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Role of Secondary Transporters and Phosphotransferase Systems in Glucose Transport by *Oenococcus oeni*▽

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Glucose uptake by the heterofermentative lactic acid bacterium *Oenococcus oeni* B1 was studied at the physiological and gene expression levels. Glucose- or fructose-grown bacteria catalyzed uptake of [^{14}C]glucose over a pH range from pH 4 to 9, with maxima at pHs 5.5 and 7. Uptake occurred in two-step kinetics in a high- and low-affinity reaction. The high-affinity uptake followed Michaelis-Menten kinetics and required energization. It accumulated the radioactivity of glucose by a factor of 55 within the bacteria. A large portion (about 80%) of the uptake of glucose was inhibited by protonophores and ionophores. Uptake of the glucose at neutral pH was not sensitive to degradation of the proton potential, Δ*p*. Expression of the genes OEOE_0819 and OEOE_1574 (here referred to as 0819 and 1574), coding for secondary transporters, was induced by glucose as identified by quantitative real-time (RT)-PCR. The genes 1574 and 0819 were able to complement growth of a *Bacillus subtilis* hexose transport-deficient mutant on glucose but not on fructose. The genes 1574 and 0819 therefore encode secondary transporters for glucose, and the transports are presumably Δ*p* dependent. *O. oeni* codes, in addition, for a phosphotransferase transport system (PTS) (gene OEOE_0464 [0464] for the permease) with similarity to the fructose- and mannose-specific PTS of lactic acid bacteria. Quantitative RT-PCR showed induction of the gene 0464 by glucose and by fructose. The data suggest that the PTS is responsible for Δ*p*-independent hexose transport at neutral pH and for the residual Δ*p*-independent transport of hexoses at acidic pH.

Oenococcus oeni can be isolated from must and grapevine and is a close relative of *Leuconostoc mesenteroides* (9, 13). *O. oeni* ferments the hexoses glucose and fructose and pentoses like ribose by the phosphoketolase pathway (9, 11, 19, 24, 32). Glucose and fructose are present in approximately equimolar concentrations in grape must, and each reaches concentrations of 100 g/liter and higher (31, 34), whereas pentoses are present only in trace amounts (1). The growth of *O. oeni* in must and wine depends largely on sugars, citric acid, or other organic acids (31). The capability of *O. oeni* to convert L-malic acid to L-lactate plus CO_2 in malolactic fermentation is used in winemaking (15, 19). Hexose metabolism of lactic acid bacteria, including *O. oeni*, has been studied in detail because of the significance of the bacteria in wine and food fermentation and the formation of lactate, acetate, ethanol, and CO_2 depending on the sugars present. The classical phosphoketolase pathway is modified in *O. oeni* and some other lactic acid bacteria when a substrate such as fructose, pyruvate, citrate, or oxygen is present in addition to glucose. The substrates are used for reoxidizing NAD(P)H derived from sugar oxidation in the phosphoketolase pathway (16, 24, 25, 30, 31, 35), resulting in the formation of undesired products, such as acetate and mannitol.

Uptake of the hexoses into the cells of *O. oeni* represents an important part of hexose metabolism that has not been studied so far. Recently, the genome of *O. oeni* strain PSU-1 was completely sequenced (19). The sequence allowed the search for genes encoding potential transporters. Altogether, the genome exhibits 40 genes for secondary carriers, 8 complete PTS (phosphotransferase systems), and 7 complete ABC (ATP-binding cassette) transport systems that could be involved in the uptake of sugars and sugar alcohols (19, 36). The heterofermentative lactic acid bacterium *Leuconostoc mesenteroides* or the facultative heterofermentative *Lactobacillus plantarum* showed a portion of genes for sugar transport in the genome similar to that for *O. oeni*: 41 secondary carriers, 7 complete PTS, and 3 complete ABC transport systems in *L. mesenteroides* and 64 secondary carriers, 25 complete PTS, and 11 complete ABC transport systems in *L. plantarum* (Table 1). The large quota of genes for transporters (4 to 5% of the total number of genes of the genome), including sugar transporters, demonstrates their significance in *O. oeni* and related bacteria (36).

The large number of genes for potential hexose transporters and the lack of information on hexose transport impeded the identification of candidate genes for hexose transport of *O. oeni*. This study aimed to characterize the properties of glucose transport of *O. oeni*. We tested whether one or more permeases are present for glucose transport, and it was differentiated from some aspects of fructose transport. The uptake of glucose was characterized with respect to energetics, kinetics,

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TABLE 1. Genes for potential secondary sugar transporters of *O. oeni*[a]

COG/Pfam	Description	No. of genes for species				
		L. lactis (31)	*L. plantarum* (64)	*P. pentosaceus* (32)	*L. mesenteroides* (41)	*O. oeni* (40)
COG0580	Glycerol uptake facilitator and related permeases (major intrinsic protein family)	4	6	4	2	1
COG0697	Permeases of the drug/metabolite transporter (DMT) superfamily	1	1	0	0	0
COG0738	Fucose permease	0	1	0	0	1
COG2211	Na^+/melibiose symporter and related transporters	3	7	5	8	4
COG2270	Permeases of major facilitator superfamily	0	1	0	0	0
COG2271	Sugar phosphate permease	2	1	1	1	0
COG2814	Arabinose efflux permease	16	33	16	23	23
COG4975	Putative glucose uptake permease	1	3	2	1	1
Pfam00083	Sugar transporter (of MFS)	1	3	3	5	5
Pfam00230	Major intrinsic protein family (e.g., glycerol facilitator protein GlfP)	4	6	4	2	1
Pfam04215	SgaT_UlaA (putative sugar specific permease)	0	2	0	1	0
Pfam06800	Sugar transport proteins	1	3	2	1	1
Pfam07690	MFS_1 (major facilitator superfamily)	22	49	24	31	34

[a] Adapted with kind permission from Springer Science+Business Media: Biology of microorganisms on grapes, in must and in wine, Transport of sugars and sugar alcohols by lactic acid bacteria, 2009, p. 149–166, To Zaunmüller and G. Unden, Table 8.1 (36). Genome search for COGs and Pfams of secondary carriers for the uptake of sugars and sugar alcohols in *Lactococcus lactis*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, and *Oenococcus oeni*. The total number (in parentheses after species name) gives the overall number of candidate genes for secondary carriers. The list shows the COGs and Pfams used for the genome screening and the number of corresponding genes found in each organism. Each gene can be assigned to one COG and/or one Pfam, and therefore neither the number of COGs or Pfams nor the sum of both adds up to the total number given.

and substrate specificity. Based on this information, the gene expression of candidate genes for glucose transporters was studied by quantitative PCR. It turned out that *O. oeni* contains at least two different transporters for glucose, which differ in expression and biochemical properties.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Subcultures of *Oenococcus oeni* strains B1 (23) were grown in modified tomato juice medium (24) at 30°C to an A_{578} of 0.8. Main cultures were inoculated with 2% (vol/vol) of a fresh exponential subculture to obtain bacteria for transport assays or for RNA isolation. Growth for transport experiments was performed at 30°C in 200 ml of anoxic modified MLD medium (23) with 40 mM glucose, fructose, or ribose under N_2 in sealed bottles. The bacteria were harvested in late logarithmic growth phase (A_{578} = 0.7). For RNA isolation, the bacteria were grown in 50 ml anoxic modified MLD medium (strain B1) at 30°C. For RNA isolation, mid-exponential subcultures of bacteria grown under the same conditions were used for inoculation. Bacteria for RNA isolation were harvested from the third subculture obtained in this way in the mid-exponential growth phase (A_{578} of about 0.5).

