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Transcriptomic profiling reveals differences in the adaptation of two *Tetragenococcus halophilus* strains to a lupine moromi model medium

Tobias Link and Matthias A. Ehrmann b

Abstract

Background *Tetragenococcus* (*T.*) *halophilus* is a common member of the microbial consortia of food fermented under high salt conditions. These comprises salty condiments based on soy or lupine beans, fish sauce, shrimp paste and brined anchovies. Within these fermentations this lactic acid bacterium (LAB) is responsible for the formation of lactic and other short chain acids that contribute to the flavor and lower the pH of the product. In this study, we investigated the transcriptomic profile of the two *T. halophilus* strains TMW 2.2254 and TMW 2.2256 in a lupine moromi model medium supplied with galactose. To get further insights into which genomic trait is important, we used a setup with two strains. That way we can determine if strain dependent pathways contribute to the overall fitness. These strains differ in the ability to utilize L-arginine, L-aspartate, L-arabinose, D-sorbitol, glycerol, D-lactose or D-melibiose. The lupine moromi model medium is an adapted version of the regular MRS medium supplied with lupine peptone instead of casein peptone and meat extract, to simulate the amino acid availabilities in lupine moromi.

Results The transcriptomic profiles of the *T. halophilus* strains TMW 2.2254 and TMW 2.2256 in a lupine peptone-based model media supplied with galactose, used as simulation media for a lupine seasoning sauce fermentation, were compared to the determine potentially important traits. Both strains, have a great overlap in their response to the culture conditions but some strain specific features such as the utilization of glycerol, sorbitol and arginine contribute to the overall fitness of the strain TMW 2.2256. Interestingly, although both strains have two non-identical copies of the tagatose-6P pathway and the Leloir pathway increased under the same conditions, TMW 2.2256 prefers the degradation via the tagatose-6P pathway while TMW 2.2254 does not. Furthermore, TMW 2.2256 shows an increase in pathways required for balancing out the intracellular NADH/NADH⁺ ratios.

Conclusions Our study reveals for the first time, that both versions of tagatose-6P pathways encoded in both strains are simultaneously active together with the Leloir pathway and contribute to the degradation of galactose. These findings will help to understand the strain dependent features that might be required for a starter strain in lupine moromi.

Keywords Tetragenococcus halophilus, Lupine MRS, Galactose metabolism, Starter culture, Transcriptomics

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Introduction

Tetragenococcus (T.) halophilus is a moderate halophilic lactic acid bacterium commonly isolated from fermented foods containing high amounts of NaCl, such as lupine seasoning sauce, soy sauce, fermented soybean paste,



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fish sauce, salted fish but also from different type of cheese [1–6]. T. halophilus contributes to the final product by production of organic acids and various volatile compounds [7]. To prevent the growth of spoilers and improve the consistency of the fermentation selected starter strains are added to the respective fermentation [8]. The ability of *T. halophilus* to growth under these conditions is due to the accumulation of compatible solutes (e. g glycine-betaine, choline and proline) and due the ability to increase the intracellular pH by degradation of amino acids such as Arginine and aspartic acid under acidic conditions [9-14]. The species is also known for its diverse carbohydrate utilization patterns, that are in most cases strain specific combinations [15]. This diversity in the carbohydrate utilization pattern might be due to an adaptation to a specific fermentation type or niche within a fermentation [16]. To select starter strains for a new type of fermentation it is important to know what traits are favorable under the given conditions. The production process of the lupine seasoning sauce is similar to the production of traditional soy sauce, but the carbohydrate composition in lupine beans is very different compared to soybeans [17, 18]. Therefore, it is unclear which genetic and biochemical traits are wanted/needed in a starter culture for a lupine seasoning sauce fermentation, especially as lupine beans are rich in galactose oligosaccharides (galactans) [19, 20]. T. halophilus has two ways of using this galactose, either via the Leloir pathway or via the tagatose-6P pathway (Fig. 1) [10]. T. halophilus is the only species within the genus that possess on a strain dependent basis duplicates or triplicates of the operon encoding for the tagatose-6P pathway, which may indicate an adaptation towards a galactose rich environment in some strains [21]. The fact that multiple gene copies could potentially increase the intracellular gene dosage and thereby increase the fitness on specific substrates was reported for *Cup1* in *Saccharomyces cerevisiae* [22].

In this study, we investigated the transcriptomic profile of two T. halophilus strains grown in a lupine peptonebased culture medium supplied with galactose, used as simulation media for a lupine seasoning sauce fermentation. We used two representative strains isolated from lupine moromi that had different growth behaviors in lupine MRS medium (LMRS). The two strains differ in their spectrum of fermentable carbohydrates as TMW 2.2254 is L-arabinose negative, D-melibiose positive and D-lactose negative and TMW 2.2256 is L-arabinose positive, D-melibiose negative and D-lactose positive. Further differences are in the ability to use arginine or aspartate for deamination/decarboxylation, TMW 2.2254 has an inactive ADI-pathway but can utilize aspartate via a specific decarboxylase (AspD) and specific transporter (AspT). TMW 2.2256 can use L-arginine via the ADI-pathway but does not encode for *AspD* or *AspT* [2]. We therefore wanted to find out if the different growth behavior in LMRS can be explained using transcriptomic analysis. Furthermore, we wanted to find out which of these traits are active and what kind of effects galactose has on the metabolism of each strain.

Results

Growth of T. halophilus in LMRS

To investigate the growth behavior of T. halophilus TMW 2.2254 and TMW 2.2256 in lupine MRS medium (LMRS) we monitored the $\mathrm{OD}_{600\mathrm{nm}}$ and the pH over 40 h (Fig. 1). Furthermore, to characterize the growth behavior when the major carbohydrate source is altered, growth in LMRS supplied with $10\,\mathrm{mM}$ D-glucose or $10\,\mathrm{mM}$ D-galactose or no added carbon sources were compared.

Both strains showed a high growth without any extra carbon sources added with an $\mathrm{OD}_{600\mathrm{nm}}$ of 1.16 or 1.33 for TMW 2.2254 or TMW 2.2256. More surprisingly, the growth was the highest with D-galactose and not with D-glucose for both strains (Fig. 1). TMW 2.2254 reached a final $\mathrm{OD}_{600\mathrm{nm}}$ of 1.72 (40 h) and TMW 2.2256 reached an end $\mathrm{OD}_{600\mathrm{nm}}$ of 1.85 after 40 h. With D-glucose TMW 2.2254 reached an end $\mathrm{OD}_{600\mathrm{nm}}$ of 1.53 and TMW 2.2256 reached an end $\mathrm{OD}_{600\mathrm{nm}}$ of 1.81 (Fig. 1A).

The pH values with no added carbon sources decreased from 5.66 to 5.49 and then rose back to 5.58 for TMW 2.2254. With the addition of D-glucose the pH decreased from 5.74 to 4.91 over 40 h. Addition of D-galactose led to a decrease from 5.8 to 5.1 of 40 h (Fig. 1B).

The pH values for TMW 2.2256 cultivated in LMRS with no added carbon sources decreased from 5.67 to 5.54 and then rose again to 5.67. The addition of D-glucose led to a decrease from 5.76 to 5.24 over 40 h. D-galactose addition decreased the pH from 5.83 to 5.25 (Fig. 1B).

Overview of the transcriptomic results

To study the difference of the metabolism of two *T. halophilus* strains when galactose is the main carbohydrate source in LMRS, the strains TMW 2.2254 and TMW 2.2256 were cultivated as biological triplicates in LMRS at 30 °C for 10 h. The growth was monitored by plating serial dilutions of the cultures on to LMRS-5% (w/v) NaCl plates after 1 h and 10 h. The cell count after 1 h was 1.85×10^8 CFU/ml and 1.50×10^8 CFU/ml for TMW 2.2254 and TMW 2.2256, respectively. After 10 h of cultivation the cell count CFU/ml was 4.27×10^8 CFU/ml for TMW 2.2254 and 3.47×10^8 CFU/ml for TMW 2.2256.

For the RNA-seq analysis libraries representing each sample transcriptome were constructed for each strain at timepoints 1h and 10h. After sequencing the raw reads were mapped to each genome and normalized after mapping. The uniquely mapped reads for all samples from

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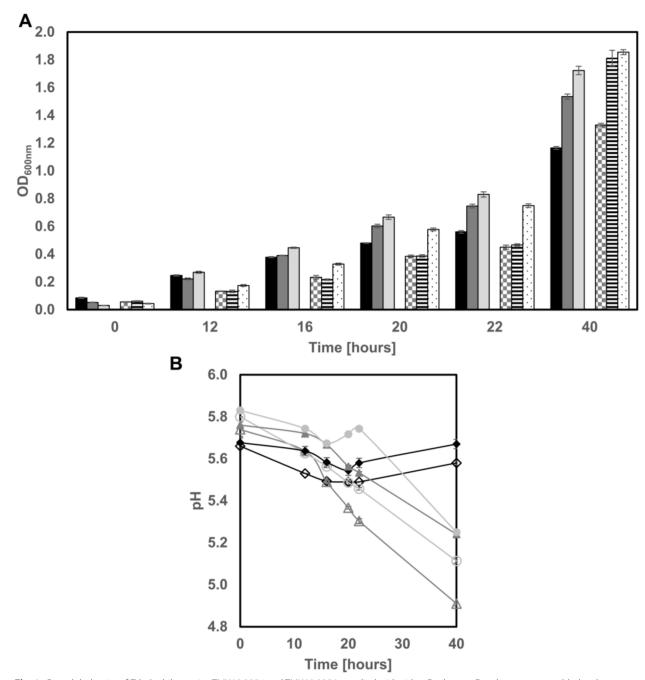


Fig. 1 Growth behavior of *T. halophilus* strains TMW 2.2254 and TMW 2.2256 supplied with either D-glucose, D-galactose or no added carbon sources. **A** OD_{600nm} of TMW 2.2254 (black = w/o added carbon sources; dark gray = 10 mM D-glucose; light gray = 10 mM D-galactose); OD_{600nm} of TMW 2.2256 (checked = w/o added carbon sources; black lines = 10 mM D-glucose; black dots = 10 mM D-galactose). **B** pH values of the culture broth of TMW 2.2254 (open symbols) and TMW 2.2256 (filled symbols) (black = w/o added carbon sources; gray = 10 mM D-glucose; light gray = 10 mM D-galactose). Standard deviation is based on 3 biological replicates

T. halophilus TMW 2.2254 ranged from 22.06 million to 43.54 million and ranged from 24.65 million to 48.13 million for TMW 2.2256. These mapped reads covered 98.3 to 99.4% and 98.7 to 99.7% of the reference genome of TMW 2.2254 and TMW 2.2256. Features with a log2

(fold change) of ≥ 2 or ≤ -2 and p-value < 0.05 and a false discovery rate (FDR) < 0.01 were considered as DEGs. 121 and 108 differentially expressed genes (DEGs) DEGs were identified for TMW 2.2254 and TMW 2.2256, respectively.

Annotation of DEGs with significant differences revealed that they are involved in biological processes and molecular pathways belonging to carbohydrate and amino acid degradation.

Differences in the metabolism of carbohydrates and sugar alcohols can mostly be traced back to the galactose utilization

The tagatose-6-P pathway of TMW 2.2254 was increased by 2 to 4.2-fold after 10h of cultivation. The same pathway was increased by 3.6 to 6.2-fold in TMW 2.2256. Both strains encode for a second version of the pathway with an additional HAD phosphatase, this pathway is here called tagatose-6-P version2. In TMW 2.2254 all enzymes and adjacent transporters were increased by 2.5 to 3.5-fold. In TMW 2.2256 this operon was increased by 2.3 to 5.5-fold. The Leloir pathway was increased in TMW 2.2254 3.4 to 3.7-fold and in TMW 2.2256 2.3 to 3.1-fold. An operon putatively responsible for the utilization of tagatose or fructose via a Tag-1P/Fruc-1P pathway was increased by 4.5 to 5.9-fold in TMW 2.2254 and 5.6 to 6.1-fold in TMW 2.2256. The operon fruAKR responsible for the utilization of fructose was 4 to 4.2 and 4.8 to 5-fold decreased in TMW 2.2254 and TWM 2.2256. A mannose-6-phosphate isomerase was increased by 3.1fold in TMW 2.2254.

