Transport and utilization of hexoses and pentoses in the halotolerant yeast *Debaryomyces hansenii*

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Running title

Pentose/hexose transport in/utilization in *Debaryomyces hansenii*

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Debaryomyces hansenii **is a yeast species well known for its halotolerance. It has seldom been mentioned as a pentose consumer. In the present work, a strain of this species was investigated with respect to the utilization of pentoses and hexoses in mixtures and as single carbon sources. Growth parameters were calculated from batch aerobic cultures with pentoses, hexoses and mixtures of both sugars. Growth on pentoses was slower than on hexoses, but the values obtained for biomass yields were very similar in both types of sugars. Furthermore, in mixtures of two sugars, the preference for one carbon source did not inhibit the consumption of the other. Glucose and xylose were transported by cells grown on glucose, via a specific low-affinity facilitated diffusion system. Cells derepressed by growth on xylose exhibited two distinct high-affinity transport systems for glucose and xylose. The sensitivity of labeled glucose and xylose transport to the dissipation of transmembranar proton gradient by the protonophore CCCP, allowed us to consider them as proton symports, although they displayed sugar associated proton uptake exclusively in the presence of NaCl or KCl. When the V_{max} of transport systems for glucose and xylose were compared with glucose and xylose specific consumption rates during growth on either sugar, transport appeared not to limit the growth rate.**

MATERIALS AND METHODS

Microorganism and media. *Debaryomyces hansenii* INETI CL18, obtained from the Instituto Nacional de Engenharia e Tecnologia Industrial, Portugal, was originally isolated from sugar cane. It was grown on YEPD (yeast extract, peptone, dextrose) slants at 28°C and mantained at 4^0C. Cells were cultivated in mineral liquid medium (21) with different carbon sources (D-glucose, D-galactose, D-mannose, D-xylose and L-arabinose), as indicated in results.

Culture conditions. Batch cultures were performed in a proportion liquid/air of 1:5, at 30°C and 160 rpm in an orbital shaker (Certomat® H, B. Braun, Melsungen A, G., West Germany). Growth was monitored by measuring the O.D. at 640 nm in a spectrophotometer (Spectronic 21, Bausch & Lomb, U.S.A.) and by dry weight determinations. Samples of 10 ml were filtered through ME 25/41 ST (mixed ester) membranes (Schleicher and Schuell, Dassel, Germany), followed by washing with identical volume of destiled water and drying at 80°C overnight. Specific growth rates during the exponential phase (μ_{max}) were determined using both O.D. measurements and dry weight determinations. Yield coefficients ($Y_{X/S}$) were based on dry weight determinations and substrate concentration in the stationary phase. Specific consumption rates for glucose or xylose were calculated as $\mu_{max}/Y_{X/S}$.

Estimation of sugar concentrations in growth media. The determination of sugar concentration in growth media were performed by High Performance Liquid Chromatography (HPLC). The system used was a pump (model Gilson 307, Villiers le Bel, France) associated with a RI detector (model Gilson 132, Villiers le Bel, France). Separation was performed on a Merck Polyspher OA KC Cat. nº 51270 column, at 50°C, using 1 mM sulphuric acid at a flow rate of 0.5 ml min^{-1} as an eluent. Quantification was performed by the internal standard method and assisted by the software……...

Measurement of initial uptake rates. Cells were harvested in exponential phase of growth (O.D. between 0.6 and 0.7) by centrifugation (centrifuge Sigma, model 4K10, West Germany) washed twice with 200 ml ice-cold distilled water (5 min. runs at a speed of 12,200 g) and resuspended to a final concentration of about 20-25 mg (d. wt) ml^{-1} in ice-cold distilled water. For estimating initial uptake rates of labeled glucose and xylose at pH 5.0 the method described earlier was used (11), with aqueous solutions of [U-^{14}C] glucose or [U-^{14}C] xylose, at a specific activity of, respectively, 8.5 and 7.4 MBq $mmol^{-1}$ (3% ethanolic solution, Amersham, Buckinghamshire, England). The concentration of the final cell

suspension was approximately 8-10 mg ml^{-1} dry weight. Sampling times used were 0, 5 and/or 10 seconds (linearity of uptake was maintained up to 20 seconds). Kinetic constants were estimated from Eadie-Hostee plots and confirmed through computer non-linear regression analysis using GraphPad PRISM^{R} (1994-97 Copyright GraphPad Software, Inc.).