Transport assay. The harvested *O. oeni* strain B1 was washed twice and resuspended in each 20 ml of ice-cold FPT buffer (100 mM Na fumarate, 100 mM K phosphate, and 100 mM Tricine, mixed in a 1:1:1 ratio, adjusted to pH 3 to 9 as needed) and suspended at an A_{578} of 3 in the same buffer. All steps were conducted under a nitrogen atmosphere in a glove bag. For energizing, the cell suspension was supplemented with 3 mM ribose and incubated at 30°C for 20 min under nitrogen in a rubber-stoppered anaerobic culture tube. If required, valinomycin (20 μM), nigericin (1 μM), CCCP (carbonyl cyanide *m*-chlorophenylhydrazone [200 μM]; Sigma) or SF6847 (3,5-di-*tert*-butyl-4-hydroxybenzylidenemalononitrile [10 μM]; Wako Chemicals) was added after 18 min of energizing and incubated for 2 min before use in transport measurements.

For the experiments, anaerobic solutions and suspensions were used, but the transport experiments were performed under air. [^{14}C]glucose (245 mCi/mmol; Moravek Biochemicals, Inc.) and [^{14}C]fructose (285 mCi/mmol; Amersham Biosciences) were diluted to 1,777 or 2,226 dpm/nmol with unlabeled glucose- and fructose-solution. For measurement of uptake activity, 180 μl of energized cell suspension was added to 20 μl of [^{14}C]glucose or [^{14}C]fructose of various concentrations at 30°C in 1.5-ml reaction tubes. After 20 s, the reaction was stopped by mixing 100 μl transport reaction mixture with 900 μl of stop solution (lithium chloride, 0.1 M, ice cold), followed by rapid vacuum filtration (FH 225V TenPlace filter manifold; Hoefer Pharmacia Biotech, San Francisco, CA) through membrane filters (mixed cellulose ester, diameter of 25 mm, 0.2-μm pore size, type ME 24; Whatman). The filters were washed three times with 1 ml of LiCl solution and transferred into scintillation vials with 4 ml of scintillation liquid (Rotiscint ecoplus; Roth), and the radioactivity was determined in a 1414 liquid scintillation counter (Wallac WinSpectral). Generally, all transport assays were performed in at least two independent experiments with two repeats each, i.e., each value was determined at least 4 times. For each concentration tested, adsorption of [^{14}C]glucose to the cells and filters was subtracted. Adsorption was measured by filtering ice-cold LiCl solution containing cells and [^{14}C]glucose at the same concentration as in the stop solution of the corresponding transport reaction.

Kinetics of uptake started with addition of 500 μl energized cell suspension to 500 μl of 200 μM [^{14}C]glucose at 30°C in 1.5-ml reaction tubes. The reaction was stopped after 10, 30, 60, and 120 s by mixing 100 μl of this reaction with 900 μl of LiCl solution as described above, followed by vacuum filtration to count the intracellular [^{14}C]glucose. For uptake at 0 s, 50 μl [^{14}C]glucose (200 μM) was mixed with 900 μl of LiCl solution, and then 50 μl energized cell suspension was added.

Transport activities were calculated from changes in the intracellular concentration of the [^{14}C]glucose or [^{14}C]fructose (1,777 and 2,226 dpm/nmol, respectively) by measuring the radioactivity of the cells. In control experiments, bacteria treated with 0.1 M $HClO_4$ after [^{14}C]glucose uptake before the filtration lost more than 90% of the radioactivity. For quantitation, the contents of [^{14}C]glucose were related to the A_{578} of the bacterial suspension. An A_{578} of 1 corresponds to 300 mg (dry weight) · liter^{-1} of *O. oeni* cells, which was determined by relating the optical density (A_{578}) values of bacterial suspensions to the dry weight after filtration and drying. The mass of 1 g dry cells corresponds to a 2.19-ml cytoplasmic volume of *O. oeni* (28). The K_i for inhibition of hexose uptake by competitive substrates was calculated as $K_i = I_{50} \times (1 + [S]/K_m)$ according to the method of Cheng and Prusoff (6); I_{50} is the concentration of the competitive inhibitor that inhibits uptake of the substrate by 50%, and S is the concentration of substrate.

Cloning of genes 1574 and 0819 and complementation of *E. coli and B. subtilis*. The genes OEOE_0819 and OEOE_1574 (here referred to as 0819 and 1574) were amplified by PCR from chromosomal DNA of *O. oeni* B1 using the primers 819-BamHI-for (TAA G**GG ATC C**TT TAA AGG AGG AAA CAA TCG TGA AAA AAA GAC AAC C), 819-HindII-rev (CCC CTT GGA AAA CGG AT**A AGC TT**A TTA GTC CTT AAA GC), 1574-SalI-for (GTA **GTC GAC** TTT AAA GGA GGA AAC AAT CAT GCA AGA AGAGAA TAT AG), and 1574-HindIII-rev (CAT CGC GTT TTG CAT **AAG CTT** TTC CCC ATT CCT GC), respectively. The primers introduce the corresponding restriction sites (printed in bold) 5′ to the coding region, and the forward primers contain in addition a Shine-Dalgarno sequence (underlined) of the *gapA* gene of *B. subtilis* as a translation start site. The products were cloned into the cloning site of the *E. coli*/*B. subtilis* shuttle vector pBQ200 (http://subtiwiki.uni-goettingen.de/wiki/index.php/PBQ200), resulting in plasmids pMW1351 with OEOE_1574 and

TABLE 2. Oligonucleotide primers for quantitative RT-PCR[a]

Primer	Sequence (5′ → 3′)	T_m (°C)	Target gene
Ldh for (HKG)*	GCC GCA GTA AAG AAC TTG ATG	57.9	OEOE_0413
Ldh rev*	TGC CGA CAA CAC CAA CTG TTT	57.9	OEOE_0413
gyrA for (HKG)*	CGC CCG ACA AAC CGC ATA AA	59.4	OEOE_0006
gyrA rev*	CAA GGA CTC ATA GAT TGC CGA A	58.4	OEOE_0006
829 Beacon for	CTC CCC GCT GGC TGA TTA TG	61.4	OEOE_0478
829 Beacon rev	TTG AGT GAC TTC TTG TTG GCT TTG	59.3	OEOE_0478
Ooe 839-5	CGC CAA TCA GCA GCT ATA TTA TG	58.9	OEOE_0819
Ooe 839-6	GAA TGA CTA GTG GTG CAA GAA C	58.4	OEOE_0819
Ooe 1047-1	GGA GCC TTC CTT GTA AAC TGG	59.8	OEOE_1574
Ooe 1047-2	CCT TCA TTT AAC AGC TGT CGT GG	60.6	OEOE_1574
1719 for	CCA TCA GGG AAT TGT CAG TCC	59.8	OEOE_1766
1719 rev	TAA GCA AAG GAT ATA TCA GGC TG	57.1	OEOE_1766
1349 for	ATT GTT ATC ACC TGT ACG ATG CTG	59.3	OEOE_1681
1349 rev	CAA CGC CGA AGT CCA TTA TCC	59.8	OEOE_1681
1629 for	TTT TAC TTT TGC CCG TCC AG	55.3	OEOE_1682
1629 rev	TGC CGA CTG AGA TCC AGA TA	57.3	OEOE_1682
E1 for	TTA TTG GAA ATT GCC GAA GG	53.2	OEOE_0647
E1 rev	TTT CTT CGC TTG ACG GAA CT	55.3	OEOE_0647
Hpr for	GCG ACG ATG CTT GTT CAA A	54.5	OEOE_0643
Hpr rev	GCG CCA AGA CTC ATT ACA C	56.7	OEOE_0643
414 for	CCA AGT GAG CTG CAA CAA AA	55.3	OEOE_0382
414 rev	GGC AGC CAA TAT CCT AGT CG	59.4	OEOE_0382
1138 for	GTG TTT CGA TGA TCG CTT CC	57.3	OEOE_0296
1138 rev	CGT AAA ACC TTC CCC ATG CAA A	58.4	OEOE_0296
808 for	GGA AAT TGC CAC TCA TAT AAT CG	57.1	OEOE_0464
808 rev	GAA TCC ACT CGA GCC AAA AC	57.3	OEOE_0464
136 Beacon for	ATC GTT CTT GTC ATT GGT GAG ATG	59.3	OEOE_0136
136 Beacon rev	CAT TCC CTG TCC TTG AAA CTG	60.3	OEOE_0136
1706 for	GCA ACA TCA AAA ACA GAA CCA A	54.7	OEOE_1706
1706 rev	TAA GCG CAT CTC CGA AGT TT	55.3	OEOE_1706
1714 for	GGA CAA CAA TTC AGA TGT GG	55.3	OEOE_1714
1714 rev	GCA TGT AGC CGA TTG TTG AA	55.3	OEOE_1714