A phosphatase part of an operon putatively involved in sucrose metabolism was increased by 2-fold in TMW 2.2256. The Cellobiose operon was only increased (2.9 to 3.5-fold) in TMW 2.2256. The alpha-galactosidase was 2.11-fold increased in TMW 2.2254. The operon *lacEGF* responsible for the lactose metabolism was increased by 6.3 to 8.1-fold in TMW 2.2256. The trehalose operon was increased by 2 to 3-fold and 2.3 to 3.5-fold in TMW 2.2254 and TMW 2.2256, respectively. In both strains a 6-phospho-beta-glucosidase with adjacent PTS-IIA was decreased, 4.2 and 4.3-fold in TMW 2.2254 and 3.9 and 4.2-fold in TMW 2.2256.

The mannitol transporter PTS-IICBA was decreased 3.6-fold in TMW 2.2256. The operon responsible for the glycerol metabolism via the glyercol-3-P pathway (glpKOF) was increased 2.2 to.2.7 fold in TMW 2.2256. Another pathway for the metabolism of glycerol is the dehydrogenation pathway via the gldA-dhaMKL operon. The dhaMKL subunits were increased by 2 to 2.3-fold in TMW 2.2256. In TMW 2.2254 the gldA was 2.3-fold decreased. Two CDs from the operons putatively responsible for the metabolism of sorbitol was increased by 2 to 2.2-fold.

TMW 2.2254 has two putative glucosamie-6-synthases increased with also increased adjacent PTS systems. The first one consisting of two SIS domain-containing proteins increased by 2.3 and 2.4-fold. The adjacent

PTS-IIABCD system was increased by 2.5–2.9-fold. The second operon consisted of a sigma 54-interacting transcriptional regulator (3-fold increase), a PTS-IIABCD (2.9 to 3.9-fold increase), an SIS domain containing protein (2.9-fold increase) and two adjacent chromate transporter (3.4 and 2.8-fold increase). A third operon with similar organization but different domains consisted of a SIS domain-containing protein and adjacent PTS-IIABCD system. The SIS domain-containing protein was increased by 5.4-fold and the PTS system by 4.9 to 5.2-fold.

Central carbon metabolism

The operon of the citrate lyase *citCDEFX* and an adjacent 2-hydroxycarboxylase transporter were increased in both strains, 2.06 to 2.2-fold in TMW 2.2254 and 2.4 to 3.7-fold in TMW 2.2256. The genomically adjacent oxaloacetate decarboxylase *oadBDGA* was increased in TMW 2.2256 by 2.6 to 3.3-fold, while in TMW 2.2254 only the *oadG* subunit was increased by 2-fold.

Multiple CDS associated with pyruvate metabolism were increased in TMW 2.2256, such as the pyruvate dehydrogenase *pdhABC* (2.3 to 2.6-fold), the pyruvate phosphate dikinase *ppdk* (2.5-fold), the lactate dehydrogenase *ldh* (2.4-fold). Furthermore, a pyruvate:ferredoxin oxidoreductase was increased by 2.2-fold in TMW 2.2256.

A lipoate-protein ligase and adjacent transporter substrate binding domain containing protein were increased by 4.2 and 3.7-fold in TMW 2.2256. An aldose 1-epimerase family protein was decreased by 2.1-fold and a SUF system *NifU* family Fe-S cluster assembly protein was increased by 2.1-fold.

Differences in the amino acid metabolism in response to LMRS

The arginine deiminase pathway (ADI) of TMW 2.2256 was increased by 4 to 5.8-fold. The 3-phosphoshikimate 1-carboxyvinyltransferase (*aroA*) was decreased by 2-fold in TMW 2.2256. An alanine dehydrogenase *alaD* was increased by 2.1-fold in TMW 2.2256.

An ABC amino acid transporter permease subunit was increased by 2.1-fold in TMW 2.2256. While another ABC amino acid permease subunit and ATP-binding protein was decreased 3.1 to 3.4-fold and 2.9 to 3.2-fold in TMW 2.2256.

Changes in the nucleotide metabolism and enzymes associated with osmotolerance are strain specific

The cluster responsible for the pyrimidine synthesis consisting of *pyrR* regulator, the uracil permease *uraA*, the small carbamoyltransferase subunit *carA* and the adjacent large carbamoyltransferase subunit *carB* were

decreased by 2.1 to 2.6-fold in TMW 2.2256. The anaerobic ribonucleotide-triphosphate reductase activating protein nrdG is increased by 2.2-fold in TMW 2.2256. While in TMW 2.2254 the only CDS associated with nucleotide metabolism is the DNA-directed RNA polymerase beta, which is decreased by 2.2-fold.

Osmostress proteins like the betaine-aldehyde dehydrogenase (*gbsA*) and the choline dehydrogenase (*gbsB*) were decreased by 3.9 and 4.3-fold in TMW 2.2254. The iron transporter *fetB* and a zinc ABC transporter substrate binding unit were decreased by 2 and 2.1-fold in TMW 2.2254.

Discussion

In this study we characterized the growth behavior in a new lupine MRS medium (LMRS) and compared the transcriptomic profile of the two *T. halophilus* strains TMW 2.2254 and TMW 2.2256 to this medium supplied with galactose as the major carbon source. These results should further characterize these strains and reveal strain specific differences that might be beneficial for the growth within the lupine moromi fermentation.

To determine the influence of the major carbon source added to the medium, the growth of TMW 2.2254 and TMW 2.2256 was analyzed when D-galactose, D-glucose or no additional carbon source was added (Fig. 1A, B). Interestingly, both strains grew best with D-galactose. Furthermore, it was also noted that the nutrient concentration in the medium was sufficient to support the growth without the addition of a major carbon source (Fig. 1A). However, it was also notable that the pH values during the cultivation were similar for both strains when no additional carbon source were added, with a small drop at 22 h and then an increase almost back to the start value. The pH values of the cultivations when glucose was added dropped the steepest for both strains. When galactose was added to the medium, the development of the pH values was comparable to the cultivation with D-glucose in TMW 2.2254 although the drop was not as steep and the final pH was higher (Fig. 1B). TMW 2.2256 cultivated with D-galactose the pH values increased after 22 H which could be due to the functional ADI pathway that could help alkalize the cultivation medium. Supporting this hypothesis is the fact that TMW 2.2256 does have a higher pH after 40h under all conditions compared to TMW 2.2254 (Fig. 1B).

As the cultivation media was supplied with 2% D-galactose as main carbohydrate-source, it was expected that this leads to an induction of the pathways responsible for the degradation of D-galactose. The Leloir pathway (galKETR) and the tagatose-6-p pathway (lacDCBAR) were highly increased in both strains, but a second

version of the tagatose-6-P with an additional phosphatase (lacDCBAR2) was also highly increased in both strains (Table 1) (Fig. 2). As the CDSs from this second version (lacDCBAR2) have a 67 to 90% sequence identity to the first version (lacDCBAR), it can be hypothesized that this cluster may have resulted from a gene duplication event (Fig. 3). As this duplicated operon lacDCBAR2 is active, it could increase the gene doses of the transporters and galactose degrading enzymes and thereby generating a higher overall galactose intake into the cell and explain the higher growth when D-galactose is supplied (Fig. 1A). Therefore, it can be assumed that T. halophilus as species in general, is adapted towards a galactose rich environment where multiple copies of galactose degrading enzymes are beneficial. This hypothesis is further strengthened by the fact that several strains from multiple origins have duplicates of this operon, the strain DSM 20338 even contains three copies of this operon [21]. The effect of the additional HAD phosphatase in T. halophilus is yet unknown, but it is known that a phosphatase activity enables the use of GAL6P via the Leloir pathway in Lactococcus lactis [23, 24]. Therefore, we hypothesis that a similar effect might be present here, although further evidence is needed to completely prove this point. Even more interesting is that TMW 2.2256 when has a higher increase in the abundance of the CDSs of the *lacDCBAR* and of the lacDCBAR2, while the Leloir pathway is not as highly increased compared to the fold-changes in TMW 2.2254 (Table 1). This indicates for a preference to utilize the galactose via tagatose-6-p pathway in TMW 2.2256, as the same effect cannot be seen in TMW 2.2254.

The strain TMW 2.2254 has three strains specific clus-(HV360 06680-HV360 06705), (HV360 09995-HV360 10015) and (HV360_11195-HV360_11230) consisting of PTS-IIABCD and at least one SIS domain containing protein (Table 1). All of these cluster are at least 2-fold increased. Using the conserved domain search from NCBI, we could assign putative substrates, glucosamine or fructolysine to the genes, but their function in T. halophilus grown in LMRS is not clear. However, only two operons are transcribed in a polycistronic manner. The genes HV360_06680 - HV360_06705 are not transcribed polycistronic and therefore can not be considered as an operon.

The operon responsible for the metabolism of cellobiose highly increased in TMW 2.2256 in contrast to TMW 2.2254 (Table 1). A potential explanation could be the fact that the PTS-IIC subunit (HV360_08980) has a premature stop codon and thereby might be not fully functional, rendering this operon inactive. This cellobiose operon might be induced due the residual cellobiose that might be present in the peptone, as the manufacturer stated that 17% residual carbohydrates are present

Table 1 Annotated DEGs from TMW 2.2254 and TMW 2.2256. Annotation of the orfs was done using the NCBI and RAST annotation, but only the NCBI is shown in column two. Substrates and putative substrates of respective genes are listed in column one. The columns three and four contain the respective locus tag in the strain, missing of the ORF is represented by an X. Column four and five show the Log2 fold change of the respective ORF, missing ORFs are represented by X. ORFs that are present in the strain but not count as DEGs are indicated as small italics numbers in white cells. FS indicates and frameshift and a resulting premature stop codon. NV indicates that the comparison of the two conditions yielded no value. Cutoffs: Log2 Foldchange of ≥2 or ≤ −2, p-value ≤0.05 and FDR ≤ 0.01

