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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30 ***Debaryomyces hansenii*** **is a yeast species well known for its halotolerance. It has**

31 **seldom been mentioned as a pentose consumer. In the present work, a strain of this**

32 **species was investigated with respect to the utilization of pentoses and hexoses in**

33 **mixtures and as single carbon sources. Growth parameters were calculated from batch**

34 **aerobic cultures with pentoses, hexoses and mixtures of both sugars. Growth on**

35 **pentoses was slower than on hexoses, but the values obtained for biomass yields were**

36 **very similar in both types of sugars. Furthermore, in mixtures of two sugars, the**

37 **preference for one carbon source did not inhibit the consumption of the other. Glucose**

38 **and xylose were transported by cells grown on glucose, via a specific low-affinity**

39 **facilitated diffusion system. Cells derepressed by growth on xylose exhibited two distinct**

40 **high-affinity transport systems for glucose and xylose. The sensitivity of labeled glucose**

41 **and xylose transport to the dissipation of transmembranar proton gradient by the**

42 **protonophore CCCP, allowed us to consider them as proton symports, although they**

43 **displayed sugar associated proton uptake exclusively in the presence of NaCl or KCl.**

44 **When the V_{max} of transport systems for glucose and xylose were compared with**

45 **glucose and xylose specific consumption rates during growth on either sugar, transport**

46 **appeared not to limit the growth rate.**

47

MATERIALS AND METHODS

48

49 **Microorganism and media.** *Debaryomyces hansenii* INETI CL18, obtained from the

50 Instituto Nacional de Engenharia e Tecnologia Industrial, Portugal, was originally isolated

51 from sugar cane. It was grown on YEPD (yeast extract, peptone, dextrose) slants at 28°C and

52 mantained at 4^0C. Cells were cultivated in mineral liquid medium (21) with different carbon

53 sources (D-glucose, D-galactose, D-mannose, D-xylose and L-arabinose), as indicated in

54 results.

55   **Culture conditions.** Batch cultures were performed in a proportion liquid/air of 1:5, at

56   30°C and 160 rpm in an orbital shaker (Certomat® H, B. Braun, Melsungen A, G., West

57   Germany). Growth was monitored by measuring the O.D. at 640 nm in a spectrophotometer

58   (Spectronic 21, Bausch & Lomb, U.S.A.) and by dry weight determinations. Samples of 10

59   ml were filtered through ME 25/41 ST (mixed ester) membranes (Schleicher and Schuell,

60   Dassel, Germany), followed by washing with identical volume of destiled water and drying at

61   80°C overnight. Specific growth rates during the exponential phase (μ_{max}) were determined

62   using both O.D. measurements and dry weight determinations. Yield coefficients ($Y_{X/S}$) were

63   based on dry weight determinations and substrate concentration in the stationary phase.

64   Specific consumption rates for glucose or xylose were calculated as $\mu_{max}/Y_{X/S}$.

65   **Estimation of sugar concentrations in growth media.** The determination of sugar

66   concentration in growth media were performed by High Performance Liquid Chromatography

67   (HPLC). The system used was a pump (model Gilson 307, Villiers le Bel, France) associated

68   with a RI detector (model Gilson 132, Villiers le Bel, France). Separation was performed on a

69   Merck Polyspher OA KC Cat. nº 51270 column, at 50°C, using 1 mM sulphuric acid at a flow

70   rate of 0.5 ml min^{-1} as an eluent. Quantification was performed by the internal standard

71   method and assisted by the software………...

72   **Measurement of initial uptake rates.** Cells were harvested in exponential phase of

73   growth (O.D. between 0.6 and 0.7) by centrifugation (centrifuge Sigma, model 4K10, West

74   Germany) washed twice with 200 ml ice-cold distilled water (5 min. runs at a speed of 12,200

75   g) and resuspended to a final concentration of about 20-25 mg (d. wt) ml^{-1} in ice-cold

76   distilled water. For estimating initial uptake rates of labeled glucose and xylose at pH 5.0 the

77   method described earlier was used (11), with aqueous solutions of [U-^{14}C] glucose or [U-

78   ^{14}C] xylose, at a specific activity of, respectively, 8.5 and 7.4 MBq mmol^{-1} (3% ethanolic

79   solution, Amersham, Buckinghamshire, England). The concentration of the final cell

80   suspension was approximately 8-10 mg ml^{-1} dry weight. Sampling times used were 0, 5

81   and/or 10 seconds (linearity of uptake was maintained up to 20 seconds). Kinetic constants

82   were estimated from Eadie-Hostee plots and confirmed through computer non-linear

83   regression analysis using GraphPad PRISM^{R} (1994-97 Copyright GraphPad Software, Inc.).

84   No quenching effects were observed in uptake experiments, not even in the presence of

85   high concentrations of NaCl.

86   The method used to estimate initial rates of proton uptake upon glucose or xylose

87   addition, in the absence or in the presence of several NaCl concentrations, was the same

88   described earlier (8). All the experiments were performed at 30°C.