[a] Labeled primers (*) are the same as in reference 6. The target gene gives the gene number as in the *O. oeni* database (www.doe.jgi.gov).

pMW1352 with OEOE_0819. The cloned fragments were verified by sequencing in the final form. The plasmids were maintained in *Escherichia coli* JM109 and *Bacillus subtilis* GP778 (a gift from J. Stülke, Göttingen, Germany) containing a Spc^r cassette replacing the *glcT-ptsGHI* region. For growth of complemented *B. subtilis* GP778 on agar plates, C-mineral medium (C-MM) (18) supplemented with 10 mM glucose or fructose was used, and the plates were incubated under aerobic conditions at 37°C for 2 to 3 days.

Quantitative PCR. Total mRNA was isolated from *O. oeni* strain B1 grown anaerobically on 40 mM glucose, fructose, or ribose over three passages to an A_{578} of 0.5 (mid-exponential growth phase) using RNAprotect bacterial reagent (Qiagen) and the RNeasy minikit (Qiagen). The remaining DNA was digested on a column with DNase (RNase-free DNase set; Qiagen). The mRNA was transcribed in cDNA with reverse transcriptase (RT) (iScript cDNA synthesis kit; Bio-Rad). Quantitative RT-PCR was carried out with three independent replicates of the growth experiments in a 96-well plate with cDNA, SYBR green (Bio-Rad), and primers by use of the iCycler system (Bio-Rad). Primers (Table 2) were designed using the software program Primer3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi), and the formation of secondary structure of primers was check with the software programs OligoAnalyzer, Oligonucleotides Properties, and mfold (38). For quantification, the amount of mRNA of candidate genes was normalized to mRNA of the housekeeping genes *gyrA* (encoding gyrase A; OEOE_0006) and *ldhD* (encoding lactate dehydrogenase; OEOE_0413) separately (7). Relative gene expression was determined by a relative quantification model, which was calculated by the $\Delta\Delta C_T$ method under inclusion of primer efficiency (E), which was calculated from dilution series of cDNA (22): ratio = $(E_T)^{\Delta CT_T(\text{control} - \text{process})}/(E_R)^{\Delta CT_R(\text{control process})}$.

The subscripts "T" and "R" stand for the target gene and reference gene, respectively. ΔC_T values are derived from the differences of the threshold cycle (C_T) values of the gene under reference (control) and test (process) conditions. For the genes of the hexose carriers, the reference condition was growth on ribose and the test condition was growth on glucose or fructose. The value of relative gene expression was determined by comparing the differences in the C_T value of a transporter gene with the differences in the C_T value of the housekeeping gene. Then, changes in gene expression were determined separately relative to the expression of *gyrA* and *ldhD*, and then the average was formed to compensate for slight variations in the expression of *gyrA* and *ldhD*, which are never completely constant (7). The C_T values were determined for each condition from three independent experiments. Growth on ribose was chosen as the reference condition. Thus, the expression level of genes from ribose culture was set to a value of 1, which resulted from the $\Delta\Delta C_T$ analysis method, model of Pfaffl (22). Values of >1 indicate induction, and values of <1 indicate repression of gene expression.

RESULTS

Two different glucose uptake systems in *O. oeni*: pH dependence. Glucose and fructose represent important substrates for most strains of *Oenococcus oeni* (3, 36), but neither the transporter nor the type and mode of sugar transport are known. Uptake of glucose by whole cells was characterized by using a filtration assay. After 30 to 40 s, the uptake of [^{14}C]glucose by *O. oeni* strain B1 (Fig. 1A) reached maximal intracellular contents, demonstrating that the reaction displays essentially uptake but not metabolism of the glucose. Bacteria that were not energized showed only slow and weak uptake. Assuming that the intracellular label corresponds to the glucose levels, the energized bacteria accumulated glucose by a factor of up to 55 starting from 10 μM extracellular glucose and by a factor of 36 when 100 μM glucose was supplied in the medium (not shown).

The pH value is an important aspect of metabolism and

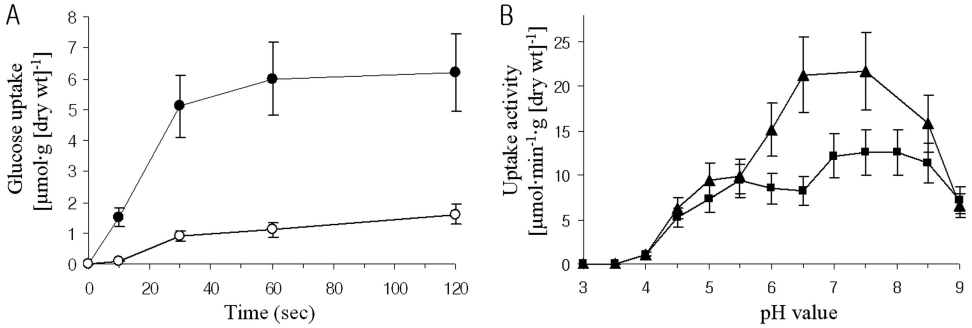

FIG. 1. Time (A) or pH (B) dependence of glucose uptake by *O. oeni* B1. Uptake of glucose over time was quantified in buffer (pH 5) with energized (●) or nonenergized (○) cells in the presence of 100 μM [^{14}C]glucose after growth on glucose at pH 5.8 (A). Uptake rates of glucose in buffer from pH 3 to pH 9 were measured with energized cells in the presence of 200 μM [^{14}C]glucose after growth on glucose at pH 5.8 (▲) or at pH 4 (■) (B). The cell suspensions of bacteria (A_{578} = 3) were energized with ribose.

cellular energetics in lactic acid bacteria (12, 14, 29). Uptake of 100 μM glucose was compared for cell suspensions of *O. oeni* that were grown at acidic (pH 4) or near neutral (pH 5.8) pH (Fig. 1B). pH 4 is closer to the ambient pH of *O. oeni* in wine, and the pH value of 5.8 is close to the pH optimum for growth. The rate of glucose uptake was tested with anaerobic cell suspensions of the pH 4- and pH 5.8-grown bacteria in buffers ranging from pH 3 to pH 9 (Fig. 1B). At pH values of ≤4, the rates for the uptake of glucose were very low. The transport rates increased rapidly at pH values of >4 for the pH 4- and pH 5.8-grown bacteria. The pH 4-grown bacteria showed two pH maxima for transport, around pHs 5.5 and 7.5, whereas the pH 5.8-grown bacteria had only one maximum, at pH 7, with a small shoulder around pH 5. The pH dependence suggests that the bacteria contain two or more uptake systems for glucose with different pH optima, and the synthesis of the transporters is affected by the pH value during growth. For further experiments, pH 5 or pH 7 was used for characterizing either type of transport.