PTS su	ose-bisphosphate aldolase ugar transporter subunit IIIA ransporter subunit IIIA ransporter subunit IIIA sugar transporter subunit IIA sugar transporter subunit	HY360 06695 HY360 06700 HY360 06705 HY360 1195 HY360 11200 HY360 11205 HY360 11215 HY360 11215 HY360 11225 HY360 11225 HY360 11220 X	HXW74 10490 HXW74 10495 HXW74 10495 HXW74 10505 HXW74 10505 HXW74 10505 HXW74 06055 HXW74 06055 HXW74 06055 HXW74 11235 HXW74 11235 HXW74 11235 HXW74 11235 HXW74 11236 HXW74 11236 HXW74 11236 HXW74 11236 HXW74 11236 HXW74 11255 HXW74 11265 HXW74 11265 HXW74 11265 HXW74 11265 HXW74 11265 HXW74 10735	4.1.2.40 2.7.1.101 2.7.1.101 5.3.1.26 5.3.1.26 5.3.1.26 2.7.1.144 4.1.2.40 4.1.2.40 4.1.2.40 5.3.1.26 5.3.1.	5.95 (4.55) (4.5	5.88 (6.13 5.80 5.8
PTS trans-	ransporter subunit EliC supar transporter subunit IIIA tuse 6-phosphate isomerase subunit LacA tuse 6-phosphate isomerase subunit LacB tuse 6-phosphate isomerase subunit LacB tuse 6-phosphate phosphate turidylytransferase galT glucose 6-pinding transcriptional regulator glucose 1-phosphate uridylytransferase galT tukinase galK - C-acetyltransferase - galactosidase actose/cellobiose transporter subunit IIA spho-beta-galactosidase subunit 1-phosphate uridylytransferase lacticse/cellobiose transporter subunit IIC tuse open repressor subunit IIB betical protein cellobiose transporter subunit IIC tuse open repressor phosphotansferase subidity fundamental subunit IIA hetical protein cellobiose transporter subunit IIB supar transporter subunit IIB supar transporter subunit IIB sugar transporter subunit IIA sugar t	HY360 03995 HY360 03995 HY360 07360 07360 HY360 07365 HY360 07365 HY360 07377 HY360 07377 HY360 07377 HY360 07377 HY360 07378 HY360 011240 HY360 11240 HY360 11240 HY360 11240 HY360 11250 HY360 11250 HY360 11260 HY360 01265 HY360 04870 HY360 04870 HY360 04890 HY360 04890 HY360 04890 HY360 09000 HY360 09100 HY360 01100 HY360 01100 HY360 11200 HY360 11200 HY360 01120	HXW74 10505 HXW74 08040 HXW74 08040 HXW74 08045 HXW74 08050 HXW74 08055 HXW74 08055 HXW74 11230 HXW74 11240 HXW74 11250 HXW74 11250 HXW74 11260 HXW74 107385 HXW74 07385 HXW74 07385 HXW74 07385 HXW74 07390 HXW74 07390 HXW74 07391 HXW74 07395 HXW74 07395 HXW74 0735 HXW74 0736	5.3.1.26 5.3.1.26 5.3.1.26 2.7.1.144 4.1.2.40 4.1.2.40 4.1.2.40 5.3.1.26 5.3.1.26 5.3.1.26 2.7.7.12 5.1.3.2 2.7.1.20 5.3.1.26 2.7.1.20 5.3.1.26 2.7.1.20 5.3.1.26 3.2.1.22 2.7.1.20 5.3.1.26 2.7.1.20 5.3.1.26 5.3.1.	4.98 (2.204 4.022	5.80 0.55 6.90 0.55 6.90 0.55 6.90 0.55 6.90 0.55 6.90 0.55 6.20 0
PTS su galactose-6P Galactose-6P Galactose-6P Galactose-6P Galactose-6P Galactose-6P Galactose-6P Galactose-6P Galactose-6P FTS su gal	ugar transporter subunit III A tose-6-phosphate isomerase subunit LacA tose-6-phosphate isomerase subunit LacB tose-6-phosphate isomerase subunit LacB tose-6-phosphate isomerase subunit LacB sos-6-phosphate kinase lacC sos-6-phosphate isomerase subunit LacB sos-6-phosphate isomerase subunit LacB2 tose-6-phosphate subunit IIB ducose 4-spinerase GalE tokinase galK - O-acetyltransferase -galactosidase actose/cellobiose transporter subunit IIB selbobose transporter subuni	HY380 07369 HY380 07365 HY380 07365 HY380 07375 HY380 07380 HY380 11250 HY380 04875 HY380 06895 HY380 06869 HY380 06869 HY380 06690 HY380 11200 HY380 11200 HY380 11210 HY380 11210 HY380 11210 HY380 11210 HY380 08255	HXWY74 08040 HXWY74 08045 HXWY74 08055 HXWY74 08055 HXWY74 08055 HXWY74 08055 HXWY74 08055 HXWY74 11235 HXWY74 11235 HXWY74 11245 HXWY74 11255 HXWY74 11255 HXWY74 11255 HXWY74 11260 HXWY74 17260 HXWY74 07385 HXWY74 08065 HXWY74 07385 HXWY74 08070 HXWY74 08085	5.3.1.26 5.3.1.26 5.3.1.26 2.7.1.144 4.1.2.40 4.1.2.40 4.1.2.40 5.3.1.26 5.3.1.26 5.3.1.26 2.7.7.12 5.1.3.2 2.7.1.20 5.3.1.26 2.7.1.20 5.3.1.26 2.7.1.20 5.3.1.26 3.2.1.22 2.7.1.20 5.3.1.26 2.7.1.20 5.3.1.26 5.3.1.	2,04 3,62 2,79 3,62 4,28 3,62 4,28 2,36 4,28 2,67 2,70 3,58 2,67 3,42 2,67 3,42 2,67 3,42 2,67 3,42 2,67 3,42 2,67 3,42 2,67 3,42 2,67 3,42 2,67 3,49 2,67 3,49 2,67 3,49 2,67 3,49 2,67 3,69 3,69 3,69 3,69 3,69 3,69 3,69 3,69	0.59 0.59 0.59 0.50 0.50 0.50 0.50 0.50
Galactose 6P FTS 64 Galactose	tose-e-phosphate isomerase subunit LacA tose-e-phosphate isomerase subunit LacB sose-e-phosphate isomerase subunit LacB sose-e-phosphate isomerase subunit LacB sose-e-phosphate idolose LacD sose-bisphosphate addolase LacD sose-bisphosphate addolase LacD sose-bisphosphate addolase LacD sose-bisphosphate isomerase subunit LacB tose-e-phosphate isomerase subunit LacB tose-e-phosphate isomerase subunit LacB tose-e-phosphate isomerase subunit LacB sugar transporter subunit IIIA ructose transporter subunit IIIB plactiot transporter subunit IIII subunit IIIIB plactiot subunit IIII subunit IIIIB plactiot subunit IIIIB plactiot subunit IIIB plaction subunit IIB plaction s	HV350 07365 HV350 07377	HXWY4 08045 HXWY4 08050 HXWY4 08050 HXWY4 08055 HXWY4 08055 HXWY4 08066 HXWY4 17230 HXWY4 17235 HXWY4 17255 HXWY4 17255 HXWY4 17255 HXWY4 17265 HXWY4 07395 HXWY4 0735 HXWY4 07395	5.3.1.26 2.7.1.144 4.1.2.40 4.1.2.40 2.7.1.144 5.3.1.26 5.3.1.26 2.7.7.12 5.1.3.2 2.7.1.20 2.7.1.20 2.7.1.20 2.7.1.20 3.2.1.20 2.7.1.20 3.2.1.20 2.7.1.20 3.2.20 3.20 3	2.79 (2.6) (3.67 5.29 5.29 5.59 5.59 5.59 5.59 5.59 5.59
Galactose-6P Inagatos Inaga	ose-6-phosphate kinase lacC ose-bisphosphate aidolase LacD2 ose-bisphosphate aidolase LacD2 ose-bisphosphate aidolase LacD2 ose-bisphosphate aidolase LacD2 ose-bisphosphate lidolase LacD2 ase-bisphosphate lidolase LacD2 ase-bisphosphate lidolase LacD2 ase-bisphosphate lidolase LacD2 lidose-bisphosphate isomerase subunit LacD2 ose-bisphosphate isomerase subunit LacD2 ose-bisphosphate lidolase lido	HY360 07375	HXWY4 08055 HXW74 08060 HXW74 11230 HXW74 11235 HXW74 11240 HXW74 11245 HXW74 11245 HXW74 11245 HXW74 11265 HXW74 11265 HXW74 11265 HXW74 17265 HXW74 07395 HXW74 0735	2.7.1.144 4.1.2.40 4.1.2.40 4.1.2.40 5.3.1.26 5.	4.02 4.28 3.27 4.28 4.28 4.28 4.28 4.28 4.28 4.28 4.28	5.29 5.30 4.25 5.30 4.25 5.30 4.25 5.30 4.25 6.25 6.81 6.81 6.81 6.81 6.82 6.81 6.82 6.83 6.83 6.83 6.83 6.83 6.83 6.83 6.83
Galactose-6P Inagatios I	ose-bisphosphate aldolase LacD ose-bisphosphate aldolase LacD2 ose-6-phosphate kinase LacD2 ose-6-phosphate kinase LacD2 ose-6-phosphate isomerase subunit LacB2 tose-6-phosphate isomerase subunit LacB2 tose-fromerase GalE tokinase galK tokinase galK to-acetyliransferase -galactosidase actose/cellobiose transporter subunit IIIA subunit LacB2 tose-fromerase CalB2 tokinase galK to-acetyliransferase -galactosidase actose/cellobiose transporter subunit IIIA subunit LacB2 tose-fromerase calB2 tokinase galK toki	HY360 07389 HY360 08385 HY360 09305 HY360 11205 HY360 11205 HY360 11205 HY360 08255	HXW74 08060 HXW74 11230 HXW74 11230 HXW74 11236 HXW74 11240 HXW74 11245 HXW74 11245 HXW74 11265 HXW74 11265 HXW74 11265 HXW74 11266 HXW74 07395 HXW74 07396	4.1.2.40 4.1.2.40 2.7.1.144 5.3.1.26 5.3.1.26 5.3.1.26 5.3.1.26 5.3.1.26 2.7.1.20 2.7.1.20 2.7.1.20 2.7.1.20 2.7.1.20 2.7.1.20 2.7.1.20 2.7.1.20 2.7.1.20 3.2.20 3.20 3	4.28 3.32 3.47 3.55 3.49 3.49 3.49 3.49 3.49 3.49 3.49 3.49	6.29 5.39 5.30 5.30 5.30 5.30 5.35 5.35 5.35 5.35
Inagatios Inag	ose-bisphosphate aldolase LacD2 ose-biposphate kinase LacD2 annily phosphatase pgbmB cose-biposphate isomerase subunit LacB2 tose-biposphate isomerase subunit lacB2 luciol transporter subunit liB amily DNA-binding transcriptional regulator glucose-bexose-i-phosphate uridylytransferase galT colored galk	HY350 11245 HY350 11245 HY350 11245 HY350 11255 HY350 11255 HY350 11255 HY350 11255 HY350 11255 HY350 11255 HY350 11270 HY350 11270 HY350 04889 HY350 04889 HY350 04889 HY350 04889 HY350 04889 HY350 04889 HY350 04890 HY350 04900 HY350 04900 HY350 04900 HY350 04900 HY350 06990 HY350 06445 HY350 06699 HY350 06700 HY350 1120 HY350 11210 HY350 11220 X X X X X X X X X X X X X X X X X X	HXW74 11235 HXW74 11246 HXW74 11246 HXW74 11250 HXW74 11250 HXW74 11250 HXW74 11250 HXW74 11250 HXW74 11260 HXW74 17260 HXW74 07385 HXW74 08075 HXW74 10735 HXW74 10735 HXW74 10735 HXW74 10735 HXW74 08226 HXW74 08226 HXW74 08226 HXW74 08226 HXW74 08226 HXW74 08227 HXW74 08227 HXW74 08227 HXW74 08228 HXW74 0828	2.7.1.144 5.3.1.26 5.3.1.26 5.3.1.26 5.3.1.26 2.7.1.2 2.7.1.2 2.7.1.207 3.2.1.25 2.7.1.207 3.2.1.85 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.201 5.3.1.8	3.47 2.26 2.27 2.27 2.27 2.27 2.27 2.27 2.2	5.30 5.30 5.30 5.37 5.31 2.39 3.40 2.97 3.40 2.97 3.40 2.97 3.40 2.97 2.35 X X X X X X X X X X X X X
HAD far gallacto ga	iranily phosphatase pgbmB tose-6-phosphate isomerase subunit LacB2 tose-6-phosphate subunit IIA ructose transporter subunit IIA analy DNA-briding transcriptional regulator glucose-4-pinerase GaIE tokinase gaIK -7-cacetyltransferase -palactosidase actose/cellobiose transporter subunit IIA spho-beta-galactosidase -palactosidase -palactos	HY360 11245	HXW74 11240 HXW74 11245 HXW74 11256 HXW74 11256 HXW74 11255 HXW74 11256 HXW74 11260 HXW74 17266 HXW74 07385 HXW74 07385 HXW74 07385 HXW74 07386 HXW74 07400 HXW74 07400 HXW74 07400 HXW74 07400 HXW74 08070 HXW74 10725 HXW74 10725 HXW74 10730 HXW74 10740	5.3.1.26 5.3.1.26 5.3.1.26 2.77.12 5.1.3.2 2.71.20 3.2.1.22 2.71.207 3.2.1.86 2.71.205 2.71.205 5.4.2.8 2.4.1.216 2.71.205 5.3.1.8	3.56 2.299 2.267 2.70 3.42 3.42 3.45 2.45 2.26 2.270 3.42 3.45 2.45 2.45 2.45 2.45 2.45 2.45 2.45 2	4.25 3.57 3.31 2.39 2.79 2.79 2.87 2.87 2.87 2.87 2.87 2.87 2.87 2.87
Galactose 6P PTS fur	tose-6-phosphate isomerase subunit Lac82 tose-6-phosphate isomerase subunit Lac82 ugar transporter subunit lilA ructose transporter subunit lilB julctiot transporter subunit lilB julction lilb lilb lilb lilb lilb lilb lilb lil	HY360 11250 HY360 11255 HY360 11255 HY360 11265 HY360 11265 HY360 11265 HY360 11265 HY360 11265 HY360 11270 HY360 11270 HY360 04875 HY360 04895 HY360 04895 HY360 04895 HY360 04895 HY360 04895 HY360 08995 HY360 08999 HY360 08999 HY360 08999 HY360 08995 HY360 06445 HY360 06455 HY360 06455 HY360 06455 HY360 06455 HY360 06455 HY360 06695 HY360 06695 HY360 06705 HY360 11200 HY360 01220 HY360 08255	HXW74 11245 HXW74 11250 HXW74 11250 HXW74 11250 HXW74 11260 HXW74 11260 HXW74 11260 HXW74 11265 HXW74 07385 HXW74 07385 HXW74 07395	5.3.1.26 2.77.12 5.1.3.2 2.7.1.6 3.2.1.22 2.7.1.20 3.2.1.85 2.7.1.205 2.7.1.205 2.7.1.205 5.4.2.6 2.4.1.216 3.3.1.8 1.3.1.805 1.3	2.99 (2.57) (2.57) (2.57) (2.57) (2.57) (2.50) (2.5	3.57 3.31 2.39 3.40 3.40 3.14 2.97 2.67 2.67 2.87 X X X 8.09 6.81 6.81 6.82 5.35 8.35 8.35 8.35 8.35 8.35 8.35 8.35