89   The effect of other sugars on uptake of glucose or xylose (11) was assayed using 200

90   mM and 20 mM of each sugar for inhibition of the low-affinity and the high-affinity uptake

91   systems, respectively. The effect of ethanol on sugar transport (20; 22) was determined by

92   incubating the cells for 2 min. in ethanol at increasing concentrations, from 5 to 15% (v/v),

93   after which uptake was assayed. The same methodology was used to assay the effect of the

94   protonophore CCCP (carbonyl cyanide m-chlorophenyl hydrazone) (50 μM – concentration

95   in the assay) on sugar transport. The effect of starvation was investigated by incubating the

96   cells in mineral medium without carbon source at 30°C for variable periods of time. Samples

97   were centrifuged, washed twice in ice-cold distilled water and assayed as described above.

98   Cycloheximide concentration used was 200 μg ml^{-1} (MIC, minimum inhibitory

99   concentration). Uptake controls were performed before starvation.

100   **Reproducibility of the results.** All the experiments were repeated at least three times,

101   unless otherwise stated.

102   **RESULTS**

103   **Growth in batch culture**. *D. hansenii* was grown on pentoses or hexoses as single

104   carbon and energy sources and growth parameters (specific growth rate, μ_{max}; yield

105   coefficient, $Y_{X/S}$; specific substrate consumption rate, $\mu_{max}/Y_{X/S}$) were calculated (Table 1).

106   Growth on glucose or mannose led to similar growth rates and in the case of galactose to a

107   sligtly lower value. On xylose or arabinose growth was slower than on hexoses. In spite of

108   these differences, final biomass yields achieved were similar for all sugars assayed.

109   Growth on mixtures of two sugars (1% w/v each) was investigated using all the possible

110   combinations between the hexoses and pentoses mentioned above. Representative results of

111   mixtures with two hexoses, two pentoses or one hexose with one pentose are presented in

112   Table 1. Diauxic growth with similar growth parameters was observed when glucose was

113   mixed with either mannose or galactose, glucose being consumed first. In all other mixtures,

114   the consumption of both sugars occured simultaneously. All the hexose/pentose mixtures

115   resulted in growth parameters identical to the example given in Table 1. Utilization of

116   hexoses was prefered to pentoses, in the order glucose, mannose, galactose, xylose and

117   arabinose. The beginning of consumption of the second substrate generally followed a lag-

118   phase. As an example, we stress the case of the glucose/ xylose mixture in which case, only

119   when glucose was below 20% the original concentration, did xylose consumption began.

120   However, the same specific growth rate was found in both phases of growth (not shown).

121   Experiments were repeated with lower concentrations (0.1%, w/v) of each sugar, but still no

122   distinct value for μ could be determined during the second growth phase. Similar results were

123   obtained for all the other mixtures mentioned and we thus consider growth on these not to be

124   diauxic.

125

126   After growth on simple sugars, the medium pH could reach values as low as 2.2. In the

127   case of sugar mixtures, and taking into consideration the changes displayed by this

128   environmental parameter during consumption of the first substrate, we examined the

129   influence of pH on consumption of the second substrate. For this we chose hexose/arabinose

130   or pentose/arabinose mixtures, in which no consumption of arabinose could be observed

131   unless medium pH was readjusted to 5.5 (initial pH of growth medium) after preferential

132   carbon source consumption. Arabinose consumption, as single carbon source, was examined

133   between pH 1.7 and 7.2. The μ_{max} value was obtained around an initial pH of 5.2. Below an

134   initial pH of 2.5 no growth was measurable.

135   **Glucose and xylose transport on glucose-grown cells.** The uptake of glucose (Fig. 1)

136   and xylose (not shown) by cells of *D. hansenii* growing on glucose and collected in mid

137   exponential phase exhibited Michaelis-Menten kinetics. Both transport systems had only low-

138   affinities for their substrates, the K_m for glucose being approximately 8 times lower than for

139   xylose (Table 2), whereas the V_{max} values for both sugar transport systems were very similar.

140   Xylose inhibited glucose uptake competitively (Fig. 2) yielding a K_i of 175 mM. Galactose,

141   arabinose, mannose and 2-deoxiglucose were also tested as potential inhibitors of glucose

142   transport, but produced no effect.

143   The protonophore CCCP did not affect significantly glucose uptake over an external pH

144   range from 3.0 to 7.0 (not shown). Ethanol inhibited the initial uptake rate of glucose and

145   xylose in a non-competitive way. V_{max} decreased exponentially with the ethanol

146   concentration, consistent with the equation published for other mediated transport systems

147   (20; 22). From these experiments, an exponential inhibition constant (k_i) for ethanol of 0.6

148   M^{-1} was estimated, being the minimal inhibitory ethanol concentration (c_{min}) approximately

149   zero.