Kinetics of glucose transport. The effect of the glucose concentration on the uptake rate was studied for cell suspensions of *O. oeni* B1 (Fig. 2). For the experiments, pH 5.8-grown bacteria were used in order to apply a situation in which the overall hexose uptake activity is at a maximum. At pH 7, the initial rates for [^{14}C]glucose uptake rapidly increased with higher glucose concentrations and yielded a first plateau at glucose concentrations of >20 μM. At high glucose concentrations (>0.5 mM), the transport rates slowly increased further, showing no obvious saturation even with 5 mM or higher glucose concentrations (not shown). When the bacteria were lysed by acid treatment after uptake of [^{14}C]glucose, most of the radioactivity was lost in the filtration assay (not shown). Therefore, the increase in intracellular [^{14}C]glucose reflects uptake rather than incorporation into cell material. The high-affinity transport at pH 7 was saturated at about 20 μM glucose and showed a V_{max} of 12 μmol · min^{-1} · g (dry weight)$^{-1}$ and a K_m value of 9 μM. The low-affinity transport showed no saturation or Michaelis-Menten behavior, and no V_{max} or K_m values can be given.

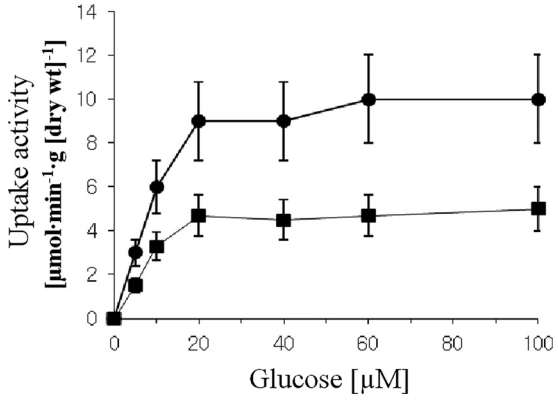

FIG. 2. Concentration-dependent uptake of [^{14}C]glucose in *O. oeni* B1. A cell suspension was incubated with increasing concentrations of [^{14}C]glucose at pH 5 (■) or 7(●), and initial rates of [^{14}C]glucose uptake were determined. The cell suspension of bacteria (A_{578} = 3) was obtained from bacteria grown at pH 5.8 on glucose and was energized with ribose.

The transport of glucose at pH 5 followed a Michaelis-Menten kinetics, with a V_{max} of 9 μmol · min^{-1} · g (dry weight)$^{-1}$ and a K_m value of 9 μM. At high glucose concentrations, the uptake rate further increased very slightly and showed no obvious saturation when concentrations of 5 mM or higher were applied (not shown). Thus, pH 5.8-grown *O. oeni* B1 contains a high-affinity transport system for the uptake of glucose, with similar activities at pHs 5 and 7. The nature and type of low-affinity uptake are not clear.

Ionophores and protonophores inhibit glucose uptake at pH 5. The effect of the proton potential on high-affinity glucose transport was studied by the use of protonophores and ionophores (Table 3). The components degrade constituents of the proton motive force Δp (or $\Delta\mu_{H+}$) over the bacterial membrane (14, 20). For the experiments, bacteria grown at pH 5.8 were used. Transport at pH 5 required energizing the bacteria by ribose or other substrates. Without energizing, the transport activities were only 24% of those of the energized bacteria (1.0 instead of 4.1 μmol glucose · min^{-1} · g [dry weight]$^{-1}$). Addition of the protonophore CCCP or SF6847, each of which collapses the proton motive force, Δp (10, 20, 33), inhibited the transport to levels for nonenergized bacteria or below. The presence of the K^+ ionophore valinomycin, which affects the membrane potential, $\Delta\psi$, partially decreased the uptake activity (59% of the energized bacteria). The ionophore nigericin is a mobile carrier which catalyzes K^+/H^+ exchange and,

TABLE 3. Effects of protonophores (CCCP or SF6847) or ionophores (valinomycin or/and nigericin) on glucose uptake at pH 5 and pH 7[a]

Addition of ionophore or protonophore to energized bacteria (concn [μM])	Glucose transport activity (μmol · min^{-1} · g [dry wt]$^{-1}$)	
	pH 5	pH 7
No addition	4.1	7.1
+ valinomycin (2)	2.4	7.9
+ nigericin (2)	0.8	7.3
+ valinomycin (1) + nigericin (1)	0.8	7.6
+ CCCP (200)	0.8	5.7
+ SF6847 (10)	0.8	5.0
Bacteria without energization	1.0	1.6

[a] The cell suspensions were obtained from *O. oeni* B1 grown on glucose at pH 5.8 and energized with ribose. Uptake of [^{14}C]glucose was tested as described for the experiments analyzed in Fig. 2 and Fig. 3, and initial transport rates are given. The average standard deviation was ±6%.

unlike valinomycin, affects only the proton gradient ΔpH. Similar to the protonophores, Nigericin decreased the transport rates to background levels. The same inhibition was observed for the combined action at low levels of valinomycin and nigericin, which degrade Δ*p* (or both ΔpH and Δψ). At pH 5, overall, energizing the bacteria and the proton motive force Δ*p* are essential for driving glucose uptake; in particular, ΔpH played a major role in driving transport whereas the role of Δψ was minor.

At neutral pH 7, the effect of ionophores on glucose transport was different. Glucose uptake still depended on energizing the bacteria, but the ionophore valinomycin or nigericin had no effect on glucose uptake. Only the protonophores CCCP and SF6847 had some effect on transport and inhibited up to 30% of the uptake activity, suggesting that Δ*p* or its constituents had only a minor or indirect effect on glucose uptake.

Glucose and fructose induce glucose uptake. *O. oeni* is able to ferment various hexoses and pentoses that are degraded by the common phosphoketolase pathway (19, 31, 35). The effects of glucose, fructose, and ribose on glucose uptake activity were tested after growing the bacteria on the alternative substrates at pH 5.8 (Table 4). The glucose uptake of glucose- or fructose-grown bacteria (4.5 and 10.1 μmol · min^{-1} · g [dry weight]$^{-1}$) exceeded the activity in the ribose-grown bacteria by factors of 2.8 and 6.3, respectively. In the same way, uptake of [^{14}C]fructose was maximal in the fructose-grown bacteria, followed by the activity in glucose-grown *O. oeni* (not shown). Therefore, both transport activities are found at the highest

TABLE 4. Transport activities for glucose and fructose after growth on different sugars[a]

Sugar used	Uptake (μmol · min^{-1} · g [dry wt]$^{-1}$) of:	
	[^{14}C]glucose	[^{14}C]fructose
Glucose	4.5	2.2
Fructose	10.1	3.1
Ribose	1.6	0.7

[a] Activities for uptake of 40 μM [^{14}C]glucose or 40 μM [^{14}C]fructose were measured at pH 5.0 in bacterial cell suspensions (A_{578} = 3.0) as described for the experiments of Fig. 2 and Fig. 3 after growth on glucose, fructose, or ribose at pH 5.8. The average standard deviation was 6%.

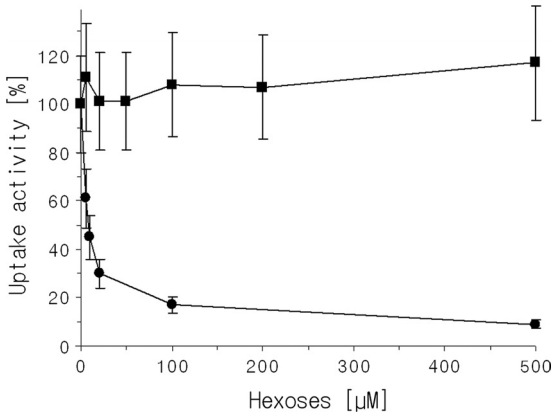

FIG. 3. Uptake of [^{14}C]glucose and [^{14}C]fructose in cells of *O. oeni* B1 in the presence of fructose and glucose. The activity of [^{14}C]glucose (200 μM) (■) or of [^{14}C]fructose (100 μM) (●) uptake was determined in the presence of increasing concentrations of the respective second unlabeled hexose. Uptake was determined in buffer at pH 5 with a cell suspension of bacteria grown on glucose or fructose, respectively, at pH 5.8 (energized with ribose).

levels after growth on fructose, but the properties of glucose transport were characterized with glucose-grown bacteria in order to use glucose-adapted bacteria and the relevant transporters.