PTS quarter PTS run ru	ugar transporter subunit IIIA ructosa transporter subunit IIIB pluctiot transporter subunit IIIB pluctiot transporter subunit IIIB pluctiot transporter subunit IIIB plucosa despirerase GalE plucosa de GalE plucosa de GalE plucosa de GalE plucosa	HY350 11260 HY350 1127 HY350 1127 HY350 04875 HY350 04875 HY350 04875 HY350 04875 HY350 04876 HY350 04877 HY350 08999 HY350 08999 HY350 08999 HY350 08989 HY350 06445 HY350 06455 HY350 06455 HY350 06595 HY350 06595 HY350 06705 HY350 11200 HY350 11200 HY350 11201 HY350 11201 HY350 11201 HY350 11201 HY350 11201 HY350 11201	HXW74 11255 HXW74 11260 HXW74 11266 HXW74 11265 HXW74 07385 HXW74 07385 HXW74 07395 HXW74 10739 HXW74 10739 HXW74 10739 HXW74 10739 HXW74 0739	2.77.12 5.1.3.2 2.71.1207 3.2.1.82 2.71.207 3.2.1.85 2.71.207 3.2.1.85 2.71.205 2.71.205 2.71.205 2.71.201 5.3.1.8	2.57 2.270 2.80 3.42 3.73 3.42 3.73 3.49 3.46 2.12 X X X X X X X X X X X X X X X X X X X	2.39 3.40 2.79 3.40 2.97 3.66 3.14 2.97 2.67 2.87 X X 8.09 4.74 6.25 6.81 6.30 6.81 6.25 6.25 8.07 2.58 X X X X X X X X X X X X X X X X X X X
PIS for PIS of	ructose transporter subunit IIB Jucisel transporter subunit IIB Jucisel transporter subunit III Jucisel transporter subunit III Jucisel despinerase GallE C-seetyltransferase -galactosidase -galact	HV350 11265 HV350 04875 HV350 04875 HV350 04875 HV350 04875 HV350 04880 HV350 04880 HV350 04880 HV350 04880 HV350 04880 HV350 04880 HV350 04890 HV350 04890 HV350 04890 HV350 04890 HV350 04890 HV350 09900 HV350 09900 HV350 08995 HV350 08995 HV350 08995 HV350 08995 HV350 06450 HV350 06450 HV350 06450 HV350 06590 HV350 06680 HV350 06680 HV350 06690 HV350 01120 HV350 11200 HV350 11200 HV350 11210 HV350 11210 HV350 11210 HV350 011210 HV350 011210 HV350 011220 HV350 08255	HXW74 11260 HXW74 11265 HXW74 07385 HXW74 07385 HXW74 07390 HXW74 07395 HXW74 07400 X X X X X X X X X X X X X X X X X X	5.1.3.2 2.7.1.6 3.2.1.22 2.7.1.207 3.2.1.85 2.7.1.207 3.2.1.85 2.7.1.207 2.7.1.205 2.7.1.205 2.7.1.205 5.4.2.6 2.7.1.201 5.3.1.8	2.70 2.80 3.42 3.73 3.49 3.46 2.47 2.12 X X X X NV NV NV NV SS 3.06 3.06 3.05 2.81 2.61 3.00 3.06 3.06 3.06 3.06 3.06 3.06 3.06	2.79 2.79 3.40 3.14 3.14 3.14 3.14 3.14 3.14 3.14 3.16 3.16 3.16 3.16 3.16 3.16 3.16 3.16
Galactose-6P PTS qi Laci far UPP-qi Galactose Galactose Galactose Galactose Galactose Galactose Galactose Galactose PTS term FTS term Galactose PTS term FTS term Galactose Galacto	plucitot transporter subunit IIA amily DNA-binding transcriptional regulator glucose-hexose-1-phosphate uridylytiransferase galT glucose-hexose-1-phosphate uridylytiransferase galT tokinase galK Co-acetyliransferase galcucase-galcucase galcucase-galcucase galcucase-galcucase galcucase-galcucase galcucase-	HY360 04875 HY360 04876 HY360 04880 HY360 04880 HY360 04880 HY360 04880 HY360 04890 HY360 04890 HY360 04890 HY360 04890 HY360 04890 HY360 04890 HY360 09900 HY360 08995 HY360 08995 HY360 08995 HY360 08985 HY360 08980 HY360 06990 HY360 06990 HY360 06990 HY360 06990 HY360 06690 HY360 0700 HY360 11200 HY360 11200 HY360 11210 HY360 11220 HY360 08255 HY360 08255 HY360 08255	HXW74_07385 HXW74_07385 HXW74_07385 HXW74_07395	5.1.3.2 2.7.1.6 3.2.1.22 2.7.1.207 3.2.1.85 2.7.1.207 3.2.1.85 2.7.1.207 2.7.1.205 2.7.1.205 2.7.1.205 5.4.2.6 2.7.1.201 5.3.1.8	3.42 3.73 3.49 3.46 2.47 2.47 2.12 X X X X V V V V V V V V V V V V V V V	3.14 2.97 2.67 2.35 X X X 8.09 6.81 6.30 4.74 6.25 6.13 6.60 6.25 3.59 2.34 7.06 X X X X X X X X X X X X X X X X X X X
UDP-git	glucose-hexose-1-phosphate uridylyttransferase galT glucose-hexose-1-phosphate uridylyttransferase galE tokinase galK - O-acetyttransferase - galactosidase - canciose-diolobiose transporter subunit III - spho-beta-galactosidase - galactosidase - galact	HY360 04898 HY360 04898 HY360 04898 HY360 04898 HY360 04890 HY360 04870 HY360 04870 HY360 04870 HY360 04870 HY360 04870 HY360 08990 HY360 08990 HY360 08990 HY360 08990 HY360 08990 HY360 08980 HY360 06449 HY360 06454 HY360 06456 HY360 06456 HY360 06456 HY360 06456 HY360 06456 HY360 06456 HY360 06990 HY360 06450 HY360 06450 HY360 06450 HY360 06990 HY360 06990 HY360 06990 HY360 07900	HXW74_07390 HXW74_07395 HXW74_07395 HXW74_07395 HXW74_07400 X X X X X X X X X X X X X X X X X X	5.1.3.2 2.7.1.6 3.2.1.22 2.7.1.207 3.2.1.85 2.7.1.207 3.2.1.85 2.7.1.207 2.7.1.205 2.7.1.205 2.7.1.205 5.4.2.6 2.7.1.201 5.3.1.8	3.73 3.49 3.46 2.47 2.12 X X X X X X X X NV NV NV NV NV L S S S S S S S S S S S S S S S S S S	2.97 2.67 2.35 X 8.09 6.81 6.30 4.74 6.25 6.13 6.60 6.25 3.59 3.57 2.58 X X X X X X X X X X X X X X X X X X X
Galactose UDP_di	glucose 4-opimerase GalE tokinase galK C-D-acetyltransferase g-galactosidase actose/cellobiose transporter subunit IIIA spsho-beta-galactosidase ransporter subunit IIIG geliobiose transporter subunit IIIB eliobiose transporter subunit IIIB eliobiose transporter subunit III helicolar porter subunit IIIC sose operon repressor phosphoglucomutase side hydrolase family 65 protein system trehalose-specific EIIBC component ose-6-phosphoglucomutase side hydrolase family 65 protein system trehalose-specific EIIBC component ose-6-phosphot somerase, class I omain-containing protein system manoses/ruclose/sorbose family transporter subunit sugar transporter subunit IIIA sugar transporter subunit IIIB sugar transporter subunit IIB sugar transporter subunit II	HY360 04895	HXW74 07395 HXW74 07400 X X X X X X X X X X X X X X X X X X	5.1.3.2 2.7.1.6 3.2.1.22 2.7.1.207 3.2.1.85 2.7.1.207 3.2.1.85 2.7.1.207 2.7.1.205 2.7.1.205 2.7.1.205 5.4.2.6 2.7.1.201 5.3.1.8	3.49 3.47 2.47 2.47 X X X X X X X X X X X X X X X X X X X	2.67 2.35 X X 8.09 6.81 6.30 4.74 6.25 6.13 6.60 6.25 3.59 3.59 3.57 2.58 X X X X X X X X X X X X X X X X X X X
Galactose Gala	tokinase galK - O-acetyltransferase - galactosidase - galacto	HV380 04870	X XY HXW74 08065 HXW74 08065 HXW74 08070 HXW74 08070 HXW74 08070 HXW74 08070 HXW74 10735 HXW74 10735 HXW74 10735 HXW74 10735 HXW74 10735 HXW74 02320 HXW74 02320 HXW74 02320 HXW74 02320 X X X X X X X X X X X X X	3.2.1.22 2.7.1.207 3.2.1.85 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.201 5.3.1.8	2.47 2.12 X X X X X X X NV NV NV NV SS 3.06 3.06 3.06 3.06 3.18 2.61 2.71 2.71 2.71 2.90 2.61 3.98 3.98 3.98 3.66 2.81 X X	X X X 8.09 6.81 6.30 4.74 6.25 6.60 6.25 3.59 2.34 7.06 X X X X X X X X X X X X X X X X X X X
Elibiose/Raffinose Initial Ini	cyalactosidase actose/cellobiose transporter subunit IIA spho-beta-galactosidase spho-beta-galactosida	HY360_04860	X HXW74_08065 HXW74_08075 HXW74_08075 HXW74_08075 HXW74_10725 HXW74_10725 HXW74_10735 HXW74_10735 HXW74_10735 HXW74_10735 HXW74_10735 HXW74_03230 HXW74_03225 HXW74_03225 HXW74_08225 HXW74_08890 X X X X X X X X X X X X X X X X X X X	2.7.1.207 3.2.1.85 2.7.1.207 3.2.1.86 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.205 5.4.2.6 2.4.1.216 2.7.1.201 5.3.1.8	2.12 X X X X X X X V -0.11 NV NV NV NV NV S S 0.66 3.05 2.81 2.71 2.71 2.71 2.71 2.71 2.71 2.71 2.7	X 8.09 6.81 6.30 4.74 6.25 6.13 6.60 6.25 3.59 3.57 2.58 X X X X X X X X X X X X X X X X X X X
PTS lac	actose/cellobiose transporter aubunit IIA spsho-beta-galectosidase ransporter subunit EliC ransporter subunit EliC spsho-beta-galectosidase (2) seliobiose transporter subunit IIB seliobiose transporter subunit IIB seliobiose transporter subunit IIA hericia protein seliobiose transporter subunit IIC sugar transporter subunit IIC sugar transporter subunit III sugar transporter subunit IIII sugar transporter subunit III sugar transporter subunit IIII sugar transporter subunit IIII sugar transporter subunit I	X X X X X X X X X X X X X X X X X X X	HXW74_08070 HXW74_08075 HXW74_10725 HXW74_10725 HXW74_10726 HXW74_10730 HXW74_10730 HXW74_10730 HXW74_10730 HXW74_10730 HXW74_10730 HXW74_10730 HXW74_10730 HXW74_03225 HXW74_03225 HXW74_08890 XX	2.7.1.207 3.2.1.85 2.7.1.207 3.2.1.86 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.205 5.4.2.6 2.4.1.216 2.7.1.201 5.3.1.8	X X X X NV NV NV NV FS 3.06 3.05 2.81 2.02 3.18 2.23 3.18 2.26 2.71 2.71 2.90 2.61 3.95 2.48 2.71 2.71 2.91 2.91 2.91 3.98	8.09 6.81 6.30 4.74 6.25 6.13 6.60 6.25 3.59 3.57 2.58 2.34 7.06 X X X X X X X X X X X X X X X X X X X
Laclose	ransporter subunit EIIC spiho-beta-glucosidase (2) seliobiose transporter subunit IIB seliobiose transporter subunit IIB seliobiose transporter subunit III seliobiose transporter subunit IIIC see operon repressor horses fransporter subunit IIC see operon repressor horses fransporter subunit IIC see operon repressor side in troises fransporter subunit IIC see operon repressor side in troises fransporter subunit IIC see operon repressor main-containing protein system manoses-fransporter subunit III sugar transporter subunit III sugar transporter subunit III sugar transporter subunit III see operon subunit III see operon subunit III see operon subunit III sugar transporter subunit IIC sugar transporter subunit III sugar transporter subunit IIC sugar	HV380 08999 HV380 08999 HV380 08989 HV380 08989 HV380 08488 HV380 08448 HV380 08448 HV380 08446 HV380 08446 HV380 08469 HV380 08698 HV380 06709 HV380 06709 HV380 11200 HV380 11200 HV380 11201 HV380 08255	HXW74 08075 HXW74 10725 HXW74 10735 HXW74 10736 HXW74 10735 HXW74 10735 HXW74 10740 HXW74 02320 HXW74 02320 HXW74 03225 HXW74 03225 HXW74 03225 X X X X X X X X X X X X X X X X X X	2.7.1.207 3.2.1.86 2.7.1.205 2.7.1.205 2.7.1.205 2.7.1.205 5.4.2.6 2.4.1.216 2.7.1.201 5.3.1.8	X -0.ff NV NV NV NV NV NV S-5 3.06 3.05 3.05 2.81 2.02 3.18 2.51 2.77 2.90 2.91 3.98 3.52 2.70 2.90 3.98 3.52 2.70 2.92 2.99 3.46 2.81 X X X	6.30 4.774 6.25 6.13 6.60 6.25 3.59 3.57 2.58 2.34 X X X X X X X X X X X X X X X X X X X
6-phose	spho-beta-glucosidase (2) espho-beta-glucosidase (2) eslebolisos transporter subunit IIB eslebolisos transporter subunit IIB hetical protein hetical protein eslebolisos transporter subunit III eslebolisos transporter subunit III eslebolisos transporter subunit III eslebolisos transporter subunit III side hydrolises family 55 protein ystem trehalose-specific EIIBC component oses-6-posphatis somerase, class I omain-containing protein omain-containing protein omain-containing protein omain-containing protein omain-containing protein omain-containing protein ystem manoses-fastion III ystem transporter subunit III sayar transporter subunit III sayar transporter subunit III ystem transporter subunit III ystem transporter subunit III ystem transporter subunit III sugar transporter subunit IIII sugar transporter subunit III sugar transporter	HV380 08999 HV380 08999 HV380 08989 HV380 08989 HV380 08488 HV380 08448 HV380 08448 HV380 08446 HV380 08446 HV380 08469 HV380 08698 HV380 06709 HV380 06709 HV380 11200 HV380 11200 HV380 11201 HV380 08255	HXW74 10725 HXW74 10730 HXW74 10730 HXW74 10735 HXW74 10740 HXW74 10745 HXW74 10745 HXW74 03225 HXW74 03225 HXW74 03225 HXW74 03225 X X X X X X X X X X X X X X X X X X	3.2.1.86 2.7.1.205 2.7.1.205 2.7.1.205 5.4.2.6 5.4.2.6 5.4.2.6 5.4.2.6 5.4.1.216 2.7.1.201 5.3.1.6	NV NV NV NV FS 3.06 3.05 2.61 2.20 2.33 2.48 2.51 2.90 2.61 3.09 3.52 2.71 2.90 2.61 3.09 3.52 2.71 2.90 2.61 3.98 3.52 2.70 2.81 3.90 3.90 3.90 3.90 3.90 3.90 3.90 3.90	4.74 6.25 6.13 6.60 6.25 3.59 3.57 2.58 X X X X X X X X X X X X X X X X X X X
