-CoA decarbonylase/synthase complex subunit beta	acetyl-CoA synthase subunit beta [Methanothermobacter marburgensis]	WP_013295124.1	-	-	-	-
EC 2.1.1.245	Desfe_0759	WP_014767556.1	acetyl-CoA decarbonylase/synthase complex subunit gamma/delta	acetyl-CoA synthase subunit delta [Methanothermobacter marburgensis]	WP_013295126.1	-	-	-	-
EC 2.1.1.258	not found		5-methyltetrahydrofolate corrinoid/iron sulfur protein methyltransferase	methyltetrahydrofolate--corrinoid/iron-sulfur protein methyltransferase [Candidatus Methanomassiliicoccus intestinalis]	WP_020447981.1	-	-	-	-
EC 2.3.1.169	not found		acetyl-CoA synthase	CO dehydrogenase/acetyl-CoA synthase complex, beta subunit [Ammonifex degensii KC4]	ACX51497.1	-	-	-	-
EC 3.5.1.49	not found		formamidase	acetamidase/formamidase [Aeropyrum camini SY1 = JCM 12091]	BAN89876.1	-	-	-	-
EC 6.3.4.3	not found		formate--tetrahydrofolate ligase	formate--tetrahydrofolate ligase [Thermoplasma acidophilum]	WP_010901878.1	-	-	-	-

EC 1.5.1.5	not found		methylenetetrahydrofolate dehydrogenase (NADP+)	Methylenetetrahydrofolate dehydrogenase (NADP(+)) [Nitrosopumilus maritimus SCM1]	ABX13013.1	-	-	-	-
EC 3.5.4.9	not found		methenyltetrahydrofolate cyclohydrolase	Methylenetetrahydrofolate dehydrogenase (NADP(+)) [Nitrosopumilus maritimus SCM1]	ABX13013.2	-	-	-	-
EC 1.5.1.20	not found		methylenetetrahydrofolate reductase (NADPH)	methylenetetrahydrofolate reductase [Haloarcula marismortui]	WP_011222746.1	-	-	-	-
Fructose & Mannose									
EC 5.3.1.5	not found		Xylose isomerase	xylose isomerase [Caldivirga maquilingensis]	WP_012185764.1	-	-	-	-
EC 5.3.1.7	no data		Mannose isomerase			-	-	-	-
EC 2.7.1.69	not found		PTS system, D-glucosaminate-specific IIA component (also IIB-IID)	PTS fructose transporter subunit IID [Serratia fonticola]	AKG71692	-	-	-	-
EC 2.7.1.1	not found		hexokinase	Sulfolobus tokodaii str. 7 ATP-dependent hexokinase	Q96Y14 (UniProt)	-	-	-	-
EC 2.7.1.7	not found		mannokinase	Inorganic polyphosphate/ATP-glucomannokinase (Fragment) OS=Arthrobacter sp.	Q7WT42 (UniProt)	-	-	-	-
EC 2.7.1.4	Desfe_0717 Desfe_0968 Desfe_0922	WP_048815869.1 WP_014767752.1 WP_014767710.1	fructokinase	ribokinase-like domain-containing protein [Caldivirga maquilingensis]	WP_012186411.1	phosphofructokinase [Desulfurococcus fermentans]phosphofructokinase [Desulfurococcus fermentans]	9882	1,00E-271,00E-14	2729

EC 2.7.7.22	not found		mannose-1-phosphate guanylyltransferase (GDP)	mannose-1-phosphate guanyltransferase [Pyrococcus horikoshii]	WP_010885015.1	-	-	-	-
EC 2.7.1.3	Desfe_0717 Desfe_0968	WP_048815869.1 WP_014767752.1	ketohexokinase	ketohexokinase [Halorhodospira halophila]	WP_011813720.1|	phosphofructokinase [Desulfurococcus fermentans]	84	6,00E-04	21
EC 2.7.1.56	Desfe_0717 Desfe_1388	WP_048815869.1 WP_048815947.1	1-phosphofructokinase	1-phosphofructokinase [Haloferax volcanii]	WP_004043435.1	glycerol-1-phosphate dehydrogenase [Desulfurococcus fermentans]	31	7,3	28
EC 2.7.1.90	not found		pyrophosphate--fructose-6-phosphate 1-phosphotransferase	pyrophosphate--fructose-6-phosphate 1-phosphotransferase [Methylomonas methanica]	WP_013817506.1	-	-	-	-
EC 2.7.1.28	no data					-	-	-	-
Galactose									
EC 5.1.3.3	not found		aldose 1-epimerase	aldose 1-epimerase [Sulfolobus solfataricus]	WP_009990266.1	-	-	-	-
EC 3.2.1.108	Desfe_0624	WP_014767426.1	lactase-phlorizin hydrolase	actase-phlorizin hydrolase [Latimeria chalumnae]	XP_006006254.1	beta-glucosidase [Desulfurococcus fermentans]	73	5,00E-36	27
EC 2.4.1.22	not found		beta-1,4-galactosyltransferase 1 + 2, alpha-lactalbumin,	beta-1,4-galactosyltransferase 2 [Latimeria chalumnae]	XP_005999968.1	-	-	-	-
EC 3.1.3.9	not found		glucose-6-phosphatase			-	-	-	-
EC 3.2.1.10	Desfe_0644	WP_014767446.1	oligo-1,6-glucosidase	oligo-1,6-glucosidase [Halorhabdus tiamatea]	WP_008526807.1	pullulanase [Desulfurococcus fermentans]	59	5,00E-20	26