No quenching effects were observed in uptake experiments, not even in the presence of high concentrations of NaCl.

The method used to estimate initial rates of proton uptake upon glucose or xylose addition, in the absence or in the presence of several NaCl concentrations, was the same described earlier (8). All the experiments were performed at 30°C.

The effect of other sugars on uptake of glucose or xylose (11) was assayed using 200 mM and 20 mM of each sugar for inhibition of the low-affinity and the high-affinity uptake systems, respectively. The effect of ethanol on sugar transport (20; 22) was determined by incubating the cells for 2 min. in ethanol at increasing concentrations, from 5 to 15% (v/v), after which uptake was assayed. The same methodology was used to assay the effect of the protonophore CCCP (carbonyl cyanide m-chlorophenyl hydrazone) (50 μM – concentration in the assay) on sugar transport**.** The effect of starvation was investigated by incubating the cells in mineral medium without carbon source at 30°C for variable periods of time. Samples were centrifuged, washed twice in ice-cold distilled water and assayed as described above. Cycloheximide concentration used was 200 μg ml^{-1} (MIC, minimum inhibitory concentration). Uptake controls were performed before starvation.

Reproducibility of the results. All the experiments were repeated at least three times, unless otherwise stated.

RESULTS

Growth in batch culture. *D. hansenii* was grown on pentoses or hexoses as single carbon and energy sources and growth parameters (specific growth rate, μ_{max}; yield

coefficient, $Y_{X/S}$; specific substrate consumption rate, $\mu_{max}/Y_{X/S}$) were calculated (Table 1). Growth on glucose or mannose led to similar growth rates and in the case of galactose to a sligtly lower value. On xylose or arabinose growth was slower than on hexoses. In spite of these differences, final biomass yields achieved were similar for all sugars assayed.

Growth on mixtures of two sugars (1% w/v each) was investigated using all the possible combinations between the hexoses and pentoses mentioned above. Representative results of mixtures with two hexoses, two pentoses or one hexose with one pentose are presented in Table 1. Diauxic growth with similar growth parameters was observed when glucose was mixed with either mannose or galactose, glucose being consumed first. In all other mixtures, the consumption of both sugars occured simultaneously. All the hexose/pentose mixtures resulted in growth parameters identical to the example given in Table 1. Utilization of hexoses was prefered to pentoses, in the order glucose, mannose, galactose, xylose and arabinose. The beginning of consumption of the second substrate generally followed a lag-phase. As an example, we stress the case of the glucose/ xylose mixture in which case, only when glucose was below 20% the original concentration, did xylose consumption began. However, the same specific growth rate was found in both phases of growth (not shown). Experiments were repeated with lower concentrations (0.1%, w/v) of each sugar, but still no distinct value for μ could be determined during the second growth phase. Similar results were obtained for all the other mixtures mentioned and we thus consider growth on these not to be diauxic.

After growth on simple sugars, the medium pH could reach values as low as 2.2. In the case of sugar mixtures, and taking into consideration the changes displayed by this environmental parameter during consumption of the first substrate, we examined the influence of pH on consumption of the second substrate. For this we chose hexose/arabinose or pentose/arabinose mixtures, in which no consumption of arabinose could be observed

unless medium pH was readjusted to 5.5 (initial pH of growth medium) after preferential carbon source consumption. Arabinose consumption, as single carbon source, was examined between pH 1.7 and 7.2. The μ_{max} value was obtained around an initial pH of 5.2. Below an initial pH of 2.5 no growth was measurable.

Glucose and xylose transport on glucose-grown cells. The uptake of glucose (Fig. 1) and xylose (not shown) by cells of *D. hansenii* growing on glucose and collected in mid exponential phase exhibited Michaelis-Menten kinetics. Both transport systems had only low-affinities for their substrates, the K_m for glucose being approximately 8 times lower than for xylose (Table 2), whereas the V_{max} values for both sugar transport systems were very similar. Xylose inhibited glucose uptake competitively (Fig. 2) yielding a K_i of 175 mM. Galactose, arabinose, mannose and 2-deoxiglucose were also tested as potential inhibitors of glucose transport, but produced no effect.