PTS ce P	cellobiose transporter subunit IIB cellobiose transporter subunit IIA hetical protein clesobjose transporter subunit IIIC lose operon repressor hopsporter subunit IIC lose operon repressor hopsporter subunit IIC lose operon repressor hopsporter subunit IIC lose operon repressor hopsporter lose from the subunit IIC lose operon repressor hopsporter lose from the subunit IIC lose operon repressor hopsporter lose from the subunit IIC lose -E-prophist losenerses, class I lomain-containing protein lose -E-prophist losenerses, class I lomain-containing protein lose -E-prophist losenerses lomain-containing protein lose -E-prophist losenerses lose -E-prophist losenerses lose -E-prophist losenerses lose -E-prophist lose lose -E-prophist lose lose -E-prophist lose lose -E-prophist lose -E	HY360_08990 HY360_08985 HY360_08985 HY360_08445 HY360_06445 HY360_06445 HY360_0645 HY360_0645 HY360_0645 HY360_06695 HY360_06695 HY360_06690 HY360_0700 HY360_0700	HXW74 10730 HXW74 10735 HXW74 10745 HXW74 10746 HXW74 10745 HXW74 02320 HXW74 03225 HXW74 03225 HXW74 03225 HXW74 03225 X X X X X X X X X X X X X X X X X X	2.7.1.205 2.7.1.205 5.4.26 2.4.1.216 2.7.1.201 5.3.1.8	NV NV NV FS 3.06 3.05 2.81 2.02 2.33 2.48 2.33 2.48 2.51 2.71 2.90 2.61 3.00 3.52 2.70 2.92 2.92 3.98 3.52 2.70 2.92 3.98	6.13 6.60 6.25 3.59 3.57 2.58 2.34 7.06 X X X X X X X X X X X X X X X X X X X
Cellobiose	infetical protein eleibolises transporter subunit IIC lose operon repressor hopsphoglucomuly 65 protein side hydrolase family 65 protein side hydrolase family 65 protein yesten trehalose-specific BiBC component ornalism	HY360 08985	HXW74 10740 HXW74 10745 HXW74 10745 HXW74 03230 HXW74 03225 HXW74 03225 HXW74 03225 HXW74 03226 HXW74 03215	2.7.1.205 5.4.2.6 2.4.1.216 2.7.1.201 5.3.1.8	NV FS 3.06 3.05 2.81 2.02 3.18 2.23 2.48 2.51 2.71 2.90 2.61 3.00 3.52 2.70 2.92 2.70 2.93 3.52 2.81 3.98 3.52 2.99 3.98 3.52 2.99 3.99 3.99 3.99 3.99 3.99 3.99 3.9	6.60 6.25 3.59 3.57 2.58 2.34 X X X X X X X X X X X X X X X X X X X
Cellobiose	selebiose transporter subunit IIC lose operan repressor phosphoglucomutase side hydrolase family 55 protein side hydrolase family side mannose fructose/sorbose family transporter subunit IIQ side family 55 protein side hydrolase family 55 protein side side side side side side side side	HY360 08980 HY360 08445 HY360 08445 HY360 08445 HY360 08445 HY360 08445 HY360 08456 HY360 08685 HY360 06686 HY360 06695 HY360 06700 HY360 0700 HY360 11200 HY360 11201 HY360 11205 HY360 11205 HY360 11205 HY360 11205 HY360 11205 HY360 11205 HY360 11225 HY360 11225 HY360 11225 HY360 11225 HY360 08255 HY360 08255	HXW74 10745 HXW74 03230 HXW74 03230 HXW74 03225 HXW74 03226 HXW74 03215 HXW74 03215 HXW74 03890 X X X X X X X X X X X X X X X X X X X	5.4.2.6 2.4.1.216 2.7.1.201 5.3.1.8	FS 3.06 3.05 3.05 2.81 2.02 2.81 2.02 3.18 2.33 2.48 2.51 2.71 2.90 2.61 3.00 3.98 3.52 2.70 2.92 2.99 3.46 2.81 X	6.25 3.59 3.57 2.58 2.34 1.06 X X X X X X X X X X X X X X X X X X X
Irehalose	lose operon repressor hopesport of the property of the propert	HY360 06435 HY360 06440 HY360 06440 HY360 06440 HY360 06445 HY360 06450 HY360 09365 HY360 09365 HY360 06680 HY360 06680 HY360 06695 HY360 06695 HY360 06705 HY360 11200 HY360 11200 HY360 11200 HY360 11210 HY360 11210 HY360 11210 HY360 11220 HY360 11220 HY360 11220 HY360 11220 HY360 11230 X X X X X X X X X X X X X X X X X X	HXW74_03230 HXW74_03225 HXW74_03225 HXW74_03225 HXW74_032215 HXW74_06890 X X X X X X X X X X X X X X X X X X X	5.4.2.6 2.4.1.216 2.7.1.201 5.3.1.8	3.06 3.05 2.81 2.02 3.18 2.23 2.48 2.51 2.71 2.90 2.61 3.90 3.98 3.52 2.70 2.92 2.99 3.46 2.81 X	3.59 3.57 2.58 2.34 1.06 X X X X X X X X X X X X X X X X X X X
Trehalose	side hydrolase family 65 protein ysytem trehalose-specific EliBC component lose-6-phosphate isomerase, class I omain-containing protein omain-containing protein omain-containing protein upger transporter subunit IIC uugar transporter subunit IIC uugar transporter subunit III nannose transporter subunit III nannose transporter subunit III nannose transporter subunit III nannose transporter subunit III uugar transporter subunit III uugar transporter subunit III nugar transporter nuturit III nuturit II	HV360 06445 HV360 06450 HV360 09365 HV360 09365 HV360 09366 HV360 06669 HV360 06669 HV360 06669 HV360 06669 HV360 06669 HV360 06669 HV360 06705 HV360 06705 HV360 07105 HV360 11200 HV360 01255 HV360 08255	HXW74 03220 HXW74 082015 HXW74 06890 X X X X X X X X X X X X X X X X X X X	2.4.1.216 2.7.1.201 5.3.1.8	2.81 2.02 3.18 2.33 2.48 2.51 2.71 2.90 3.98 3.98 3.52 2.70 2.92 2.99 3.46 2.81 X	2.58 2.34 7.06 X X X X X X X X X X X X X X X X X X X
Trehalose	ystem trehalose-specific EIIBC component oses-E-phosphate isomerase, class is omain-containing protein omain-containing protein ystem mannose/fructose/sorbose family transporter subunit to upper transporter subunit IIC upper transporter subunit IIA III III III III III III III III III	HV380 08455 HV380 08255 HV380 08255 HV380 08255 HV380 08255 HV380 08698 HV380 08698 HV380 08699 HV380 08700 HV380 08700 HV380 08700 HV380 01200 HV380 011200 HV380 011200 HV380 01120 HV380 011225 HV380 011225 HV380 011225 HV380 011230 HV380 011225 HV380 011230 HV380 0112	HXW74_02215 HXW74_06890 X X X X X X X X X X X X X X X X X X X	2.7.1.201 5.3.1.8	2.02 3.18 2.33 2.48 2.51 2.71 2.90 2.61 3.00 3.52 2.70 2.92 2.92 2.93 3.46 2.81 X	2.34 1.06 X X X X X X X X X X X X X
Mannose mannose Mannose Sis dor Sis	cose-6-phosphate Isomerase, class I manin-containing protein omain-containing protein omain-containing protein omain-containing protein super transporter subunit III super transporter subunit III a 54-Interacting transcriptional regulator ugger transporter subunit III a 54-Interacting transcriptional regulator ugger transporter subunit III ugger transporter subunit III ugger transporter subunit III auger transporter subuni	HY360 09365 HY360 093680 HY360 06689 HY360 06689 HY360 06699 HY360 06699 HY360 06699 HY360 06699 HY360 0700 HY360 11200 HY360 11200 HY360 11210 HY360 11210 HY360 11210 HY360 11220 HY360 11230 KY360 11230 HY360 01250 	HXW74 06890 X X X X X X X X X X X X X X X X X X X	1.1.1.140	2.33 2.48 2.51 2.71 2.90 2.61 3.00 3.98 3.52 2.70 2.92 2.99 3.46 2.81 X	X X X X X X X X X X X X X
SIS dofu-	omain-containing protein yistem mannosetriuctose/sorbose family transporter subunit ugar transporter subunit IIC ugar transporter subunit IIR ugar transporter subunit IIB mannose transporter subunit IIA 19-1-Interacting transcriptional regulator ugar transporter subunit IIA ugar transporter subunit IIA ugar transporter subunit IIC ugar transporter subunit IIC ugar transporter subunit IIC main-containing protein tel transporter nate transporter	HV380 06699 HV380 06699 HV380 06699 HV380 06699 HV380 06699 HV380 06700 HV380 06700 HV380 06700 HV380 1120 HV380 11205 HV380 11205 HV380 11205 HV380 11215 HV380 11215 HV380 11225 HV380 11225 HV380 11225 HV380 01225 HV380 01250 HV380 01250 HV380 01255 HV380 08255	X X X X X X X X X X X X X X		2.48 2.51 2.71 2.90 2.61 3.00 3.98 3.52 2.70 2.92 2.99 3.46 2.81 X	X X X X X X X X X X X X X X X X X X X
PTS sy PTS sy	ystem manose/fructose/sorbose family transporter subunit uguar transporter subunit III uguar transporter manuser uguar transporter manuser uguar transporter mate transporter material uguar	HV360_06690 HV360_06695 HV360_06705 HV360_06705 HV360_0705 HV360_11200 HV360_11200 HV360_11210 HV360_11210 HV360_11225 HV360_11230 KV360_11230 KV360_11230 KV360_11230 KV360_11230 KV360_08255 HV360_08255	X X X X X X X X X X X X X X		2.51 2.71 2.90 2.61 3.00 3.98 3.52 2.70 2.92 2.99 3.46 2.81 X	X X X X X X X X X X X X
PTS su PTS su	ugar transporter subunit IIC ugar transporter subunit IIA mannose transporter subunit IIA 3-4-Interactini (ranscriptional regulator ugar transporter subunit IIA ugar transporter subunit IIA ugar transporter subunit IIA ugar transporter subunit IIC ugar transporter subunit IIC main-containing protein main-containing protein tel transporter mate	HV350_06695	X X X X X X X X X X X X X X X X X X X		2.90 2.61 3.00 3.98 3.52 2.70 2.92 2.99 3.46 2.81 X	X X X X X X X X X X
(putatively)	mannose transporter subunit IIA 3 5-Interacting transcriptional regulator ugar transporter subunit IIA ugar transporter subunit IIIA ugar transporter subunit IIIA ugar transporter subunit IIIC wistem nannoserinuctose/sorbose family transporter subunit omain-containing protein sale transporter nate transporter nate transporter antity oxidoreductase domain-containing protein dependent difly/droxyacetone kinase phosphotransferase sub troxyacetone kinase subunit L rok kinase dipK [a lyeverol-3-phosphate oxidase	HV380 08255 HV380 08255 HV380 01200 HV380 11200 HV380 11210 HV380 11210 HV380 11210 HV380 11220 HV380 11220 X X HV380 08255 HV380 08255	X X X X X X X X X X X X X X X X X X X		2.61 3.00 3.98 3.52 2.70 2.92 2.99 3.46 2.81 X	X X X X X X X X
Samma Samma FTS su FTS	a 54-interacting transcriptional regulator upgar transporter subunit IIA upgar transporter subunit IIB upgar transporter subunit IIB upgar transporter subunit IIC upgar transporter subunit IIC mainter transporter mate transporter mate transporter mate transporter mate transporter mate transporter and transporter and transporter mate transporter and transporter mate transporter mate transporter mate transporter mate transporter mate transporter mate transporter mate transporter mate transporter material subunit transporter to delytransporter to delytransp	HV360 11195 HV360 11200 HV360 11205 HV360 11210 HV360 11210 HV360 11220 HV360 11225 HV360 11225 HV360 11230 X X HV360 08255 HV360 08255 HV360 08245	X X X X X X HXW74_09615 HXW74_06190		3.00 3.98 3.52 2.70 2.92 2.99 3.46 2.81 X	X X X X X X