Pyruvate									
EC 1.1.1.28	Desfe_1189 Desfe_0945 Desfe_1212	WP_014767955.1 WP_014767730.1 WP_014767977.1	D-lactate dehydrogenase	hydroxyacid dehydrogenase [Methanoculleus bourgensis]	WP_014868069.1	2-hydroxyacid dehydrogenase [Desulfurococcus fermentans] D-glycerate dehydrogenase [Desulfurococcus fermentans] lactate dehydrogenase [Desulfurococcus fermentans]	86 80 82	2,00E-60 1,00E-42 7,00E-23	38 35 28
EC 1.1.2.3	not found		L-lactate dehydrogenase (cytochrome)	alpha-hydroxy-acid oxidizing enzyme [Myxococcus stipitatus]	WP_015348264.1	-	-	-	-
EC 1.1.2.4	Desfe_0392	WP_014767203.1	D-lactate dehydrogenase (cytochrome)	FAD-linked oxidase [Metallosphaera sedula]	WP_012021068.1	pyruvate ferredoxin oxidoreductase [Desulfurococcus fermentans]	20	1,2	22
EC 1.1.2.5	no data		D-lactate dehydrogenase (cytochrome c-553)	FAD-linked oxidase [Metallosphaera sedula]	WP_012020401.1	-	-	-	-
EC 5.1.2.1	no data		lactate racemase			-	-	-	-
EC 1.13.12.4	not found		lactate 2-monooxygenase	lactate 2-monooxygenase [Picrophilus torridus]	WP_011177813.1	-	-	-	-
EC 1.2.4.1	not found		pyruvate dehydrogenase E1 component alpha subunit , etc	pyruvate dehydrogenase E1 component, beta subunit [Aeropyrum pernix K1]	BAA80675.1	-	-	-	-
EC 2.3.1.12	not found		pyruvate dehydrogenase E2 component (dihydrolipoamide acetyltransferase)	pyruvate dehydrogenase complex, E2 component [Aeropyrum pernix K1]	BAA80672	-	-	-	-
EC 1.2.5.1	not found		pyruvate dehydrogenase (quinone)	pyruvate dehydrogenase [Sulfolobus]	WP_009989226.1	-	-	-	-

EC 1.2.3.3	not found		pyruvate oxidase	pyruvate dehydrogenase [Methanobacterium lacus]	WP_013645606.1	-	-	-	-
EC 2.7.2.1	not found		acetate kinase	Acetate kinase [Methanosarcina thermophila CHTI-55]	AKB16585.1	-	-	-	-
EC 2.3.1.8	not found		phosphate acetyltransferase, putative phosphotransacetylase	phosphotransacetylase [Methanosarcina acetivorans]	WP_011023515.1	-	-	-	-
EC 3.6.1.7	Desfe_0339	WP_014767151.1	acylphosphatase	acylphosphatase [Desulfurococcus mucosus DSM 2162]	ADV64765.1	hydrogenase maturation protein HypF [Desulfurococcus fermentans]	87	1,00E-07	39
EC 3.1.2.1	not found		acetyl-CoA hydrolase	acetyl-CoA hydrolase [Metallosphaera sedula]	WP_012021165.1	-	-	-	-
EC 6.2.1.1	not found		acetyl-CoA synthetase	acetyl-coenzyme A synthetase [Aeropyrum pernix K1]	BAA80340.2	-	-	-	-
EC 4.1.2.36	no data		lactate aldolase			-	-	-	-
EC 1.2.1.3	Desfe_0067	WP_014766885.1	aldehyde dehydrogenase (NAD+), aldehyde dehydrogenase family 7 member A1, etc	aldehyde dehydrogenase [Sulfolobus acidocaldarius]	WP_011278508.1	aldehyde dehydrogenase [Desulfurococcus fermentans]	83	3,00E-48	29
EC 1.2.1.-	Desfe_0067	WP_014766885.1	aldehyde dehydrogenase	aldehyde dehydrogenase [Deinococcus radiodurans R1]	NP_285671.1	aldehyde dehydrogenase [Desulfurococcus fermentans]	93	3,00E-47	27
EC 1.2.5.2	not found		aldehyde dehydrogenase (quinone)	Quinohemoprotein alcohol dehydrogenase ADH IIB OS=Pseudomonas putida	Q8GR64 (UniProt)	-	-	-	-
EC 1.2.99.6	no data		carboxylate reductase			-	-	-	-