Competition and substrate specificity of the hexose carriers. In order to differentiate the supposed glucose transport from that of fructose, the high-affinity uptake of glucose or fructose was tested at pH 5 in the presence of increasing amounts of the second hexose (Fig. 3). [^{14}C]glucose uptake by glucose-grown bacteria was not inhibited by the presence of fructose (Fig. 3) even at a 50-fold excess of fructose (not shown). On the other hand, uptake of [^{14}C]fructose was severely inhibited by glucose, even though fructose-grown bacteria were used. The presence of 10 μM glucose inhibited the uptake of 100 μM [^{14}C]fructose by approximately 50%, and glucose concentrations equimolar to the [^{14}C]fructose inhibited approximately 80% of fructose uptake. The site or mechanism of fructose transport inhibition by glucose is not known, and even regulation by inducer exclusion cannot be ruled out.

O. oeni is able to use various other hexoses, pentoses, and oligosaccharides as substrates for growth (3, 31, 37). Competition experiments were used to test whether alternative substrates compete for the uptake of 100 μM [^{14}C]glucose at pH 5 (Fig. 4). The competing sugars were added in 100-fold excess over the labeled glucose. Pentoses (ribose and xylose), polyols (mannitol and sorbitol), most of the disaccharides (lactose, saccharose, and trehalose), and fructose decreased [^{14}C]glucose uptake only to a low extent (<36%). Excess cellobiose and maltose more severely inhibited [^{14}C]glucose uptake (68 to 81%). Glucose, 2-deoxyglucose, and mannose inhibited the uptake of [^{14}C]glucose completely or nearly completely. Overall, the data of Fig. 3 and Fig. 4 suggests that *O. oeni* B1 grown at pH 5.8 on glucose contains a high-affinity carrier for glucose with a preference for glucose, mannose, 2-deoxyglucose, and maltose.

Relative gene expression of sugar transport genes (secondary transporters). *O. oeni* contains a considerable number of

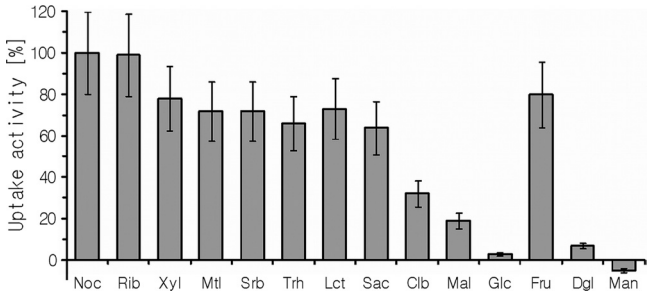

FIG. 4. Inhibition of glucose uptake by other sugars. Uptake of 100 μM [^{14}C]glucose into cells of *O. oeni* B1 was measured at pH 5 in the presence of various sugars (10 mM). The cell suspensions were obtained from bacteria grown at pH 5.8 with glucose and energized with ribose. Noc, no competitor; Rib, ribose; Xyl, xylose; Mtl, mannitol; Srb, sorbitol; Trh, trehalose; Lct, lactose; Sac, saccharose; Clb, cellobiose; Mal, maltose; Glc, glucose; Fru, fructose; Dgl, 2-deoxyglucose; Man, mannose.

candidate genes for sugar transporters (17, 19, 36). Altogether, genes for 40 secondary carriers of the major facilitator superfamily (MFS), 8 complete phosphotransferase systems (PTS), and 7 complete ABC transport systems were identified (36). Among them, nine secondary carriers and three PTS represent the best candidates for hexose transport based on their sequence similarity to hexose permeases and their clustering with genes of hexose metabolism (Table 5). Glucose uptake at pH 5 had shown the characteristics of transport by secondary carriers. At pH 7, however, the transport was mostly Δp independent (Table 3), suggesting the function of a PTS. *O. oeni* also encodes ABC-type transporters. A catabolic role for ABC transporters in glucose catabolism by heterofermentative lactic acid bacteria is unlikely since the transporter would consume all of the ATP produced in glucose fermentation by the phosphoketolase pathway (1 ATP/hexose) (36).

In order to narrow down the number of candidate genes for glucose transport, the expression of the best candidate genes was studied after mRNA isolation and quantitative RT-PCR after growing the bacteria on glucose versus ribose as a non-inducing condition and with fructose as an alternative hexose. Candidate genes of secondary carriers were selected by the following criteria: (i) Similarity of corresponding protein sequence to those of known secondary transporters for sugars, (ii) protein sequence with 400 to 800 amino acids, (iii) predicted secondary structure with 12 to 14 transmembrane helices, and (iv) location of the gene in a genomic context in relation to genes for hexose metabolism. By the criteria, the genes 0136, 0478, 0819, 1574, 1681, 1682, 1706, 1714, and 1766 for secondary transporters were selected (Table 5).

The relative gene expression of candidates for secondary transporters was determined by quantitative RT-PCR with *O. oeni* strain B1 after growth on the sugars at pH 5.5 (Fig. 5A). Relative expression of each candidate gene after growth on a sugar was calculated by comparing the ΔC_T values of candidate genes and the housekeeping genes *gyrA* and *ldhD* after growth on different sugars, indicating the changes in mRNA content of the candidate gene after addition of the sugars. For *gyrA* and *ldhD*, constant expression has been shown (7) and was confirmed here (not shown), supporting their usefulness as reference genes. Growth on ribose was chosen as a reference condition, for which no significant uptake of glucose or fructose was measurable.

TABLE 5. Candidate genes for uptake of hexoses in *O. oeni* strain PSU-1[a]

Candidate gene	Annotation	Component	No. of aa in product	No. of predicted TMH
Secondary carriers				
0136	Permease of MFS		401	12
0478	D-Xylose/proton symporter		480	2 × 6
0819	Cyanate permease		395	2 × 6
1574	Permease of MFS		403	2 × 6
1681	D-Xylose/proton symporter		464	2 × 6
1682	D-Xylose/proton symporter		458	2 × 6
1706	Predicted membrane protein		255	6
1714	Possible glucose uptake permease		315	9[b] or 10[c]
1766	D-Xylose/proton symporter		462	2 × 6
Phosphotransferase systems				
647[d]	PEP protein kinase	EI	575	0
643[d]	Phosphotransferase system	HPr	87	0
464[d]	PTS, mannose/fructose-specific component	EIIAB	331	0
465	PTS, mannose/fructose-specific component	EIIC	271	7
466	PTS, mannose/fructose-specific component	EIID	304	5
382[d]	PTS, mannose/fructose/(*N*-acetylgalactosamine)-specific component	EIIA	139	0
379	PTS, mannose/fructose/(*N*-acetylgalactosamine)-specific component	EIIB	163	0
380	PTS, mannose/fructose/(*N*-acetylgalactosamine)-specific component	EIIC	311	7
381	PTS, mannose/fructose/(*N*-acetylgalactosamine)-specific component	EIID	272	5
296	PTS, specific component[d]	EIIA	134	0
297	PTS, specific component	EIIBC	236	4

[a] The gene numbers and annotations are according to the sequenced and annotated genome of *O. oeni* strain PSU-1 in Joint Genome Institute (www.doe.jgi.gov). aa, amino acids; TMH, transmembrane helices.
[b] Prediction of transmembrane helices from TMHMM server.
[c] Prediction of transmembrane helices from Transport Classification Database (TCDB) (www.tcdb.org).
[d] Genes of PTS that were tested for relative expression.