PTS su P	sugar transporter subunit IIB ugar transporter subunit IIC system mannosefructose/sorbose family transporter subunit main-containing protein nate transporter hanity oxidoreductase domain-containing protein rol dehydrogenase jegendent diflyroxyacetone kinase phosphotransferase sub roxyacetone kinase subunit L rol kinase dipK rol kjueroi-2-phosphate oxidase	HY360 11205 HY360 11210 HY360 11215 HY360 11220 HY360 11220 HY360 11225 HY360 11230 X X HY360 08255 HY360 08255 HY360 08245	X X X X X X HXW74_09615 HXW74_06190		3.52 2.70 2.92 2.99 3.46 2.81 X	X X X X X
PTS su SIS dor	uugar transporter subunit IIC ystem nannoseriructose/sorbose family transporter subunit omain-containing protein nate transporter nate transporter nate transporter nate transporter omain-containing protein omain-containing omain-cont	HV360_11210 HV360_11215 HV360_11220 HV360_11225 HV360_11225 HV360_11230 X X X HV360_08255 HV360_08255 HV360_08245	X X X X X X HXW74_09615 HXW74_06190		2.70 2.92 2.99 3.46 2.81 X	X X X X
Sist of chromatic Sist of chromatic	omain-containing protein nate transporter nate transporter nate transporter namily oxidoreductase domain-containing protein ot delyrigorganey oxidoreductase to delyrigorganey oxidoreductase to delyrigorganey oxidoreductase to delyrigorganey oxidoreductase troxyacetone kinase subunit L roxyacetone kinase subunit L rot kinase dipK t qilyereri3-phosphate oxidase	HV360_11220 HV360_11225 HV360_11230 X X X HV360_08255 HV360_08250 HV360_08245	X X X HXW74_09615 HXW74_09620 HXW74_06190		2.99 3.46 2.81 X	X X X
Glucosamine chroms chrom	nate transporter family oxidoreductase foomain-containing protein rol dehydrogenase fependent difhydroxyacetone kinase phosphotransferase sub roxyacetone kinase subunit L rol kinase dipK _ [alyeren's-phosphate oxidase	HV360_11225 HV360_11230 X X X HV360_08255 HV360_08250 HV360_08245	X X HXW74_09615 HXW74_09620 HXW74_06190		3.46 2.81 X	X
(putatively) chroman	nate transporter Immily oxidoreductase domain-containing protein Ordelytrogener O	X X HV360_08255 HV360_08250 HV360_08245	HXW74_09615 HXW74_09620 HXW74_06190		X	
Sorbitol	Jomain-containing protein rol dehydrogenase lependent dihydroxyacetone kinase phosphotransferase sub roxyacetone kinase subunit Dhak roxyacetone kinase subunit L rol kinase GIpK gilycerol-3-phosphate oxidase	HV360_08245	HXW74_09620 HXW74_06190		Х	2.24
Glycerol	rol dehydrogenase dependent dhydroxyacetone kinase phosphotransferase sub roxyacetone kinase subunit DhaK roxyacetone kinase subunit L rol kinase GlpK (glycerol-3-phosphate oxidase	HV360_08245	HXW74_06190	1116		2.07
Glycerol dilhydro dilhydro glycerol dilhydro glycerol dilhydro glycerol gly	Iroxyacetone kinase subunit DhaK Iroxyacetone kinase subunit L rol kinase GIpK 1 glycerol-3-phosphate oxidase	HV360_08245		11.1.1.0	-2.37	1.78
Glycerol dilhydric Glycerol Gephos Gephos Glycosamino Glycosamino Glycosamino Glycosamino Glycosamino Glycosamino Glycosamino Glycosamino FTS fix FTs su Glycosamino	Iroxyacetone kinase subunit L rol kinase GIpK 1 glycerol-3-phosphate oxidase		HXW74_06195 HXW74_06200	2.7.1.121	-0.70 -0.89	2.19
Sye 1 Sye	1 glycerol-3-phosphate oxidase		HXW74 06205	2.7.1.121	-0.84	2.31
Glycerol aquapo Agricol		HV360_09185 HV360_09180	HXW74_09205 HXW74_09210	2.7.1.30	0.54	2.21
Mannitol PTS mr	porin family protein	HV360_09175	HXW74_09210	1.1.3.21	0.69	2.77
(putatively) FTS git PTS sit Fructose Fructose Glucosamine Glutosamine Glutosamine FTS fru PTS sru PTS sru PTS su	nannitol transporter subunit IICBA	HV360_05275	HXW74_04665	2.7.1.197	-1.59	-3.65
FTS su 1-phosp 1-pho	pspho-beta-glucosidase (1) glucose transporter subunit IIA	HV360_08285 HV360_08280	HXW74_06160 HXW74_06165	3.2.1.86	-4.25 -4.31	-3.98 -4.27
Fructose	sugar transporter subunit IIA	HV360_00385	HXW74_00510	2.7.1.202	-4.22	-4.89
Glucosamine glutami PTS fru PTS su PTS su PTS su PTS su PTS su PTS sy Fructolysin SiS dor ucrose (putatively) endonu 2-hydre [citrate i citrate i citrate i	esphofructokinase /GIpR transcriptional regulator	HV360_00390 HV360_00395	HXW74_00505 HXW74_00500	2.7.1.56	-4.00 -4.20	-4.90 -5.07
PTS fru PTS su PTS su PTS su PTS sy Fructolysin SIS dor sucrose (putatively) endonu elfrate citrate citrate i	minefructose-6-phosphate transaminase (isomerizing)	HV360_00345	HXW74_00550	2.6.1.16	-1.01	-2.96
Fructolysin SIS dor Sucrose (putatively) endonu 2-hydra [citrate citrate i	ructose transporter subunit IIA	HV360_09995	Х		4.95	Х
Fructolysin SIS dor sucrose (putatively) endonu 2-hydro [citrate citrate	sugar transporter subunit IIB sugar transporter subunit IIC	HV360_10000 HV360_10005	X		4.94 5.27	X
Fructolysin SIS dor fucrose (putatively) endonu 2-hydro [citrate citrate			X		4.96	x
2-hydro [citrate citrate citrate	omain-containing protein	HV360_10015	Х		5.43	Х
[citrate citrate	nuclease/exonuclease/phosphatase family protein Iroxycarboxylate transporter family protein	HV360_03845 HV360_05475	HXW74_06245 HXW74_02240		1.75	2.09
citrate (te (pro-3S)-lyase] ligase citC	HV360_05490	HXW74_02255	6.2.1.22	2.19	2.93
	e lyase acyl carrier protein citD	HV360_05495	HXW74_02260		2.27	3.36
	e (pro-3S)-lyase subunit beta CitE e lyase subunit alpha citF	HV360_05500 HV360_05505	HXW74_02265 HXW74_02270	4.1.3.34 2.8.3.10	2.10	3.52
Citrate citrate I	e lyase holo-[acyl-carrier protein] synthase CitX	HV360_05510	HXW74_02275	2.7.7.61	2.23	3.72
sodium	m ion-translocating decarboxylase subunit beta	HV360_10380	HXW74_02215	7.2.4.2	1.87	3.15
OadG f	v/lipoyl-binding protein i family protein	HV360_10385 HV360_10390	HXW74_02220 HXW74_02225	7.2.4.2 7.2.4.2	1.68	3.10
Oxalacetate oxaload	acetate decarboxylase subunit alpha	HV360_10395	HXW74_02230	7.2.4.2	1.91	2.62
alpha	rate, phosphate dikinase Irolipoyllysine-residue acetyltransferase	HV360_08015 HV360_00115	HXW74_08830 HXW74_00795	2.3.1.12	0.73	2.50 2.31
	-ketoacid dehydrogenase subunit beta	HV360_00110	HXW74_00800	1.2.4.1	1.02	2.52
pyruvat	rate dehydrogenase (acetyl-transferring) E1 component subur	HV360_00105	HXW74_00805	1.2.4.1	1.00	2.65
	ne dehydrogenase tate dehydrogenase	HV360_07735 HV360_07515	HXW74_07135 HXW74_08205	1.4.1.1	0.40	2.15
Pyruvate aldose	e 1-epimerase family protein	HV350_07250	HXW74_07925		-1.89	-2.11
pyruvat	rate:ferredoxin (flavodoxin) oxidoreductase	HV360_01935 HV360_01615	HXW74_01520 HXW74_03680	1.2.7.1	-0.31 0.88	2.26
	system NifU family Fe-S cluster assembly protein teprotein ligase family protein	HV360_01615 HV360_04570	HXW74_03680 HXW74_10860		-0.01	4.26
Co-factors transpo	porter substrate-binding domain-containing protein	HV360_04575	HXW74_10865		0.70	3.72
	ine deiminase	X	HXW74_08090 HXW74_08095	3.5.3.6 2.1.3.3	X	5.73 5.87
carbam	nine carbamoyltransferase mate kinase	X	HXW74_08095 HXW74_08100	2.7.2.2	x	5.65
Crp/Fnr	nr family transcriptional regulator	X	HXW74 08105		х	5.39
L-arginine YfcC fa	family protein	X	HXW74_08110 HXW74_07015		X	4.02 2.13
	a sold ARC transporter perman	X HV360 11155	HXW74_07015 HXW74_05020		-3.45	-3.20
acids amino a	o acid ABC transporter permease transporter permease subunit	HV360_11160	HXW74_05025		-3.11	-2.95
matic amino acids 3-phos	transporter permease subunit o acid ABC transporter ATP-binding protein		HXW74_00200	2.5.1.19	-0.61	-2.10
DNA-di	transporter permease subunit o acid ABC transporter ATP-binding protein osphoshikimate 1-carboxyvinyltransferase aroA	HV360_00675		4.05	-2.29 0.24	-1.21 2.30
bifuncti	transporter permease subunit a acid ABC transporter ATP-binding protein ssphoshikimate 1-carboxyvinyItransferase aroA directed RNA polymerase subunit beta'	HV360_06645	HXW74_10050 HXW74_01150	11.97 1 4	-0.36	0.4=
uracil p	transporter permease subunit o acid ABC transporter ATP-binding protein osphoshikimate 1-carboxyvinyltransferase aroA	HV360_06645 HV360_06095 HV360_00770	HXW74_01150 HXW74_00105	1.97.1.4 2.4.2.9	1-0.30	-2.17
	transporter permease subunit a acid ABC transporter ATP-binding protein sephoshikimate 1-carboxyvinyltransferase aroA directed RNA polymerase subunit beta' oblic ribbnucleoside-triphosphate reductase activating protei ctional pyr operon transcriptional regulator/uracii phosphorit permease uraA.	HV360_06645 HV360_06095 HV360_00770 HV360_00765	HXW74_01150 HXW74_00105 HXW74_00110	2.4.2.9	-0.78	-2.66
aniyara	transporter permease subunit a oid ABC transporter ATP-binding protein sphoshikimate 1-carboxyvinytransferase aroA directed RNA polymerase subunit bleti obic ribonucleoside-triphosphate reductase activating protei tional pyr openor transcriptional regulator/uracil phosphorib permease uraA tate carbamoytransferase catalytic subunit, pyrB	HV360_06645 (HV360_06095 HV360_00770 HV360_00765 HV360_00760	HXW74_01150 HXW74_00105 HXW74_00110 HXW74_00115	2.4.2.9	-0.78 -1.06	-2.66 -2.85
Carbani	transporter permease subunit a oid dBC transporter ATP-binding protein sphoshikimate 1-carboxyvinyltransferase aroA directed RNA polymerase subunit bets! obic ribnucleoside-triphosphate reductase activating protei clional pyr openor transcriptional regulator/uracil phosphorib permease uraA tate carbamyvitransferase catalytic subunit, pyrB troorotase, pyrC movy phosphate synthase small subunit, carA	HV360_06645 HV360_06095 HV360_00770 HV360_00765 HV360_00760 HV360_00755 HV360_00750	HXW74_01150 HXW74_00105 HXW74_00110 HXW74_00115 HXW74_00120 HXW74_00125	2.4.2.9 2.1.3.2 3.5.2.3 6.3.5.5	-0.78 -1.06 -0.90 -0.85	-2.66 -2.85 -2.72 -2.43
Nucleotides carbam	transporter permease subunit a caid ABC transporter ATP-binding protein sphoshikimate 1-carboxyvinytransferase areA directed RNA polymerase subunit beat oble ribonucleoside-triphosphate reductase activating protei clional pry operor transcriptional regulator/uracil phosphorit permease uraA permease uraA transferase catalytic subunit, pyrB terorotase, pyrC movi phosphate synthase large subunit, carA moyl phosphate synthase large subunit, carB	HV360_06645 HV360_06095 HV360_00770 HV360_00765 HV360_00760 HV360_00750 HV360_00750 HV360_00745	HXW74_01150 HXW74_00105 HXW74_00110 HXW74_00115 HXW74_00120 HXW74_00125 HXW74_00130	2.4.2.9	-0.78 -1.06 -0.90 -0.85 -0.91	-2.66 -2.85 -2.72 -2.43 -2.19
Nucleotides carbam ferrous	transporter permease subunit a oid dBC transporter ATP-binding protein sphoshikimate 1-carboxyvinyltransferase aroA directed RNA polymerase subunit bets! obic ribnucleoside-triphosphate reductase activating protei clional pyr openor transcriptional regulator/uracil phosphorib permease uraA tate carbamyvitransferase catalytic subunit, pyrB troorotase, pyrC movy phosphate synthase small subunit, carA	HV360_06645 HV360_06095 HV360_00770 HV360_00765 HV360_00760 HV360_00755 HV360_00750	HXW74_01150 HXW74_00105 HXW74_00110 HXW74_00115 HXW74_00120 HXW74_00125	2.4.2.9 2.1.3.2 3.5.2.3 6.3.5.5	-0.78 -1.06 -0.90 -0.85	-2.66 -2.85 -2.72 -2.43