The protonophore CCCP did not affect significantly glucose uptake over an external pH range from 3.0 to 7.0 (not shown). Ethanol inhibited the initial uptake rate of glucose and xylose in a non-competitive way. V_{max} decreased exponentially with the ethanol concentration, consistent with the equation published for other mediated transport systems (20; 22). From these experiments, an exponential inhibition constant (k_i) for ethanol of 0.6 M^{-1} was estimated, being the minimal inhibitory ethanol concentration (c_{min}) approximately zero.

Glucose and xylose transport on xylose-grown cell. We also measured transport of glucose and xylose in cells of *D. hansenii* growing on xylose. In these cells, the Eadie-Hofstee plots of the initial uptake rates of glucose and xylose were biphasic. Fig. 1 shows the results obtained for glucose uptake. The lower affinity component presented kinetic parameters similar to the ones obtained for the low-affinity glucose-xylose uptake observed in glucose-grown cells (Table 2). It can also be seen that besides the low-affinity component

found in glucose-grown cells, a higher affinity system for glucose seems to operate in xylose-grown cells. Similar results were obtained for xylose transport (not shown). The kinetic parameters estimated for these systems are presented in Table 2. The K_m and V_{max} values for the higher-affinity transport of glucose were different from those for xylose uptake. Mannose competitively inhibited the high-affinity glucose transport, (K_i 0.38 mM) whereas galactose, xylose and arabinose did not. On the other hand, the xylose uptake was not competitively inhibited by any of these sugars (not shown).

The K_m values for both high-affinity glucose and xylose transport systems were unaffected by the extracellular pH (from 3.0 to 7.0), while V_{max} for either glucose or xylose uptake decreased slightly for pH below 5.0 (not shown). Both the glucose and the xylose transport systems were strongly inhibited by the protonophore CCCP (82 and 67% decrease in V_{max}, respectively). Both glucose and xylose uptake were inhibited by ethanol in a non-competitive way. Similar as in glucose-grown cells, the V_{max} values decreased exponentially with the ethanol concentration yielding the following characteristics: K_i for ethanol of 0.98 M^{-1} and 0.80 M^{-1} and c_{min} of 860 mM and near zero for glucose and xylose transport, respectively.

Regulation of glucose and xylose transport systems. Carbon source starvation of glucose-grown cells in mineral medium for 2h, resulted in a gradual increase in the activity of the high affinity transport system for glucose (Fig. 3), which was inhibited by the presence of cycloheximide.

Transfer of glucose grown-cells to mineral medium containing 2 % xylose, resulted within 10 min. in the formation of both the high-affinity system for glucose as well as that for xylose (Fig. 3), which were again prevented by cycloheximide.

H^+ movements associated with sugar uptake in glucose-grown cells. In many cases, when the mechanism of sugar transport in yeasts is a H^+-symport, a transient alkalinization of

an aqueous cell suspension occurs during the initial uptake of the substrate (10). In cells of *D. hansenii,* grown on either a hexose or a pentose as carbon source, the addition of glucose, mannose, galactose, xylose or arabinose did not result in an alkalinization of the medium. However, using xylose-grown cells, the addition of glucose, mannose, galactose or xylose elicited alkalinization if the cells had previously been incubated in 1 M NaCl or KCl, (but not LiCl, $MgCl_2$ or $CaCl_2$). The initial proton uptake rates followed saturation kinetics and the corresponding parameters, for glucose and xylose, calculated from Eadie-Hofstee plots, are presented in Table 3. The K_m values were the same to the correspondent ones estimated with radiolabeled sugars, but V_{max} values are considerably lower than those presented in Table 2. The K_m of glucose and xylose uptake for cells incubated in 1M NaCl did not differ from the ones determined in the absence of NaCl (Table 2), but V_{max} decreased, reaching values close to those for proton uptake. Hence one proton per glucose or xylose molecules is transported in the presence of 1M NaCl.

The minimum incubation period in 1M NaCl for the detection of lowered V_{max} was determined. As can be seen in Fig. 3, the lowest incubation period possible to assay for technical reasons, 30 seconds, was already enough to determine the observed decrease in V_{max}. The V_{max} of proton uptake increased with increasing salt concentrations. The proton-sugar stoichiometry of 1:1 (see above) was only valid for salt concentrations above 600-800 mM (Fig. 4).