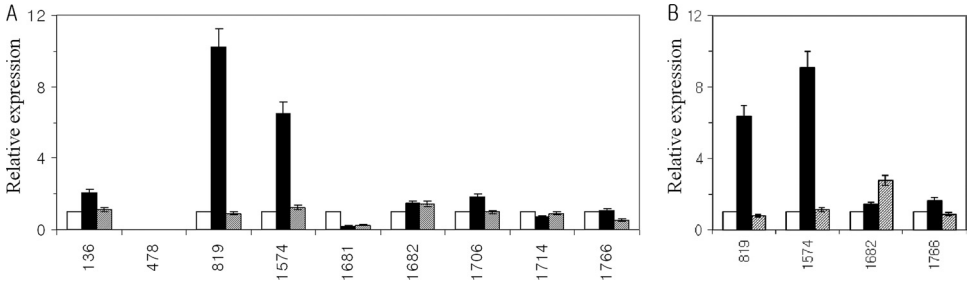

FIG. 5. Relative expression of genes for secondary hexose transporters in *O. oeni* B1. Bacteria were grown on glucose (black), fructose (striped), or ribose (white) at pH 5.5 (A) or at pH 4 (B). mRNA of exponentially growing bacteria was isolated after growing the bacteria for three passages under exponential conditions. The relative contents of the mRNA of the genes were determined by the $\Delta\Delta C_T$ method using the contents of *gyrA* and *ldhD* as the reference. The gene expression on ribose (white) was selected as a control condition, and the expression level was set to 1. The error bars show the standard deviations.

The genes 0819 and 1574 of *O. oeni* B1 showed a marked increase in expression in response to glucose at pH 5.5 by factors of 10.2 and 6.5, respectively. The gene 0819 was annotated as a potential cyanate permease; it consists of 395 amino acids and 2 × 6 transmembrane domains, which represents a typical arrangement of transmembrane helices for many secondary transporters for sugars. The gene 1574 is a supposed permease of 403 amino acids with 2 × 6 putative transmembrane domains (http://www.jgi.doe.gov/, http://www.cbs.dtu.dk/services/TMHMM/, and http://www.tcdb.org/). The mRNA contents of the genes 0136 and 1706 increased slightly after growth on glucose, whereas the expression of the other genes showed no characteristic response or was even repressed by glucose. For the gene 0478, no significant amounts of mRNA were detected under the conditions tested. The same primers were used successfully for amplification of genes from chromosomal DNA, indicating that the cells were devoid of detectable or sufficiently stable mRNA of the gene.

The relative expression of the genes after growth at pH 4 was similar overall to expression in the pH 5.5-grown bacteria (Fig. 5B). Again, the genes 0819 and 1574 showed the strongest induction by glucose (factors of 6.3 and 9.1, respectively) among all genes tested. The relative expression of both genes was reversed compared to results at pH 5.5. As for pH 5.5, fructose caused no significant induction compared to growth on ribose. Only gene the 1682 showed the highest induction with fructose (factor of 2.7) compared to growth on ribose.

Relative gene expression of sugar transport (PTS). For studies of the role of PTS in glucose uptake, the relative gene expression of five genes of potentially hexose-related PTS was investigated (Fig. 6). The PTS are composed of an integral membrane protein (EIIC) (or permease of the system) and cytoplasmic proteins (EIIB, EIIA, HPr, and EI) for phosphoryl transfer from PEP to the EIIC proteins (for a review, see reference 8). HPr and EI are the universal proteins of the phosphoryl cascade of the PTS of a cell, whereas the EII proteins are specific for individual transport systems. The genes 0647 and 0643 (Table 5) are supposed to encode EI and HPr, whereas the genes 0296, 0382, and 0464 code for specific components of three PTS. The genes 0382 and 0464 encode the proteins EIIA and EIIAB of two supposed mannose/fructose PTS, and the gene 0296 encodes the protein EIIA of a further PTS. The genes are representatives of three clusters with the genes 0379-0380-0381-0382, 0464-0465-0466, and 0296-0297 for three PTS.

The genes 0647 and 0643, coding for the EI and HPr proteins, showed relatively high expression and were slightly induced by glucose (1.6- and 1.3-fold) and by fructose (factors of 1.9 and 2.8). The high expression of the universal components of the PTS indicates that PTS are produced and have a function in *O. oeni* B1. Expression of the genes 0296 and 0382 for the specific PTS remained nearly constant. Significant induction was measured for the gene 0464 with glucose and with fructose (factors of 3.5 and 5.1, respectively). Therefore, the potential genes for the PTS of *O. oeni* are expressed, and the components of a putative mannose/fructose-specific PTS encoded by the genes 0464, 0465, and 0466 are induced by hexoses and might be involved in hexose transport, including that of glucose or fructose, across the membrane under specific conditions.

Complementation of glucose growth by transporters 1574 and 0819 in *B. subtilis*. The genes 0819 and 1574 represented the best available candidates coding for secondary glucose transporters due to clear induction by glucose. To probe the capacity of the transporters 0819 and 1574 for glucose uptake, complementation of *B. subtilis* and *E. coli* mutants that were

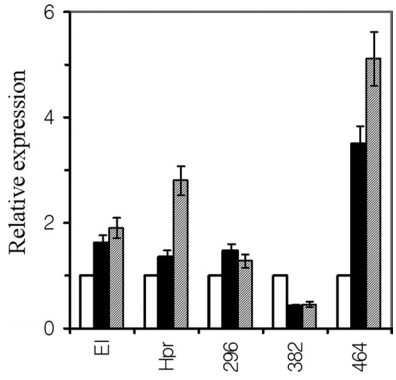

FIG. 6. Relative expression of components of PTS in *O. oeni* B1. The mRNA contents after growth on glucose (black) or fructose (striped) were compared by analyzing the mRNA after growth on ribose (white) at pH 5.5. The expression on ribose was selected as a control condition, and their expression level was set to 1. Other parameters were as for Fig. 5.

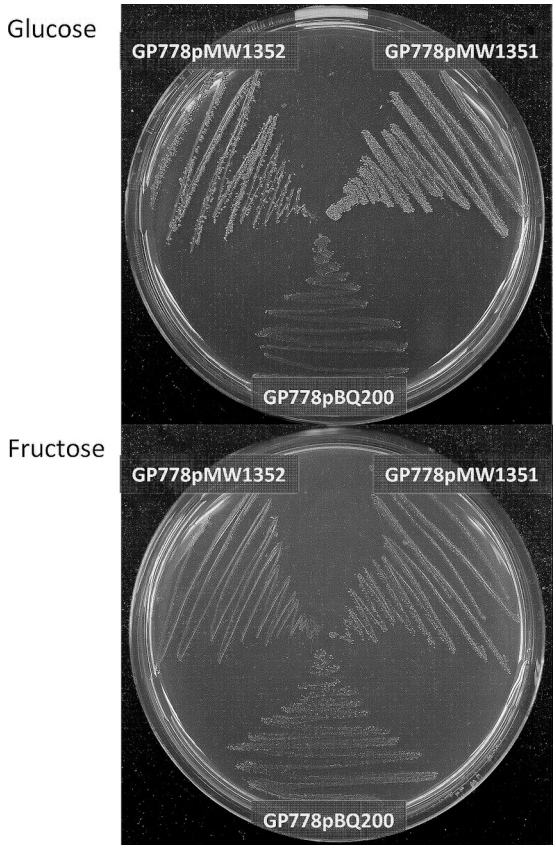

FIG. 7. Growth of *B. subtilis* GP778 on glucose or fructose after complementation with the transporter genes 0819 and 1574. *B. subtilis* GP778 (Δ*glcT-ptsGHI*) was transformed with plasmid pBQ200 (vector only), pMW1351 (gene 1574), or pMW1352 (gene 0819) on C-MM agar plates with 10 mM glucose or fructose.