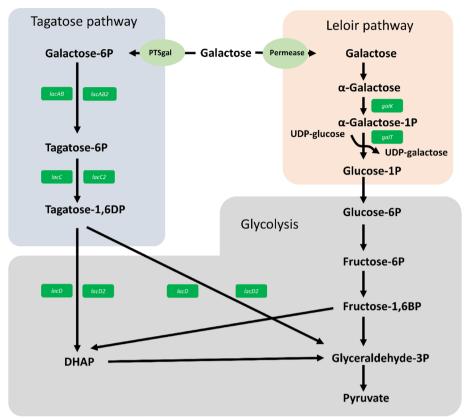


Fig. 2 Schematic overview of the significantly upregulated genes of the galactose utilization pathways of *T. halophilus* when cultivated in LMRS supplied D-galactose. Enyzme abbreviations: *galK* = galactokinase; *galT* = UDP-hexose-1-phosphate uridyltransferase; *lacAB/lacAB2* = galactose-6-phosphate isomerase subunit A or B; *lacC/lacC2* = tagatose 6-phosphate kinase; *lacD/lacD2* = tagatose 1,6-diphosphate aldolase; Permease = unidentified permease; PTSgal = Galactose specific PTS systems. Based on Table 1. A KEGG based map including the upregulated genes showing the connection of the genes and substrates can be found in Fig.S1

(A230100, Solabia group, France). As the α -galactosidase (α -gal) and β -galactosidase (lacG) as well as the adjacent CDSs are strain dependent but increased in the respective strains, the expression might be induced by intracellular galactose levels (Table 1).

The operon responsible for the metabolism trehalose was increased in both strains to a similar degree. However, as trehalose is not added to the medium, we hypothesis that this monocistronic operon is induced by salt stress and trehalose is produced by the trehalose-6-phosphorylase trePP, as this reversible reaction to the formation of trehalose can be catalyzed by this enzyme [25]. This is in line with the current knowledge that T. halophilus accumulates trehalose intracellularly as a compatible solute under salt stress [26]. Another pathway known for its importance under salt stress in T. halophi*lus* is the ADI pathway [10]. This pathway is encoded by the arcABCRD operon in TMW 2.2256 but is inactive in TMW 2.2254 as several genes as missing. This pathway generates ATP and NH₃ by breaking down arginine a thereby increases the intracellular pH. This operon is highly increased with fold-changes up to 5.8-fold as it is induced by salt stress and one of the main repressors, glucose is absent [11].

Strain specific responses can also be found in the pyrimidine synthesis operon pyrRPBC-carAB (Table 1), as TMW 2.2256 has the complete operon decreased by at least 2-fold and no such changes can be seen in TMW 2.2254. As the bottleneck enzyme pyrB in TMW 2.2254 is rendered inactive due to a premature stop codon, the strain completely relies on the uptake of uracil via transporters such as the uracil permease uraA and subsequent formation of UMP via the pyrimidine-nucleoside phosphorylase pdp and the uridine kinase udk. As these enzymes are not regulated by pyrR, no changes can be found. However, the decrease of this operon in TMW 2.2256 is most likely due to the regulation of pyrR. In L. lactis pyrR senses intracellular UMP levels by binding UMP and repressing the correct transcription of the operon under high UMP levels, a similar regulation could be affecting the transcription in *T. halophilus* [27].