No extracellular alkalinization was elicited by either glucose or xylose in glucose-grown cells in the presence of NaCl and KCl.

DISCUSSION

Our results show that growth of *D. hansenii* on glucose and mannose occurs with approximately the same μ_{max} and yield of biomass. On the other hand, the growth rate on xylose or arabinose was slower, whereas rather similar biomass yields were achieved. In

206 sugar mixtures, diauxy or sequential sugar consumption did not hinder the consumption of a
207 second substrate. Sequential consumption of mixtures of various pentoses or pentoses and
208 hexoses has been reported in the case glucose/xylose for *P. tannophilus* (7). On the other
209 hand, no improvement on biomass yield could be obtained using sugar mixtures when
210 compared to using the same amount of one sugar alone (no residual sugar was detected). This
211 indicates that in *D. hansenii*, pentose metabolism, as well as hexose metabolism, proceeds
212 without any particular drawbacks, unlike with what has been published for *S. cerevisiae* (19;
213 23). Our data suggest that mixtures of hexoses and pentoses, as present in hemicellulose
214 hydrolysates, will probably be fully consumed by *D. hansenii*, as long as pH of the medium
215 can be maintained close to 4-5. Hemicellulose extracts for industrial utilization usually
216 undergo acid hydrolysis, but the pH of the solution is normally neutralized with $CaCO_3$.

217 *D. hansenii* when grown on glucose formed a low-affinity glucose transport system that
218 transports xylose with an approximately 8 times higher K_m. The absence of simultaneous
219 proton uptake, the insensitivity of glucose uptake to the CCCP and to changes in the external
220 pH, as well as the relatively low inhibition by ethanol, led us to conclude that this glucose
221 uptake occurs by facilitated diffusion.

222 In contrast, *D. hansenii* cells derepressed by growth on xylose presented an altogether
223 different situation. Radiolabeled glucose and xylose exhibited uptake kinetic parameters of
224 much higher affinity than in glucose-grown cells and did not act as mutual inhibitors,
225 indicating that these sugars are transported by different permeases. Both sugar transport
226 systems from these cells inhibited by the protonophore CCCP, and the inhibition by ethanol
227 was characterized by exponential inhibition constants comparable to results published for
228 active transporters of proton symport type (20; 22). Uptake of mannose also occurred via the
229 glucose transport system, while the xylose transport system was not shared by any of the
230 other monosaccharides and thus apparently specific for this sugar. Also in *C. shehatate,*
231 facilitated diffusion and sugar proton symports have distinct specificities for different

pentoses and hexoses (11).

The specific consumption rate for glucose by *D. hansenii* growing on glucose, was lower than its glucose transport capacity (V_{max}) (Tables 1 and 2). This suggests that glucose transport is not limiting growth on this sugar. For cells growing on xylose, the specific consumption rate for this sugar was considerably higher than the V_{max} of the high-affinity transport system, indicating that the glucose-xylose facilitated diffusion could also play an important role to sustain growth on xylose. Consistent with these interpretations, no diauxic growth was observed in mixtures of glucose and xylose. As soon as a low concentration of glucose in the growth medium was reached, xylose may compete with glucose transport by the facilitated diffusion system, and then allow the induction of the high-affinity transport, still in the presence of glucose.

The accumulative monosaccharide transport systems usually have been described as proton symports, driven by the proton motive force generated by the plasma membrane H^+/ATPase, e. g. the H^+/xylose symport described in *E. coli* (17) and sugar transport in different yeasts (3; 5; 10; 11). Surprisingly, in *D. hansenii*, no proton uptake could be detected upon the addition of glucose or xylose to xylose-grown cell suspensions. Taking into consideration that (i) *D. hansenii* is a halotolerant yeast (1; 16), (ii) a Na^+/glycerol symport has been postulated in this yeast (12) and that (iii) this yeast has been described as regulating K^+ and Na^+ intracellular contents as an even interchange, substituting one for the other and generating ion potential from high intracellular sodium contents (13; 16), it is not unlikely that glucose and xylose high-affinity transport systems are affected by a salt gradient over the plasma membrane. Apparently, the presence of salt did not require time to induce the reduction in V_{max} of radiolabeled sugar uptake that allows stoicheiometry determination. But, on the other hand, a minimum salt concentration was required for proton uptake detection. These results favoured the recognition of glucose and xylose high-affinity transport systems

as proton symports, possibly indirectly dependent on salt presence to determine sensible variations on p.m.f. which can be critical for proton uptake detection.