deficient of glucose and fructose transport was tested. *B. subtilis* strain GP778 carries a deletion in the *glcT-pts* region and is not able to grow on hexoses in mineral medium. The gene 0819 or 1574 was cloned together with a Shine-Dalgarno sequence into plasmid pBQ200 and transferred into *B. subtilis* GP778. The strain transformed with plasmid pMW1351, encoding the gene 1574, gained significant growth on glucose (Fig. 7), which was lacking in the strain transformed with the empty plasmid pBQ200. Plasmid pMW1352, encoding the gene 0819, also conferred the capability for growth on glucose but to a significantly lesser extent than plasmid pMW1351. Growth on fructose was very low for all strains and was not significant compared to the background level (Fig. 7). Overall, the gene 1574 and to a lesser extent the gene 0819 mediate growth on glucose in the *B. subtilis* mutant GP778 but only to very low levels on fructose. When a comparable complementation approach was applied to *E. coli* LJ141, which lacks all PTS and secondary transporters for hexoses, no complementation of the growth deficiency on glucose or fructose was obtained (not shown).

Degradation of hexoses that are taken up by secondary carriers requires phosphorylation of the sugars by hexokinases. Enzymes of this type are not needed when hexose uptake is catalyzed by PTS. The genome of *O. oeni* encodes putative glucokinase (gene 0920) and fructokinase (gene 1708) genes. Cell extracts of *O. oeni* contained a fructose-inducible fructokinase (57 U/g dry weight), and a glucokinase (up to 176 U/g dry weight) that was present in glucose- and fructose-grown bacteria. The presence of the genes and the corresponding enzyme activities are compatible with the use of secondary transporters for hexoses.

DISCUSSION

Glucose uptake by the secondary transporter proteins 1574 and 0819. Glucose-grown *O. oeni* B1 contains a high-affinity secondary transporter for glucose uptake with a low K_m value for glucose (K_m = 9 μM). Transport by this carrier is competitively inhibited by 2-deoxyglucose and mannose but not by fructose. The transport required the proton potential Δp for function, and the activity was maximal around pH 5.5. Quantitative RT-PCR showed that the candidate genes 0819 and 1574, encoding secondary transporters, are strongly induced by glucose (but not by fructose) at acidic pH (4 and 5.5). Heterologous expression of the gene 1574 and to some extent also of the gene 0819 in a *B. subtilis* hexose transport mutant complemented growth on glucose (but not on fructose), demonstrating that both genes indeed encode glucose transporters. Therefore, *O. oeni*, which degrades sugars by the phosphoketolase pathway, obviously uses secondary transporters for glucose uptake, in contrast to many glycolytic bacteria, such as *E. coli*, *B. subtilis*, and homofermentative lactic acid bacteria (26). However, there is no direct proof that the transporters 0819 and 1574 are the major carriers responsible for growth under the test conditions, and other glucose transporters might be present as well. In this respect, it has to be considered that expression of the genes 0819 and 1574 was maximal after growth on glucose whereas the transport activity was maximal after growth on fructose. This discrepancy might indicate that further glucose carriers are used by *O. oeni*.

Transporters 1574 and 0819 supplied only slow growth to the *B. subtilis* transport mutant, which prevented a more detailed analysis of the transporter in *B. subtilis*. Low heterologous expression is an inherent problem for many membrane proteins, in particular for transporters with a high number of transmembrane helices. Studies of the glucose transporters by gene inactivation also do not appear to be a promising approach, since *O. oeni* contains several supposed genes for hexose transporters (compare Table 5 and reference 36). Under these conditions, a loss of single carriers is often overcome by alternative carriers. Thus, in *E. coli*, *B. subtilis*, or *Saccharomyces cerevisiae*, multiple deletions of specific carriers are required to achieve a negative phenotype for hexose transport (5, 21, 26, 27).

The protein 1574 is a predicted secondary transporter of the MFS family, with 12 transmembrane domains and 403 amino acids. It consists of a sugar transporter motif (amino acid residues 43 to 185) and the MFS motif (amino acid residues 221 to 389), according to the KEGG Sequence Similarity DataBase. The former motif belongs to Pfam category PF00083, which is found in sugar (and other) transporters, such as the arabinose AraE, the galactose GalP, the xylose XylE MFS transporters, and other MFS transporters for organic acids.

The gene 0819 encodes the other major glucose-inducible secondary carrier of *O. oeni*, and the protein is able to com-

plement growth of a hexose-deficient mutant of *B. subtilis* with low activity. It is suggested that the 0819 protein is a glucose transporter as well. The low activity could indicate that glucose is only a poor substrate of carrier 0819 or that the 0819 protein is only poorly or not functionally expressed in *B. subtilis.* Possibly other carriers, such as those encoded by the genes 0136 and 1706, represent additional glucose transporters of *O. oeni.*

Overall, high-affinity transport of glucose in *O. oeni* is effected in a Δp-dependent mode by one or more secondary carriers. In addition, there was a significant portion of Δp-independent high-affinity transport. At acidic pH, the Δp-independent transport amounted to about 20% of the total transport (Table 3).

At acidic pH, fructose-grown *O. oeni* catalyzed high-affinity fructose uptake in a Δp-dependent manner (not shown). It is not known whether fructose transport is a side activity of the glucose transporters or is effected by different secondary transporters.

The transporters 1574, 0819, and others, such as the protein 1706, were induced by glucose, whereas the secondary carrier 1682 was induced by fructose at acidic pH. Therefore, carrier 1682 or other carriers not tested might be important for fructose transport.

Hexose transport by a phosphotransferase system. A considerable portion of the high-affinity glucose uptake by *O. oeni* at neutral pH was not sensitive to protonophores and ionophores. At pH 7, this type of transport was predominant whereas the Δp-driven transport was nearly absent. This type of transport may be represented by a PTS that is driven by phosphoenolpyruvate (PEP) or by an ABC-type transporter. The latter transport system would consume the total ATP yield of hexose fermentation by the phosphoketolase pathway (1 ATP/hexose) for uptake of the hexose. The rapid growth of the bacteria on hexoses at pH 7 strongly argues for the function of a PTS under these conditions.

The genes 0647 and 0643, encoding the general components of the PTS (the proteins E1 and HPr), were strongly expressed and further induced by the hexoses. The gene 0464, encoding substrate-specific component of a PTS, was strongly induced by glucose and in particular by fructose. The predicted protein 0464 is similar in sequence to the components of the mannose/fructose- and mannose/fructose/sorbose-specific PTS from Gram-positive bacteria (*Enterococcus faecium*, *Bacillus thuringiensis*, *Lactobacillus johnsonii*, and *Streptococcus pneumoniae*). The gene 0464, which codes for a EIIAB transport protein, is clustered with the genes 0465 and 0466, encoding the membrane components EIIC and EIID, suggesting the presence of a complete (fructose- and glucose-inducible) PTS of the EIIABCD type.

Overall, it is obvious that *O. oeni* encodes a complete hexose-specific PTS (genes 0464 to 0466) that is induced by glucose and fructose. This type of transport is the major system at neutral pH, but the residual transport activity at acidic pH in the presence of protonophores might be effected by this type of transport as well. At neutral pH, the Δp of lactic acid bacteria is low due to the lack of ΔpH (2, 4, 29); accordingly, Δp-independent transport systems like the PTS might become more important.