EC 1.2.1.10			acetaldehyde dehydrogenase (acetylating),	acetaldehyde dehydrogenase [Halobacterium sp. DL1]	WP_009487249.1	-	-	-	-
EC 2.3.1.54			formate C-acetyltransferase	glycyl radical enzyme [Thermococcus kodakarensis]	WP_011249244.1	-	-	-	-
EC 2.7.9.1	Desfe_0879	WP_014767668.1	pyruvate, orthophosphate dikinase	pyruvate, phosphate dikinase [Nitrosopumilus maritimus SCM1]	ABX12847	phosphoenolpyruvate synthase [Desulfurococcus fermentans]	97	1,00E-26	24
EC 4.1.1.31	not found		phosphoenolpyruvate carboxylase	phosphoenolpyruvate carboxylase [Ignicoccus hospitalis]	WP_011998376.1	-	-	-	-
EC 41.1.149			phosphoenolpyruvate carboxykinase (ATP)	phosphoenolpyruvate carboxykinase [ATP] [Aeropyrum pernix K1]	BAA78942.2	-	-	-	-
EC 1.1.1.38	not found		malate dehydrogenase (oxaloacetate-decarboxylating)	malate dehydrogenase [Aeropyrum pernix K1]	BAA79347.1	-	-	-	-
EC 1.1.1.39	not found		malate dehydrogenase (decarboxylating)	malate dehydrogenase [Lactobacillus paracasei ATCC 334]	YP_806019.1	-	-	-	-
EC 1.1.1.40	not found		malate dehydrogenase (oxaloacetate-decarboxylating)(NADP+)	malate dehydrogenase [Metallosphaera sedula]	WP_048060215.1	-	-	-	-
EC 1.1.1.37	not found		malate dehydrogenase	malate dehydrogenase [Aeropyrum pernix K1]	BAA79645.2	-	-	-	-
EC 1.1.1.82	not found		malate dehydrogenase (NADP+)	Aeropyrum pernix K1 malate dehydrogenase	Q9YEA1 (Uniprot)	-	-	-	-
EC 1.1.5.4	Desfe_0284	WP_014767098.1	malate dehydrogenase (quinone)	FAD-dependent oxidoreductase [Natronomonas moolapensis]	WP_015409055.1	FAD-dependent oxidoreductase [Desulfurococcus fermentans]	47	0,065	22

EC 1.3.5.4	Desfe_0380 Desfe_0770 Desfe_0500	WP_014767191.1 WP_014767576.1 WP_014767305.1	fumarate reductase flavoprotein subunit	fumarate reductase iron-sulfur subunit [Helicobacter pylori 26695]	NP_206990.1	(2Fe-2S)-binding protein [Desulfurococcus fermentans] (2Fe-2S)-binding protein [Desulfurococcus fermentans] 2-oxoglutarate ferredoxin oxidoreductase subunit delta [Desulfurococcus fermentans]	13 11 7	2,00E-04 0,18 0,75	50 44 58
EC 4.2.1.2	Desfe_0494	WP_014767299.1	fumarate hydratase, class I + II, fumarate hydratase subunit alpha + beta	fumarate hydratase class II [Aeropyrum pernix K1]	BAA80819.2	adenylosuccinate lyase [Desulfurococcus fermentans]	77	4,00E-11	28
EC 2.3.3.9	not found		malate synthase	malate synthase [Sulfolobus solfataricus]	WP_010923329.1	-	-	-	-
Glyoxylate and Dicarboxylate									
EC 1.2.1.2	Desfe_1134	WP_014767902.1	formate dehydrogenase, beta subunit, etc	formate dehydrogenase nitrate-inducible iron-sulfur subunit [Hyperthermus butylicus]	WP_011822213.1	formate dehydrogenase [Desulfurococcus fermentans]	60	6,00E-22	31
	Desfe_1297	WP_014768057.1				ferredoxin [Desulfurococcus fermentans]	23	2,00E-05	36
	Desfe_0335	WP_014767147.1				flavoprotein [Desulfurococcus fermentans]	21	7,00E-04	29

	Desfe_0282	WP_014767096.1				4Fe-4S ferredoxin [Desulfurococcus fermentans]	13	2,40E-01	43
	Desfe_1073	WP_014767841.1				NADH dehydrogenase subunit I [Desulfurococcus fermentans]	23	3,50E-01	32
	Desfe_1140	WP_014767908.1				4Fe-4S ferredoxin [Desulfurococcus fermentans]	17	3,50E-01	32
EC 1.2.2.1	no data		formate dehydrogenase (cytochrome)			-	-	-	-
EC 4.1.1.2	not found		oxalate decarboxylase	bicupin, oxalate decarboxylase family [Candidatus Nitrososphaera evergladensis SR1]	AIF83230.1	-	-	-	-
EC 1.2.3.4	no data		oxalate oxidase			-	-	-	-
EC 1.2.3.5	no data		glyoxylate oxidase			-	-	-	-
EC 1.1.99.14	not found		glycolate dehydrogenase	PCC 6803 hypothetical protein [Synechocystis sp.]	P74033 (UniProt)	-	-	-	-
EC 1.1.3.15	not found		glycolate oxidase	FAD-linked oxidase [Sulfolobus solfataricus]	WP_009989971.1	-	-	-	-
Starch and Sucrose									
EC 3.2.1.91	not found		cellulose 1,4-beta-cellobiosidase	cellulase [Streptomyces coelicolor A3(2)]	NP_630629.1	-	-	-	-
EC 3.2.1.26	not found		beta-fructofuranosidase	sucrose-6-phosphate hydrolase [Haloferax gibbonsii]	WP_050459246.1	-	-	-	-
EC 2.7.7.34	no data		glucose-1-phosphate			-	-	-	-