Starvation led to the gradual induction of the high-affinity glucose-proton symport whereas transfer of glucose-grown cells to xylose led to the gradual appearance of both high-affinity glucose and xylose proton symports. From these results we concluded that the glucose-proton symport was subject to glucose repression while the xylose-proton symport needs induction by the substrate. This type of transport regulation is similar to what has been published for glucose and xylose transport in *C. shehatae* (11) and *P stipitis* (5) as well as for glucose transport in *C. utilis* (6). Furthermore, the results obtained from the transport studies were consistent with the pattern observed for the consumption of mixed substrate and showed that, in *D. hansenii*, in contrast to other more well studied pentose fermenting species (18), xylose consumption was not prevented by the presence of other sugars, but just delayed. As concluding remarks, we would like to stress that the results here obtained, reinforced that *D. hansenii* could be a good candidate for the biodegradation of hemicellulose hydrolysates, and therefore for further biochemical engineer with the scope of xylose consumption and xylitol production improvement.

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REFERENCES

1. **Adler, L., and L. Gustafsson.** 1980. Polyhydric alcohol production and intracellular amino acid pool in relation to halotolerance of the yeast *Debaryomyces hansenii*. Arch. Microbiol. **124**:123-130.
2. **Duarte, L., C. Nobre, A. P., Gírio, F. M., and M. T. Amaral-Colaço.** 1994. Determination of the kinetic parameters in continuous cultivation by *Debaryomyces*

hansenii grown in D-xylose. Biotechnol. Techniques **8** (12):859-864.

3. **Gasnier, B.** 1987. Characterisation of low- and high-affinity glucose transports in the yeast *Kluyveromyces marxianus*. Biochim. Biophys. Acta **903**:425-433.
4. **Hahn-Hägerdal, B., H. Jeppsson, K. Skoog, and B. Prior.** 1994. Biochemistry and physiology of xylose fermentation by yeasts. Enzyme Microb. Technol. **16**:933-943.
5. **Kilian, S. G., and N. van Uden.** 1988. Transport of xylose and glucose in the xylose-fermenting yeast *Pichia stipitis*. Appl. Microbiol. Biotechnol. **27**:545-548.
6. **Kilian, S. G., B. A. Prior, and J. C. du Preez.** 1993. The kinetics and regulation of D-xylose transport in *Candida utilis*. World J. Microbiol. Biotechnol. **9**:357-360.
7. **Kruse, B., and K. Schügerl.** 1996. Investigation of ethanol formation by *Pachysolen tannophilus* from xylose and glucose/xylose co-substrates. Process Biochem. **31** :389-407.
8. **Lages, F.; and C. Lucas.** 1995. Characterization of a glycerol/H^+ symport in the halotolerant yeast *Pichia sorbitophila*. Yeast **11**:111-119.
9. **Lam, V. M. S., K. R. Daruwalla, P. J. F. Henderson, and M. C. Jones-Mortimer.** 1980. Proton-linked D-xylose transport in *Escherichia coli*. J. Bacteriol. **143** (3):396-402.
10. **Loureiro-Dias, M. C.** 1988. Movements of protons coupled to glucose transport in yeasts. A comparative study among 248 yeast strains. Antonie van Leeuwenhoeck **54**:331-343.
11. **Lucas, C., and N. van Uden.** 1986. Transport of hemicellulose monomers in the xylose-fermenting yeast *Candida shehatae*. Appl. Microbiol. Biotechnol. **23**:491-495.
12. **Lucas, C., M. da Costa, and N. van Uden.** 1990. Osmoregulatory active sodium-glycerol co-transport in the halotolerant yeast *Debaryomyces hansenii*. Yeast **6**:187-191.
13. **Neves, M. L., R. P. Oliveira, and C. M. Lucas.** 1997. Metabolic flux response to salt-induced stress in the halotolerant yeast *Debaryomyces hansenii*. Microbiol. **143**:1133-1139.
14. **Parajó, J. C., H. Dominguez, and J. M. Dominguez.** 1995. Production of xylitol from

raw wood hydrolysates by *Debaryomyces hansenii* NRRL Y-7426. Bioprocess Engineering **13**:125-131.