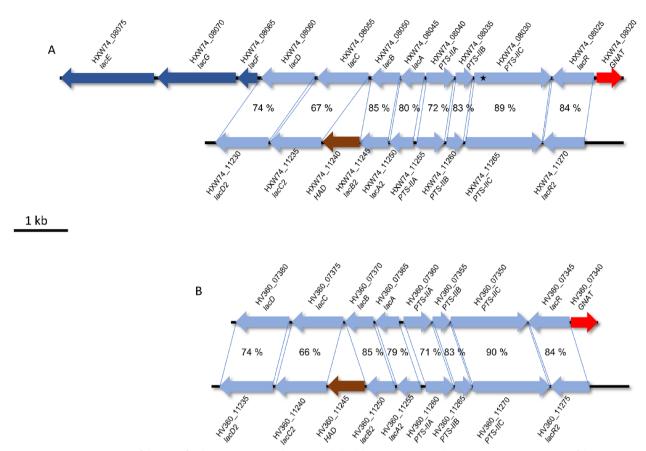


Fig. 3 Genetic organization of the CDSs for the tagatose-6P-Pathways in *T. halophilus* TMW 2.2254 and TMW 2.2256. **A** Organization of the tagatose-6P-Pathway *lacCDBAR* and *lacCDBAR2* in TMW 2.2256 with the amino acid identity shown in percent over the entire sequence of the respective CDS; *=indicates a frameshift with a resulting premature stop codon. **B** Organization of the tagatose-6P-Pathway *lacCDBAR* and *lacCDBAR2* in TMW 2.2256 with the amino acid identity shown in percent over the entire sequence. *lacAB/lacAB2* = galactose-6-phosphate isomerase subunit A or B; *lacC/lacC2* = tagatose 6-phosphate kinase; *lacD/lacD2* = tagatose 1,6-diphosphate aldolase; *lacG* = 6-phospho-β-galactosidase *LacEF* = lactose specific PTS system; *PTS-llABC/PTS-llABC2* = Galactose specific PTS systems; *lacR/lacR2* = putative regulator; *HAD* = HAD family phosphatase; GNAT = GNAT acetyltransferase. The scalebar represent 1 Kb. The sequence similarities are displayed in precent. Alignment was performed using the ncbi blastP program on the ncbi website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp& PAGE_TYPE=BlastSearch)

The metabolism of sugar alcohols in TMW 2.2254 is negligible as only the glycerol dehydrogenase *gldA* is decreased (Table 1). In contrast to that is the response of TMW 2.2256, in which the entire dihydroxyacetone kinase *dhaMKL* of the dehydrogenation pathway for glycerol as well as glycerol-3-phosphate *glpKOF* pathway is at least 2-fold increased (Table 1). Furthermore, TMW 2.2256 has a SDR family oxidoreductase responsible for the metabolism of sorbitol via a sorbitol-6P 2-dehydrogenase (EC:1.1.1.140) with adjacent PTS-IIA increased for at least 2-fold.

As citrate was added to the LMRS medium in the form of di-ammonium hydrogen citrate (CAS no. 3012-65-5), both strains have the CDSs encoding for the citrate lyase *citCDEFX* increased. Notably, the fold changes in TMW 2.2256 are slightly higher with 2.4 to 3.7-fold compared to TMW 2.2254 with only 2.0 to 2.2-fold changes. As

the reaction catalyzed by citrate lyase produces oxaloacetate and acetate, the genomically adjacent oxaloacetate decarboxylase *oadBDGA*, which catalyzes the reaction of oxaloacetate to pyruvate and extrudes sodium ions out of the cell was also increased in both strains [28, 29]. However, in TMW 2.2256 the increase was also higher compared to TMW 2.2254, as in TMW 2.2254 only the gamma subunit was increased by at least 2-fold. As the levels of the citrate lyase are also higher in TMW 2.2256, these differences in the abundance of the CDSs of the *oadBDGA* were expected, as theses enzymes are linked on a metabolic level.

The responses of the strains greatly differ in the pyruvate metabolism, as TMW 2.2256 has the pyruvate-phosphate dikinase *ppdk*, subunit ABC of the pyruvate dehydrogenase, the alanine dehydrogenase *alaD* and a l-lactate dehydrogenase increased by at least 2-fold, while

TMW 2.2254 does not. As TMW 2.2256 has more CDSs increased that are linked with the production of pyruvate than TMW 2.2254, the pathways of utilizing pyruvate must then be also increased to a similar degree to keep up with the increased flux of pyruvate. A Portion of pyruvate is most certainly converted to PEP using the ppdk to ensure a high level of PEP in the cell that can be used for the *dhaKML* or for PTS systems. Another portion of pyruvate is converted to acetyl-CoA by the pyruvate dehydrogenase and pyruvate:ferredoxin oxidoreductase. This acetyl-CoA can then be used for formation of acetate or used for the biosynthesis of lipids. The alanine dehydrogenase converts alanine to pyruvate, NH3 and NADH+ H+. This further increases the pyruvate flux and contributes to shifting the balance between NAD+ and NADH+ H+ state inside the cell to side of NADH+ H+. Therefore, an increase of the l-lactate dehydrogenase is needed to regenerate NAD+ to rebalance the redox status (NADH/NAD+ ratio), as the level of the NADH oxidase (HXW74_6500) is constant in TMW 2.2256, the activity of it may be not sufficient to keep up with the increased NADH flux.

Furthermore, the increased transcription and thereby potentially subsequent production of the pyruvate dehydrogenase and pyruvate:ferredoxin oxidoreductase results in an increased need for Fe-cluster assembly proteins and for lipoic acid, as an increase of the assembly protein and lipoic acid transporter can be seen Table 1.

Conclusion

For the first time it could be shown that all of the operons, in this case two, encoding for the tag-6P in T. halophilus are active, which clearly indicates an adaptation towards an environment rich in galactose such as lupine bean moromi or soy sauce moromi. It could be shown that *T. halophilus* is utilizing galactose via two pathways simultaneously and that a preference for either pathway can be a strain dependent feature. The studied strains revealed a different transcriptomic profile, that can be linked to their genomic differences and partially explain the different growth behaviors. Furthermore, the transcriptomic data suggests that TMW 2.2256 has a higher pyruvate flux due more energy active catabolic pathways but also greater redox stress from the resulting shift in the NADH/NAD+ ratio, compared to TMW 2.2254. One of the reasons could potentially be, that by the utilization of sorbitol and glycerol a lot of NADH is generated. TMW 2.2254 is unable to catalyze these reactions and therefore a similar reaction is not required in the same way. Although this study only reveals the difference in two specific strains it shows that the transcriptomic profile can differ greatly between strains. A similar approach might be feasible for other species to determine important traits for growth/persistence in a specific environment.

Methods

Strains and cultivation conditions

The strains used in this study were *T. halophilus* TMW 2.2254 and TMW 2.2256. All strains were cultivated in a modified MRS [30] medium, in which the meat extract (10g/L) and casein peptone (10g/L) were substituted by the addition of 20g/L lupine peptone (Solabia, Pantin, France). Then D-glucose or D-galactose or no additional carbon source were added, this medium was given the name Lupine-MRS (LMRS). To simulate the lupine moromi conditions the NaCl concentration were set to 13.5% (w/v) and a pH of 5.7.

Growth monitoring in LMRS

The growth was characterized by inoculating 15 mL of LMRS, supplied with 10 mM D-glucose or 10 mM D-galactose or without any added carbon sources, with precultures of the respective strains to a starting OD_{600nm} of \approx 0.05. The growth was monitored over the course of 40 h at 30 °C. The experiment was done in biological triplicates for TMW 2.2254 and TMW 2.2256.

Cultivation of the cells for the transcriptome analysis

Precultures were grown by inoculating LMRS with 13.5% NaCl (w/v) with single colonies of either strain and incubated statically in a 50 mL falcon tube (Sarstedt, Nümbrecht, Germany) at 30 °C for 48 h. To obtain main cultures for the experiment, 2% of either precultures were used to inoculated 100 mL of LMRS with 13.5% NaCl (w/v) and grown for 24 h under the same conditions.

On the next day, the $\mathrm{OD_{600nm}}$ of the main cultures was measured and the volumes required to inoculate $100\,\mathrm{mL}$ of LMRS with 13.5% NaCl with approximately 1×10^{8} CFU/ml of *T. halophilus* were transferred into a 50 mL falcon tube. Then the cells were harvested by centrifugation at $10.000~\mathrm{x}$ g for $10~\mathrm{min}$ at RT and resuspended in fresh $50~\mathrm{mL}$ LMRS. The resuspended solution was then mixed with $50~\mathrm{ml}$ of LMRS in a $125~\mathrm{ml}$ Erlenmeyer flask sealed with a cotton plug and incubated at $25~\mathrm{°C}$ statically for $10~\mathrm{h}$. The cultures were sampled after $1~\mathrm{h}$ and after $10~\mathrm{h}$ of incubation. All cultivations were done in biological triplicates.

The growth was determined by plating of serial dilutions of a respective sample on to LMRS agar plates with 13.5% NaCl. Therefore, $100\,\mu l$ of each dilution was streaked out using sterile glass beads (Carl Roth, Karlsruhe, Germany). The plates were then incubated in an anaerobic jar with AnaeroGenTM (Fisher scientific, Waltham, MA, USA) packs at 30°C. Plates with 20 to 200

colonies were considered for the determination of the cell count.

The cells of the sampled cultures were harvested by centrifugation at $10.000 \times g$ for 10 min at $4 \,^{\circ}\text{C}$ and then mixed with 2 mL of RNA-later solution (Thermo Fisher Scientific, Waltham, MA, USA) and kept on ice for 5 min before flash freezing in liquid nitrogen. Samples were then stored at $-80 \,^{\circ}\text{C}$ until RNA-isolation. Samples of the supernatant were immediately transferred to a fresh 50 ml tube and then stored at $-80 \,^{\circ}\text{C}$.

RNA isolation and purification

To isolate RNA from cells the RNAeasy mini kit (Qiagen, Hilden, Germany) was used following the instructions from the manufacturers with some modifications. Frozen samples in RNA-later solution were thawed and the RNA-later solution was discharged without disruption of the cell pellet. Then, the cells were resuspended in 200 μL TE buffer pH 8.5 supplied with 50 mg/ml lysozyme (24,000 kU/mL, SERVA Electrophoresis GmbH, Heidelberg, Germany) for 25 min at RT. The partially lysed cells were then mixed with 700 µL RLT supplied with 2 M DTT and transferred to a fresh 2ml Eppendorf tube containing acid-washed glass beads 212-300 µm (Sigma Aldrich, St. Louis, MO, USA) and transferred to a homogenizer (MP Fastprep-24, Fisher scientific, Waltham, MA, USA) with a shaking frequency set to 6.5 m/s for 25 s with 5 s pause afterwards, this process was done three times. Next, the lysate was centrifuged at 10.000 rpm for 10 min at RT and the supernatant was transferred to a fresh 2 ml tube and mixed with 500 µl 96%(v/v) ethanol. After that, 700 µL of the mixture was transferred onto a RNAeasy spin column and proceeded following the manufacturer's instructions. Quantitative measurement of the RNA content was determined with a Nanodrop spectrophotometer (Nanodrop 1000 3.6.0, PeQLab Biotechnologie GmbH, Erlangen, Germany).

RNA integrity, sequencing and bioinformatic analyses

RNA integrity analyses, library preparation and sequencing were done by Eurofins genomics. The sequencing was done using an Illumina Hiseq 2500 machine.

Raw read counts were created using featurecounts [31] counting only overlapping "Gene" features with a unique mapping position and minimum mapping quality score of 10. Paired-end reads were only counted if the mapped to the same contig and were counted only once. In the case of reads with multiple mapping results, the reads were assigned to the feature with the highest number of matching bases. The mapped reads were normalized using the Trimmed Mean of M-values (TMM) using edgeR package (version 3.16.5) [32].

The genomes of TMW 2.2254 (GCF_024137165.1) and TMW 2.2256 (GCF_024137145.1) were used as reference for the mapping of the reads using the program BWA-MEM (version 0.7.12-r1039) [33]. String-Tie (v. 2.2.1) [34], the annotation using the NCBI PGAP and the proximity of the genes were used for the grouping of genes into operons or clusters. StringTie was used with all settings set to standard.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-023-02760-w.

Additional file 1.

Acknowledgements

Not applicable.

Authors' contributions

TL performed all laboratory experiments, performed the transcriptomic analyses and wrote the manuscript. MAE supervised the laboratory experiments, participated in the discussion of the results and helped writing the manuscript. Both authors read and approved the final manuscript.

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Availability of data and materials

The raw reads data from the transcriptomic experiment are accessible within the Bioproject number PRJNA872913. The genomes of TMW 2.2254 and TMW 2.2256 are accessible under the accession number GCF_024137165.1 and GCF_024137145.1.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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