			guanylyltransferase						
EC 2.7.7.33	Desfe_1244	WP_048815929.1	glucose-1-phosphate cytidylyltransferase	glucose-1-phosphate cytidylyltransferase [Methanococcus aeolicus]	WP_011973103.1	nucleotidyltransferase [Desulfurococcus fermentans]	94	1,00E-29	31
	Desfe_0189	WP_014767004.1				nucleotidyltransferase [Desulfurococcus fermentans]	91	9,00E-18	25
	Desfe_1429	WP_014768181.1				nucleotidyltransferase [Desulfurococcus fermentans]	74	7,00E-15	28
	Desfe_0962	WP_014767746.1				sugar-phosphate nucleotidyltransferase [Desulfurococcus fermentans]	94	1,00E-14	27
EC 3.6.1.21	no data		ADP-sugar diphosphatase			-	-	-	-
EC 2.4.1.29	no data		cellulose synthase (GDP-forming)			-	-	-	-
EC 2.7.1.41	no data		glucose-1-phosphate phosphodismutase			-	-	-	-
EC 2.7.1.10	no data		phosphoglucokinase			-	-	-	-
EC 2.7.1.106	Desfe_0195 Desfe_0111	WP_014767010.1 WP_014766929.1	glucose-1,6-bisphosphate synthase	PREDICTED: glucose 1,6-bisphosphate synthase [Latimeria chalumnae]	XP_006010873.2	phosphoglucosamine mutase [Desulfurococcus fermentans] phosphoglucomutase [Desulfurococcus fermentans]	64 38	1,00E-11 2,00E-08	25 30
EC 3.1.3.90	not found		maltose 6'-phosphate phosphatase	endonuclease/exonuclease/phosphatase [Enterococcus faecalis V583]	NP_814697.1	-	-	-	-
EC 3.2.1.122	not found		maltose-6'-phosphate glucosidase	maltose-6'-phosphate glucosidase [Enterococcus	WP_002303716.1	-	-	-	-

				faecium]					
EC 2.4.1.25	not found		4-alpha-glucanotransferase	4-alpha-glucanotransferase [Haloferax volcanii]	WP_049914763.1	-	-	-	-
EC 3.2.1.20	not found		alpha-glucosidase , lysosomal alpha-glucosidase, maltase-glucoamylase, etc	alpha-glucosidase [Sulfolobus]	WP_009988431.1	-	-	-	-
EC 3.2.1.3	not found		glucoamylase, maltase-glucoamylase	glucan 1,3-alpha-glucosidase [Sulfolobus solfataricus]	WP_010923878.1	-	-	-	-
EC 2.7.1.175	Desfe_0406	WP_014767217.1	maltokinase	hypothetical protein [Thermofilum pendens]	WP_011752472.1	alpha-amylase [Desulfurococcus fermentans]	95	8,00E-36	27
EC 2.4.1.245	not found		trehalose synthase	trehalose synthase [Staphylothermus marinus]	WP_011838389.1	-	-	-	-
EC 2.4.1.8	not found		maltose phosphorylase	maltose phosphorylase [Dyadobacter fermentans]	WP_015812221.1	-	-	-	-
EC 3.2.1.2	not found		beta-amylase	glycoside hydrolase [Thermococcus cleftensis]	WP_014788708.1	-	-	-	-
EC 2.4.99.16	Desfe_0644	WP_014767446.1	starch synthase (maltosyl-transferring)	alpha-amylase [Mycobacterium smegmatis str. MC2 155]	YP_889171.1	pullulanase [Desulfurococcus fermentans]	30	9,00E-03	25
EC 2.4.1.7	not found		sucrose phosphorylase	sucrose phosphorylase [Leuconostoc mesenteroides]	Q14EH6 (UniProt)	-	-	-	-
EC 2.7.7.27	Desfe_0189	WP_014767004.1	glucose-1-phosphate adenylyltransferase	glucose-1-phosphate adenylyltransferase [Thermotoga maritima MSB8]	NP_228053.1	nucleotidyltransferase [Desulfurococcus fermentans]	94	2,00E-14	26
	Desfe_0962	WP_014767746.1				sugar-phosphate nucleotidyltransferase [Desulfurococcus fermentans]	69	9,00E-09	25

EC 2.7.7.9	Desfe_0189	WP_014767004.1	UTP-glucose-1-phosphate uridylyltransferase	UTP-glucose-1-phosphate uridylyltransferase [Pyrococcus furiosus]	WP_011012502.1	nucleotidyltransferase [Desulfurococcus fermentans]	86	2,00E-15	29
EC 2.4.1.4	Desfe_0644	WP_014767446.1	amylosucrase	amylosucrase [Halorhabdus tiamatea]	WP_008526809.1	pullulanase [Desulfurococcus fermentans]	23	7,00E-15	32
EC 2.4.1.5	not found		dextransucrase	hydrolase [Oenococcus oeni]	WP_011677514.1	-	-	-	-
EC 2.4.1.10	not found		levansucrase	levansucrase [Haloferax gibbonsii]	WP_050459247.1	-	-	-	-
EC 2.4.1.13	Desfe_0403 Desfe_0185 Desfe_0527	WP_014767214.1 WP_014767000.1 WP_014767332.1	sucrose synthase	sucrose synthase [Nitrosomonas europaea]	WP_011111802.1	glycosyl transferase family 1 [Desulfurococcus fermentans] glycosyl transferase [Desulfurococcus fermentans] group 1 glycosyl transferase [Desulfurococcus fermentans]	17 11 8	4,00E-06 7,00E-05 3,00E-04	27 29 33
EC 2.4.1.14	Desfe_0403	WP_014767214.1	sucrose-phosphate synthase	Glycosyltransferase [Prochlorococcus marinus subsp. marinus str. CCMP1375]	NP_876271.1	glycosyl transferase family 1 [Desulfurococcus fermentans]	42	9,00E-07	25
EC 3.1.3.24	no data		sucrose-phosphate phosphatase			-	-	-	-
EC 3.2.1.48	Desfe_0611	WP_014767413.1	sucrase-isomaltase / oligo-1,6-glucosidase	phosphate ABC transporter substrate-binding protein [Pyrococcus furiosus]	WP_048059070.1	hypothetical protein [Desulfurococcus fermentans]	97	2,00E-143	46
Citrat									