15. **Prior, B. A., S. G. Kilian, and J. C. du Preez.** 1989. Fermentation of D-xylose by the yeasts *Candida shehatae* and *Pichia stipitis*. Process Biochem. Feb. 89:21-32.
16. **Prista, C., A. Almagro, M. C. Loureiro-Dias, and J. Ramos.** 1997. Physiological basis for high salt tolerance of *Debaryomyces hansenii*. Appl. Environ. Microbiol. **63** (10):4005-4009
17. **Roseiro, J. C., M. A. Peito, and M. T. Amaral-Colaço.** 1991. The effects of the oxygen transfer coefficient and substrate concentration on the xylose fermentation by *Debaryomyces hansenii*. Arch. Microbiol. **156**:484-490.
18. **Spencer-Martins, I**. 1994. Transport of sugars in yeasts: implications in the fermentation of lignocellulosic materials. Bioresource Technology **50**:51-57.
19. **van Dijken, J. P., and W. A. Scheffers**. 1986. Redox balances in the metabolism of sugars by yeasts. FEMS Microbiol. Rev. **32**:199-224.
20. **van Uden, N.** 1967. Transport limited fermentation and growth of *Saccharomyces cerevisiae* and its competitive inhibition. Arch. Microbiol. **58**:155-168.
21. **van Uden, N.** 1985. Ethanol toxity and ethanol tolerance in yeasts. Annual Reports on Fermentation Processes **8**:11-58.
22. **van Uden, N.** 1989. Alcohol Toxicity in Yeasts and Bacteria. N.Y.: CRC Press, Inc.
23. **van Zyl, C., B. A. Prior, S. G. Kilian, and V. Brandt.** 1993. Role of D- ribose in D-Xylose metabolism by *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. **59**:1487-1494.
24. **van Zyl, C., B. A. Prior, S. G. Kilian, and J. L. Kock.** 1989. D-Xylose utilization by *Saccharomyces cerevisiae*. J. Gen. Microbiol. **135**:2791-2798.

LEGENDS

Fig. 1. Eadie-Hofstee plot and direct plot (insert) of initial uptake rates of labeled glucose in glucose (E) and xylose-grown cells (J).

Fig. 2. Inhibition of low-affinity glucose transport in glucose-grown cells by addition of xylose (B no xylose; E 300mM; C 400mM; P 500mM). *Insert:* Effect of xylose concentration on K_m for glucose.

Fig. 3. (A) Effect of starvation of glucose-grown cells in mineral medium without carbon source, on the formation of the high-affinity transport system for glucose: [U-^{14}C]glucose (J,E) and [U-^{14}C]xylose (H). (B) Appearence of the high-affinity transport systems for glucose and xylose: [U-^{14}C]glucose (J,E) and [U-^{14}C]xylose (H,C) upon transfer of glucose-grown cells to medium with 2% xylose. White symbols indicate the incubations in the presence of cycloheximide.

Fig. 4. Effect of incubation with 1M NaCl on V_{max} of the high-affinity transport system for glucose (J) and xylose (E).

Fig. 5. V_{max} of glucose (J) and xylose (E) and proton uptake upon glucose (H) and xylose addition (C) as a function of NaCl concentration in suspensions of xylose-grown cells. *Insert:* ratio between V_{max} from proton uptake and radiolabeled glucose (J) or xylose (E) uptake as a function of NaCl concentration in the assay.

350 **Table 1.** Growth parameters of *D. hansenii* on single
351 or mixed carbon sources (hexoses and pentoses).*
352

Carbon source	μmax (h^{-1})	$Y_{X/S}$ ($g.g^{-1}$)	μmax/$Y_{X/S}$ (mmol. h
Single carbon source			
glucose	0.447 ± 0.047 (4)	0.448 ± 0.093 (3)	5.519
mannose	0.466 ± 0.030 (4)	0.477 ± 0.054 (4)	5.419
galactose	0.369 ± 0.020 (4)	0.437 ± 0.004 (4)	4.671
xylose	0.279 ± 0.022 (4)	0.451 ± 0.062 (4)	4.103
arabinose	0.270 ± 0.022 (4)	0.459 ± 0.119 (4)	3.857
Mixed carbon source			
glucose - mannose	0.404 ± 0.023 (4)	0.323 ± 0.061 (3)	n.d.
xylose - arabinose	0.334 ± 0.027 (3)	0.469 ± 0.050 (3)	n.d.
glucose - xylose	0.405 ± 0.032 (4)	0.368 ± 0.035 (4)	n.d.