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REFERENCES

1. **Amerine, M. A., and C. S. Ough.** 1980. Methods for analysis of musts and wines. John Wiley & Sons, Inc., New York, NY.
2. **Augagneur, Y., et al.** 2007. Dual effect of organic acids as a function of external pH in *Oenococcus oeni*. Arch. Microbiol. **188:**147–157.
3. **Beelman, R. B., A. Gavin, and R. N. Keen.** 1977. A new strain of *Leuconostoc oenos* for induced malolactic fermentation in eastern wines. Am. J. Enol. Vitic. **28:**159–170.
4. **Belguendouz, T., R. Cachon, and C. Diviés.** 1997. pH homeostasis and citric acid utilization: differences between *Leuconostoc mesenteroides* and *Lactococcus lactis*. Curr. Microbiol. **35:**233–236.
5. **Boles, E., and C. P. Hollenberg.** 1997. The molecular genetics of hexose transport in yeasts. FEMS Microbiol. Rev. **21:**85–111.
6. **Cheng, Y., and W. H. Prusoff.** 1973. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. **22:**3099–3108.
7. **Desroche, N., C. Beltramo, and J. Guzzo.** 2005. Determination of an internal control to apply reverse transcription quantitative PCR to study stress response in the lactic acid bacterium *Oenococcus oeni*. J. Microbiol. Methods **60:**325–333.
8. **Deutscher, J., C. Francke, and P. W. Postma.** 2006. How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. Microbiol. Mol. Biol. Rev. **70:**939–1031.
9. **Garvie, E. I.** 1986. Genus *Leuconostoc*, p. 1071–1075. *In* P. H. A. Sneath, N. S. Mair, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. Williams & Wilkins, Baltimore, MD.
10. **Hayashi, M., M. Haga, S. Yatsushiro, A. Yamamoto, and Y. Moriyama.** 1999. Vesicular monoamine transporter 1 is responsible for storage of 5-hydroxytryptamine in rat pinealocytes. J. Neurochem. **73:**2538–2545.
11. **Kandler, O.** 1983. Carbohydrate metabolism in lactic acid bacteria. Antonie Van Leeuwenhoek **49:**209–224.
12. **Kashket, E. R.** 1987. Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance. FEMS Microbiol. Lett. **46:**233–244.
13. **König, H., and J. Fröhlich.** 2009. Lactic acid bacteria, p. 3–30. *In* H. König, G. Unden, and J. Fröhlich (ed.), Biology of microorganisms on grapes, in must and in wine. Springer-Verlag, Berlin, Germany.
14. **Konings, W. N., et al.** 1997. The role of transport processes in survival of lactic acid bacteria. Antonie Van Leeuwenhoek **71:**117–128.
15. **Lonvaud-Funel, A.** 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie Van Leeuwenhoek **76:**317–331.
16. **Maicas, S., S. Ferrer, and I. Pardo.** 2002. NAD(P)H regeneration is the key for heterolactic fermentation of hexoses in *Oenococcus oeni*. Microbiology **148:**325–332.
17. **Makarova, K., et al.** 2006. Comparative genomics of the lactic acid bacteria. Proc. Natl. Acad. Sci. U. S. A. **103:**15611–15616.
18. **Martin-Verstraete, I., M. Débarbouillé, A. Klier, and G. Rapoport.** 1994. Interactions of wild-type and truncated LevR of *Bacillus subtilis* with the upstream activating sequence of the levanase operon. J. Mol. Biol. **241:**178–192.
19. **Mills, D. A., H. Rawsthorne, C. Parker, D. Tamir, and K. Makarova.** 2005. Genomic analysis of *Oenococcus oeni* PSU-1 and its relevance to winemaking. FEMS Microbiol. Rev. **29:**465–475.
20. **Nicolls, D. G., and S. J. Ferguson.** 2002. Bioenergetics3. Academic Press, London, United Kingdom.
21. **Paulsen, I. T., S. Chauvaux, P. Choi, and M. H. Saier, Jr.** 1998. Characterization of glucose-specific catabolite repression-resistant mutants of Bacillus subtilis: identification of a novel hexose:H^+ symporter. J. Bacteriol. **180:**498–504.
22. **Pfaffl, M. W.** 2001. A new mathematical model for relative quantification on real-time RT-PCR. Nucleic Acids Res. **29:**2002–2007.
23. **Richter, H., D. Vlad, and G. Unden.** 2001. Significance of pantothenate for glucose fermentation by *Oenococcus oeni* and for suppression of the erythritol and acetate production. Arch. Microbiol. **175:**26–31.
24. **Richter, H., I. Hamann, and G. Unden.** 2003. Use of the mannitol pathway in fructose fermentation of *Oenococcus oeni* due to limiting redox regeneration capacity of the ethanol pathway. Arch. Microbiol. **179:**227–233.
25. **Richter, H., A. A. De Graaf, I. Hamann, and G. Unden.** 2003. Significance of phosphoglucose isomerase for the shift between heterolactic and mannitol fermentation of fructose by *Oenococcus oeni*. Arch. Microbiol. **180:**465–470.
26. **Robillard, G. T., and J. Broos.** 1999. Structure/function studies on the bac-

terial carbohydrate transporters, enzymes II, of the phosphoenolpyruvate-dependent phosphotransferase system. Biochim. Biophys. Acta **1422:**73–104.
27. **Saier, M. H., et al.** 2002. Transport capabilities encoded within the *Bacillus subtilis* genome. J. Mol. Microbiol. Biotechnol. **4:**37–67.
28. **Salema, M., B. Poolman, J. S. Lolkema, M. C. Loureiro Dias, and W. N. Konings.** 1994. Uniport of monoanionic L-malate in membrane vesicles from *Leuconostoc oenos*. Eur. J. Biochem. **225:**289–295.
29. **Salema, M., J. S. Lolkema, M. V. San Romao, and M. C. Loureiro Dias.** 1996. The proton motive force generated in *Leuconostoc oenos* by L-malate fermentation. J. Bacteriol. **178:**3127–3132.
30. **Stolz, P., R. F. Vogel, and W. P. Hammes.** 1995. Utilization of electron acceptors by lactobacilli isolated from sourdough. Z. Lebensm. Unters. Forsch. **201:**402–410.
31. **Unden, G., and T. Zaunmüller.** 2009. Metabolism of sugar and organic acids by lactic acid bacteria from wine and must, p. 135–148. *In* H. König, G. Unden, and J. Fröhlich (ed.), Biology of microorganisms on grapes, in must and in wine. Springer-Verlag, Berlin, Germany.
32. **Veiga-da-Cunha, M., H. Santos, and E. Van Schaftingen.** 1993. Pathway and regulation of erythritol formation in *Leuconostoc oenos*. J. Bacteriol. **175:** 3941–3948.
33. **Veyrat, A., V. Monedero, and G. Perez-Martinez.** 1994. Glucose transport by the phosphoenolpyruvate:mannose phosphotransferase system in *Lactobacillus casei* ATCC 393 and its role in carbon catabolite repression. Microbiology **140:**1141–1149.
34. **Würdig, W., and R. Woller.** 1989. Handbuch der Lebensmitteltechnologie-Chemie des Weins. Verlag Eugen Ulmer, Stuttgart, Germany.
35. **Zaunmüller, T., M. Eichert, H. Richter, and G. Unden.** 2006. Variations in the energy metabolism of biotechnologically relevant heterofermentative lactic acid bacteria during growth on sugars and organic acids. Appl. Microbiol. Biotechnol. **72:**421–429.
36. **Zaunmüller, T., and G. Unden.** 2009. Transport of sugars and sugar alcohols by lactic acid bacteria, p. 149–166. *In* H. König, G. Unden, and J. Fröhlich (ed.), Biology of microorganisms on grapes, in must and in wine. Springer-Verlag, Berlin, Germany.
37. **Zhang, D. S., and R. W. Lovitt.** 2005. Studies on growth and metabolism of *Oenococcus oeni* on sugars and sugar mixtures. J. Appl. Microbiol. **99:**565–572.
38. **Zuker, M.** 2003. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. **31:**3406–3415.