EC 1.1.1.286	not found		isocitrate--homoisocitrate dehydrogenase	323aa long hypothetical 3-isopropylmalate dehydrogenase [Pyrococcus horikoshii OT3]	BAA30836.1	-	-	-	-
EC 6.2.1.4	Desfe_1156	WP_048815914.1	succinyl-CoA synthetase alpha/beta subunit	succinyl-CoA ligase [GDP-forming] beta-chain [Trypanosoma cruzi strain CL Brener]	XP_818583.1	-	-	-	-
	Desfe_1155	WP_014767922.1	succinyl-CoA synthetase alpha/beta subunit	succinyl-CoA synthetase alpha subunit [Trypanosoma cruzi strain CL Brener]	XP_820834.1	-	-	-	-
EC 2.8.3.18	not found		succinyl-CoA:acetate CoA-transferase	acetyl-CoA hydrolase [Aeropyrum camini SY1 = JCM 12091]	BAN89702.1	-	-	-	-
EC 2.3.1.61	not found		2-oxoglutarate dehydrogenase E2 component (dihydrolipoamide succinyltransferase)	branched-chain alpha-keto acid dehydrogenase dihydrolipoamide acyltransferase (E2) component [Nitrospira defluvii]	WP_013249630.1	-	-	-	-
EC 1.2.4.2	not found		2-oxoglutarate dehydrogenase E1 component	2-oxoglutarate dehydrogenase E1 component [Deinococcus radiodurans R1]	NP_294010.1	-	-	-	-
						-	-	-	-
ABC Transporter									
MalE	Desfe_0354	WP_014767165.1	maltose/maltodextrin transport system substrate-binding protein	maltose ABC transporter substrate-binding protein MalE [Opitutaceae bacterium TAV5]	WP_009509605.1	ABC transporter substrate-binding protein [Desulfurococcus fermentans]	52	9,00E-09	26

MalF	Desfe_0355	WP_014767166.1	maltose/maltodextrin transport system permease protein	binding-protein-dependent transport system inner membrane protein [Dictyoglomus turgidum DSM 6724]	YP_002352610.1	sugar ABC transporter permease [Desulfurococcus fermentans]	47	2,00E-21	29
MalG	Desfe_0356	WP_014767167.1	maltose/maltodextrin transport system permease protein	maltose transporter permease [Hahella chejuensis]	WP_011394429.1	-	-	-	-
LacE	not found		lactose/L-arabinose transport system substrate-binding protein	hypothetical protein [Haloferax gibbonsii]	WP_050460451.1	-	-	-	-
LacF	Desfe_0355	WP_014767166.1	lactose/L-arabinose transport system permease protein	hypothetical protein ABY42_18625 (plasmid) [Haloferax gibbonsii]	AKU09873.1	sugar ABC transporter permease [Desulfurococcus fermentans]	78	3,00E-16	28
LacG	Desfe_0356	WP_014767167.1	lactose/L-arabinose transport system permease protein	lactose ABC transporter permease [Thermosipho africanus]	WP_004102309.1	sugar ABC transporter permease [Desulfurococcus fermentans]	77	5,00E-11	27
LacK	Desfe_1188 (0869, 0553, 0661, 0289, 0365, 0310, 0017, etc)	WP_048815919.1	lactose/L-arabinose transport system ATP-binding protein	sugar ABC transporter ATP-binding protein [Pseudovibrio sp. FO-BEG1]	WP_014284088.1	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans]	99	2,00E-93	45
ThuE	not found		trehalose/maltose transport system substrate-binding protein	ABC transporter periplasmic substrate-binding protein [Deinococcus radiodurans R1]	NP_295161.1	-	-	-	-
ThuF	Desfe_0355	WP_014767166.1	trehalose/maltose transport system permease protein	ABC transporter permease [Thermus thermophilus]	WP_014628823.1	sugar ABC transporter permease [Desulfurococcus fermentans]	83	2,00E-25	29