353 Number of independent experiments is given in brackets.
354 n.d. Not determined.
355 * Initial sugar concentration: 10 g. l^{-1} each.
356

356 **Table 2.** Kinetic parameters of glucose and xylose transport
357 systems in *D. hansenii* grown on glucose or xylose.
358

Carbon source for growth	[^{14}C] substrate uptake parameters			
	GLUCOSE		XYLOSE	
	K_m (mM)	V_{max} (mmol.h^{-1} g^{-1} [d.wt.])	K_m (mM)	(mn
GLUCOSE	18.5 ± 2.3 (4)	8.6 ± 0.7 (4)	140.0 ± 17.0 (3)	
XYLOSE	0.2 ± 0.03 (4) 25.0 (2)	2.2 ± 0.4 (4) 7.6 (2)	0.8 ± 0.2 (4) n.d.	

359
360 Number of independent experiments is given in brackets.
361 n.d. Not determined.
362 d.wt. dry weight.
363
364

364 **Table 3.** Kinetic parameters of proton and sugar uptake rates in *D. hansenii*.
365

Assays performed in 1M of:	Proton uptake accompanying by the addition of: GLUCOSE K_m (mM)	GLUCOSE V_{max} ($mmol.h^{-1}g^{-1}$[d.wt.])	XYLOSE K_m (mM)	XYLOSE V_{max} ($mmolh^{-1}g^{-1}$[d.wt.])	GL K_m (mM)	GL (
NaCl	0.12 ± 0.04 (7)	1.08 ± 0.21 (7)	0.78 ± 0.18 (5)	0.82 ± 0.17 (8)	0.16 ± 0.04 (3)	
KCl	0.26 ± 0.07 (4)	1.28 ± 0.11 (5)	0.85 ± 0.08 (3)	0.86 ± 0.21 (5)	n.d.	

366
367 Number of independent experiments is given in brackets.
368 n.d. Not determined
369 d.wt. dry weight.
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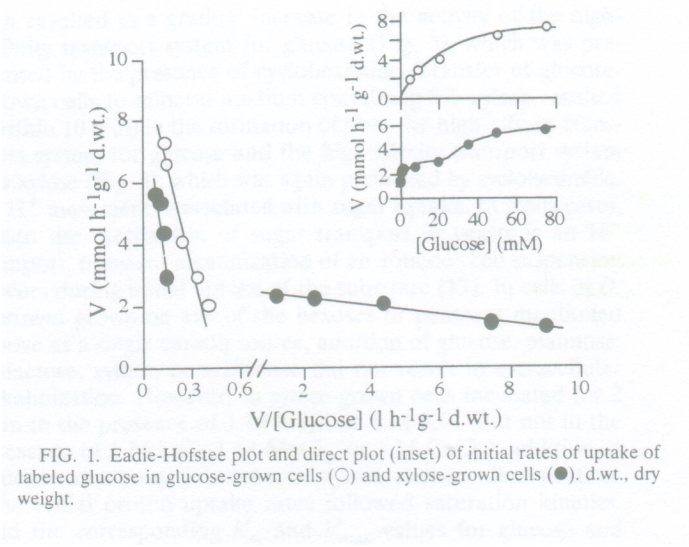

FIG. 1. Eadie-Hofstee plot and direct plot (inset) of initial rates of uptake of labeled glucose in glucose-grown cells (○) and xylose-grown cells (●). d.wt., dry weight.

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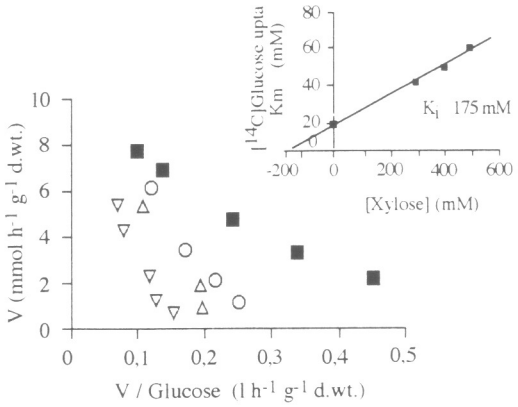

FIG. 2. Inhibition of low-affinity glucose transport in glucose-grown cells by xylose. Symbols: ■, no xylose; ○, 300 mM xylose; △, 400 mM xylose; ▽, 500 mM xylose. (Inset) Effect of xylose concentration on K_m for glucose. d.wt., dry weight.