ThuG	Desfe_0356	WP_014767167.1	trehalose/maltose transport system permease protein	sugar ABC transporter permease [Oceanithermus profundus]	WP_013457136.1	sugar ABC transporter permease [Desulfurococcus fermentans]	75	9,00E-23	30
ThuK	Desfe_1188 (0869, 0553, 0661, 0289, 0365, 0310, 0017, etc)	WP_048815919.1	multiple sugar transport system ATP-binding protein [EC:3.6.3.-]	sugar ABC transporter ATP-binding protein [Pseudomonas fluorescens]	WP_014718701.1	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans]	96	4,00E-90	41
CebE	not found		cellobiose transport system substrate-binding protein	ABC-type sugar transport system, periplasmic component [Anoxybacillus flavithermus WK1]	ACJ34716.1	-	-	-	-
CebF	Desfe_0355	WP_014767166.1	cellobiose transport system permease protein	sugar ABC transporter permease [Thermobacillus composti]	WP_015256126.1	sugar ABC transporter permease [Desulfurococcus fermentans]	85	7,00E-13	26
CebG	not found		cellobiose transport system permease protein	ABC-type sugar transport system, permease component [Thermobacillus composti KWC4]	AGA59398.1	-	-	-	-
AraN	not found		arabinosaccharide transport system permease protein	ABC transporter substrate-binding protein [Thermoanaerobacter mathranii]	WP_013150631.1	-	-	-	-
AraP	Desfe_0355	WP_014767166.1	arabinosaccharide transport system permease protein	arabinose transporter permease [Thermoanaerobacter mathranii]	WP_013150630.1	sugar ABC transporter permease [Desulfurococcus fermentans]	87	1,00E-12	25
AraQ	Desfe_0356	WP_014767167.1	arabinosaccharide transport system permease protein	arabinose transporter permease [Caldicellulosiruptor owensensis]	WP_013411881.1	sugar ABC transporter permease [Desulfurococcus	70	7,00E-11	26

						fermentans]			
GlcS	Desfe_0870	WP_014767659.1	glucose/arabinose transport system substrate-binding protein	ABC transporter substrate-binding protein [Sulfolobus solfataricus]	WP_010923998.1	-	-	-	-
GlcT	not found		glucose/arabinose transport system permease protein	sugar ABC transporter substrate-binding protein [Sulfolobus solfataricus]	WP_009990876.1	-	-	-	-
GlcU	not found		glucose/arabinose transport system permease protein	alpha-glucoside ABC transporter permease [Picrophilus torridus]	WP_011178193.1	-	-	-	-
GlcV	Desfe_1188 (0869, 0553, 0661, 0289, 0365, 0310, 0017, etc)	WP_048815919.1	glucose/arabinose transport system ATP-binding protein	sugar ABC transporter ATP-binding protein [Acidilobus saccharovorans]	WP_013266901.1	sugar ABC transporter ATP-binding protein [Desulfurococcus fermentans]	67	4,00E-62	45
GtsA	Desfe_0354	WP_014767165.1	glucose/mannose transport system substrate-binding protein	ABC transporter substrate-binding protein [Staphylothermus marinus]	WP_011839435.1	ABC transporter substrate-binding protein [Desulfurococcus fermentans]	29	0,16	24
GtsB	not found		glucose/mannose transport system permease protein	ABC transporter permease [Pyrococcus horikoshii]	WP_010885302.1				
GtsC	Desfe_0356	WP_014767167.1	glucose/mannose transport system permease protein	ABC transporter permease [Haloferax volcanii]	P_004041119.1	sugar ABC transporter permease [Desulfurococcus fermentans]	39	3,00E-08	32
AraF	not found		L-arabinose transport system substrate-binding protein	sugar ABC transporter substrate-binding protein [Spirochaeta smaragdinae]	WP_013255640.1	-	-	-	-

AraH	not found		L-arabinose transport system permease protein	hypothetical protein [Verrucomicrobia bacterium IMCC26134]	WP_046297585.1	-	-	-	-
AraG	same as FrcA		L-arabinose transport system ATP-binding protein	ABC transporter [Sphaerochaeta pleomorpha]	WP_014270961.1	-	-	-	-
FrcB	not found		fructose transport system substrate-binding protein	sugar ABC transporter [Deinococcus gobiensis]	WP_014683532.1	-	-	-	-
FrcC	not found		fructose transport system permease protein	sugar ABC transporter permease [Deinococcus geothermalis]	WP_011525726.1	-	-	-	-
FrcA	Desfe_0017	WP_014766837.1	fructose transport system ATP-binding protein	sugar ABC transporter ATP-binding protein [Deinococcus geothermalis]	WP_011525725.1	-	-	-	-
	Desfe_0721	WP_014767521.1				-	-	-	-
	Desfe_0553	WP_014767355.1				-	-	-	-
	Desfe_1188	WP_048815919.1				-	-	-	-
	Desfe_0187	WP_014767002.1				-	-	-	-
	Desfe_0446	WP_014767253.1				-	-	-	-
	Desfe_0184	WP_014767852.1				-	-	-	-
	Desfe_0754	WP_014767551.1				-	-	-	-
	Desfe_0620	WP_014767422.1				-	-	-	-
	Desfe_0639	WP_014767441.1				-	-	-	-
	Desfe_0640	WP_014767442.1				-	-	-	-

Supplementary Table 3 – Results of H_2 and CO_2 gas production. Calculations of HER, CER, qH_2, qCO_2 and $Y_{(H_2/CO_2)}$ are shown.

Substrate	**Time**	**pH**	**T [°C]**	**Cells ml^{-1}**	**μ [h^{-1}]**	**c(H_2) [Vol %]**	**c(CO_2) [Vol %]**	**HER [mmol L^{-1} h^{-1}]**	**CER [mmol L^{-1} h^{-1}]**	**qH_2 [mmol g^{-1} h^{-1}]**	**qCO_2 [mmo g^{-1} h^{-1}]**	**$Y_{(H_2/CO_2)}$**
Fructose_1_BR3	0	5,97	81,45	100.000	0,0000	0,0000	0,0000	0,0000	0,0000	0,000E+00	0,000E+00	0,0000
	57	5,99	80,02	120.000	0,0032	0,0180	0,0222	0,0309	0,0382	2,573E-10	3,181E-10	0,8090
	102	5,98	79,98	250.000	0,0149	0,0094	0,0214	0,0163	0,0368	6,502E-11	1,471E-10	0,4422
	127	5,98	80,14	330.000	0,0117	0,0047	0,0959	0,0080	0,1654	2,430E-11	5,014E-10	0,0485
	173	5,98	80,18	490.000	0,0100	0,0046	0,0257	0,0073	0,0408	1,498E-11	8,325E-11	0,1800
	267	5,98	80,15	550.000	0,0010	0,0188	0,0199	0,0298	0,0315	1,498E-14	5,725E-11	0,9454
	290	6,02	80,17	780.000	0,0152	0,0000	0,0080	0,0000	0,0126	1,498E-14	1,616E-11	0,0000
	318	6,01	80,12	1.200.000	0,0690	0,0090	0,0345	0,0143	0,0549	1,498E-14	4,571E-11	0,2616
	337	5,96	80,27	3.000.000	0,0482	0,0111	0,0093	0,0177	0,0148	1,498E-14	4,934E-12	1,1938
	409	6,02	80,25	5.000.000	0,0071	0,0037	0,0131	0,0060	0,0208	1,498E-14	4,160E-12	0,2862
	435	6,07	80,02	7.000.000	0,0129	0,0017	0,0149	0,0027	0,0238	1,498E-14	3,400E-12	0,1134
	456	6,03	80,44	8.500.000	0,0113	0,0127	0,0103	0,0204	0,0164	1,498E-14	1,929E-12	1,2418
	577	5,96	80,29	13.750.000	0,0035	0,0028	0,6068	0,0046	0,9709	1,498E-14	7,061E-11	0,0047
	627	5,99	79,94	17.750.000	0,0058	0,0016	0,0091	0,0026	0,0146	1,498E-14	8,221E-13	0,1810
	675	6,00	80,32	18.500.000	0,0004	0,0058	0,0071	0,0093	0,0114	1,498E-14	6,156E-13	0,8166
	746	6,02	80,34	19.250.000	0,0004	0,0000	0,0000	0,0000	0,0000	1,498E-14	0,000E+00	0,0000
Fructose_2_BR3	0	6,60	77,70	100.000	0,0000	NA	NA	NA	NA	NA	NA	NA
	143	5,98	80,02	440.000	0,0104	NA	NA	NA	NA	NA	NA	NA
	184	5,97	79,79	815.000	0,0150	NA	NA	NA	NA	NA	NA	NA
	327	5,98	80,02	6.750.000	0,0148	NA	NA	NA	NA	NA	NA	NA
	448	5,97	79,87	13.500.000	0,0057	0,0086	0,0224	0,0022	0,0056	1,600E-13	4,148E-13	0,3857
	475	5,98	79,92		0,0000	0,0079	0,0223	0,0020	0,0056	1,466E-13	4,146E-13	0,3537

	617	5,98	79,83	13.500.000 11.000.000	-0,0014	6,1629	4,8159	1,3877	1,0844	1,262E-10	9,858E-11	1,2797
Fructose_2_BR4	0	6,40	77,47	100.000	0,0000	NA	NA	NA	NA	NA	NA	NA
	143	5,98	80,08	675.000	0,0134	NA	NA	NA	NA	NA	NA	NA
	184	5,98	79,94	830.000	0,0050	NA	NA	NA	NA	NA	NA	NA
	327	5,98	80,19	2.625.000	0,0081	NA	NA	NA	NA	NA	NA	NA
	448	5,97	79,90	5.375.000	0,0059	0,0100	0,0191	0,0025	0,0047	4,571E-13	8,754E-13	0,5222
	475	5,96	79,98	5.375.000	0,0000	0,0068	0,0150	0,0017	0,0037	3,113E-13	6,884E-13	0,4521
	617	5,97	79,80	4.875.000	-0,0007	0,0442	0,0573	0,0109	0,0141	2,234E-12	2,896E-12	0,7716
Cellulose_1_BR3	6	6,95	79,08	350.000	0	0,0000	0,1917	0,0000	0,0543	0,000E+00	1,551E-10	0,0000
	90	7,10	80,08	400.000	0,0015	0,0000	0,0115	0,0000	0,0198	0,000E+00	4,944E-11	0,0000
	116	6,97	79,94	3.250.000	0,0806	0,0000	0,0095	0,0000	0,0164	0,000E+00	5,055E-12	0,0000
	164	7,06	80,03	4.250.000	0,0055	0,0000	0,0083	0,0000	0,0144	0,000E+00	3,380E-12	0,0000
	189	7,13	80,10	2.750.000	-0,0174	0,0000	0,0000	0,0000	0,0000	0,000E+00	0,000E+00	0,0000
	243	7,42	79,95	750.000	-0,0241	0,0000	0,0000	0,0000	0,0000	0,000E+00	0,000E+00	0,0000
Cellulose_1_BR4	6	6,16	79,32	350.000	0,0000	0,0000	0,2392	0,0000	0,0679	0,000E+00	1,941E-10	0,0000
	90	7,23	80,09	460.000	0,0030	0,0000	0,0408	0,0000	0,0701	0,000E+00	1,524E-10	0,0000
	116	6,99	80,02	2.500.000	0,0651	0,0000	0,0044	0,0000	0,0075	0,000E+00	3,020E-12	0,0000
	164	7,12	79,90	2.500.000	0,0000	0,0000	0,0041	0,0000	0,0070	0,000E+00	2,810E-12	0,0000
	189	7,25	80,00	2.500.000	0,0000	0,0000	0,0000	0,0000	0,0000	0,000E+00	0,000E+00	0,0000
	243	7,65	79,88	750.000	-0,0223	0,0000	0,0000	0,0000	0,0000	0,000E+00	0,000E+00	0,0000
Cellulose_3_BR3	1	6,53	79,36	255.000	0	0,0000	1,7031	0,0000	3,6258	0,000E+00	1,422E-08	0,0000
	21	6,38	79,89		-	0,0113	0,0366	0,0183	0,0595	7,975E-11	2,588E-10	0,3082