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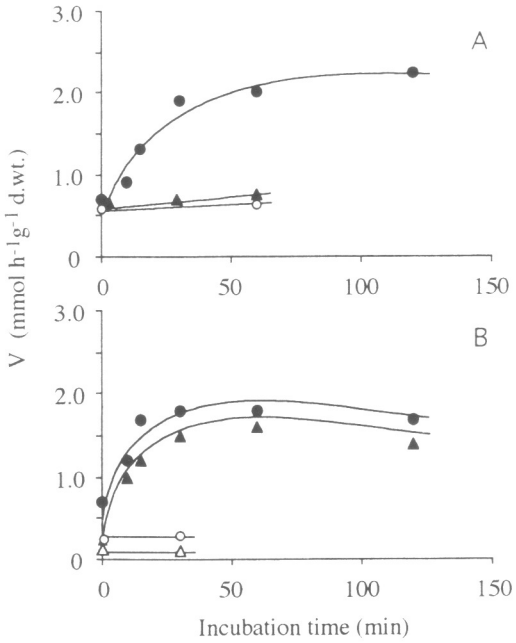

FIG. 3. (A) Effect of starving glucose-grown cells in mineral medium without a carbon source on the formation of the high-affinity transport system for glucose. Symbols: ● and ○, [U-^{14}C]glucose; ▲, [U-^{14}C]xylose. (B) Appearance of the high-affinity transport systems for glucose and xylose: V_{max} values for [U-^{14}C]glucose (● and ○) and [U-^{14}C]xylose (▲ and △) after glucose-grown cells were transferred to medium containing 2% xylose. Open symbols, cell suspensions incubated in the presence of cycloheximide; solid symbols, cell suspensions incubated in the absence of cycloheximide. d.wt., dry weight.

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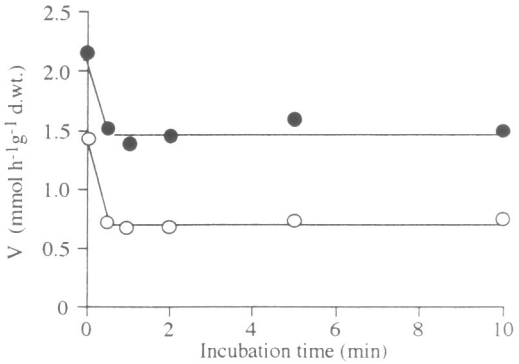

FIG. 4. Effects of incubation with 1 M NaCl on the V_{max} values of the high-affinity transport systems for radiolabeled glucose (●) and xylose (○). d.wt., dry weight.

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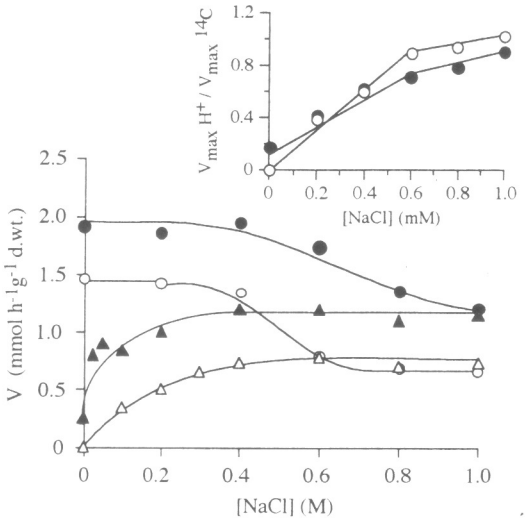

FIG. 5. V_{max} values for [U-^{14}C]glucose (●) and [U-^{14}C]xylose (○) and proton uptake after glucose (▲) and xylose (△) were added as a function of NaCl concentration in suspensions of xylose-grown cells. (Inset) Ratio between V_{max} from proton uptake and labeled glucose (●) or xylose (○) uptake as a function of NaCl concentration in the assay mixture. d.wt., dry weight.

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