				230.000	0,0052							
	43	6,36	80,18	255.000	0,0047	0,0030	0,0038	0,0049	0,0062	1,940E-11	2,434E-11	0,7971
	75	6,40	79,66	295.000	0,0046	0,0030	0,0050	0,0048	0,0081	1,638E-11	2,737E-11	0,5984
	120	6,38	80,13	525.000	0,0125	0,0028	0,0049	0,0045	0,0081	8,657E-12	1,543E-11	0,5612
	143	6,41	79,61	510.000	-0,0013	0,0022	0,0035	0,0037	0,0058	7,204E-12	1,130E-11	0,6378
	165	6,43	80,44	1.750.000	0,0587	0,0021	0,0034	0,0035	0,0056	1,996E-12	3,212E-12	0,6214
	186	6,42	79,09	11.500.000	0,0897	0,0025	0,0053	0,0040	0,0087	3,509E-13	7,591E-13	0,4623
	214	6,45	80,20	21.500.000	0,0216	0,0016	0,0056	0,0027	0,0092	1,254E-13	4,264E-13	0,2940
	288	6,41	80,15	21.500.000	0,0000	0,0083	0,0037	0,0135	0,0061	6,290E-13	2,846E-13	2,2104
	314	6,39	79,50	19.500.000	-0,0038	0,0000	0,0050	0,0000	0,0041	0,000E+00	2,090E-13	0,0000
	330	6,40	79,74	21.375.000	0,0057	0,0000	0,0083	0,0000	0,0068	0,000E+00	3,185E-13	0,0000
	356	6,41	80,20	15.000.000	-0,0136	0,0000	0,0228	0,0000	0,0187	0,000E+00	1,249E-12	0,0000
	385	6,41	79,82	10.500.000	-0,0119	0,0038	0,1152	0,0031	0,0946	2,997E-13	9,007E-12	0,0333
Cellulose_4_BR4	1	5,76	84,87	230.000	0	0,0056	0,1493	0,0091	0,2419	3,943E-11	1,052E-09	0,0375
	21	5,73	84,88	245.000	0,0032	0,0098	0,0367	0,0158	0,0593	6,456E-11	2,421E-10	0,2667
	43	5,78	85,15	350.000	0,0162	0,0020	0,0035	0,0032	0,0057	9,071E-12	1,621E-11	0,5595
	75	5,83	84,60	465.000	0,0089	0,0021	0,0038	0,0034	0,0061	7,398E-12	1,311E-11	0,5643
	120	5,77	85,10	550.000	0,0036	0,0000	0,0182	0,0000	0,0295	0,000E+00	5,358E-11	0,0000
	143	5,79	84,65	760.000	0,0141	0,0000	0,0055	0,0000	0,0088	0,000E+00	1,160E-11	0,0000
	165	5,82	85,39	425.000	-0,0277	0,0019	0,0038	0,0031	0,0062	7,239E-12	1,448E-11	0,4999
	186	5,81	83,35	9.125.000	0,1460	0,0045	0,0083	0,0073	0,0134	8,044E-13	1,468E-12	0,5478
	214	2,19	85,11	4.875.000	-0,0216	0,0029	0,0601	0,0045	0,0919	9,193E-13	1,885E-11	0,0488
	288	6,30	85,12	6.750.000	0,0045	0,0044	0,0040	0,0067	0,0062	9,947E-13	9,197E-13	1,0816
	314	6,64	84,80	7.000.000	0,0014	0,0000	0,0049	0,0000	0,0013	0,000E+00	1,793E-13	0,0000

330	6,67	85,00	4.125.000	-0,0331	0,0000	0,0261	0,0000	0,0066	0,000E+00	1,601E-12	0,0000
356	6,52	85,29	2.875.000	-0,0139	0,0000	0,0252	0,0000	0,0064	0,000E+00	2,222E-12	0,0000
385	6,60	84,97	1.125.000	-0,0313	0,0000	0,1139	0,0000	0,0288	0,000E+00	2,564E-11	0,0000

Predicted Metabolism of *Desulfurococcus fermentans*