150   **Glucose and xylose transport on xylose-grown cell.** We also measured transport of

151   glucose and xylose in cells of *D. hansenii* growing on xylose. In these cells, the Eadie-

152   Hofstee plots of the initial uptake rates of glucose and xylose were biphasic. Fig. 1 shows the

153   results obtained for glucose uptake. The lower affinity component presented kinetic

154   parameters similar to the ones obtained for the low-affinity glucose-xylose uptake observed in

155   glucose-grown cells (Table 2). It can also be seen that besides the low-affinity component

156 found in glucose-grown cells, a higher affinity system for glucose seems to operate in xylose-

157 grown cells. Similar results were obtained for xylose transport (not shown). The kinetic

158 parameters estimated for these systems are presented in Table 2. The K_m and V_{max} values for

159 the higher-affinity transport of glucose were different from those for xylose uptake. Mannose

160 competitively inhibited the high-affinity glucose transport, (K_i 0.38 mM) whereas galactose,

161 xylose and arabinose did not. On the other hand, the xylose uptake was not competitively

162 inhibited by any of these sugars (not shown).

163   The K_m values for both high-affinity glucose and xylose transport systems were

164 unaffected by the extracellular pH (from 3.0 to 7.0), while V_{max} for either glucose or xylose

165 uptake decreased slightly for pH below 5.0 (not shown). Both the glucose and the xylose

166 transport systems were strongly inhibited by the protonophore CCCP (82 and 67% decrease in

167 V_{max}, respectively). Both glucose and xylose uptake were inhibited by ethanol in a non-

168 competitive way. Similar as in glucose-grown cells, the V_{max} values decreased exponentially

169 with the ethanol concentration yielding the following characteristics: K_i for ethanol of 0.98 M$^-$

170 ^{1} and 0.80 M^{-1} and c_{min} of 860 mM and near zero for glucose and xylose transport,

171 respectively.

172   **Regulation of glucose and xylose transport systems.** Carbon source starvation of

173 glucose-grown cells in mineral medium for 2h, resulted in a gradual increase in the activity of

174 the high affinity transport system for glucose (Fig. 3), which was inhibited by the presence of

175 cycloheximide.

176   Transfer of glucose grown-cells to mineral medium containing 2 % xylose, resulted

177 within 10 min. in the formation of both the high-affinity system for glucose as well as that for

178 xylose (Fig. 3), which were again prevented by cycloheximide.

179   **H$^+$ movements associated with sugar uptake in glucose-grown cells.** In many cases,

180 when the mechanism of sugar transport in yeasts is a H$^+$-symport, a transient alkalinization of

181   an aqueous cell suspension occurs during the initial uptake of the substrate (10). In cells of *D.*

182   *hansenii,* grown on either a hexose or a pentose as carbon source, the addition of glucose,

183   mannose, galactose, xylose or arabinose did not result in an alkalinization of the medium.

184   However, using xylose-grown cells, the addition of glucose, mannose, galactose or xylose

185   elicited alkalinization if the cells had previously been incubated in 1 M NaCl or KCl, (but not

186   LiCl, $MgCl_2$ or $CaCl_2$). The initial proton uptake rates followed saturation kinetics and the

187   corresponding parameters, for glucose and xylose, calculated from Eadie-Hofstee plots, are

188   presented in Table 3. The K_m values were the same to the correspondent ones estimated with

189   radiolabeled sugars, but V_{max} values are considerably lower than those presented in Table 2.

190   The K_m of glucose and xylose uptake for cells incubated in 1M NaCl did not differ from the

191   ones determined in the absence of NaCl (Table 2), but V_{max} decreased, reaching values close

192   to those for proton uptake. Hence one proton per glucose or xylose molecules is transported in

193   the presence of 1M NaCl.

194   The minimum incubation period in 1M NaCl for the detection of lowered V_{max} was

195   determined. As can be seen in Fig. 3, the lowest incubation period possible to assay for

196   technical reasons, 30 seconds, was already enough to determine the observed decrease in

197   V_{max}. The V_{max} of proton uptake increased with increasing salt concentrations. The proton-

198   sugar stoichiometry of 1:1 (see above) was only valid for salt concentrations above 600-800

199   mM (Fig. 4).

200   No extracellular alkalinization was elicited by either glucose or xylose in glucose-

201   grown cells in the presence of NaCl and KCl.

202   **DISCUSSION**

203   Our results show that growth of *D. hansenii* on glucose and mannose occurs with

204   approximately the same μ_{max} and yield of biomass. On the other hand, the growth rate on

205   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

232   pentoses and hexoses (11).

233   The specific consumption rate for glucose by *D. hansenii* growing on glucose, was

234   lower than its glucose transport capacity (V_{max}) (Tables 1 and 2). This suggests that glucose

235   transport is not limiting growth on this sugar. For cells growing on xylose, the specific

236   consumption rate for this sugar was considerably higher than the V_{max} of the high-affinity

237   transport system, indicating that the glucose-xylose facilitated diffusion could also play an

238   important role to sustain growth on xylose. Consistent with these interpretations, no diauxic

239   growth was observed in mixtures of glucose and xylose. As soon as a low concentration of

240   glucose in the growth medium was reached, xylose may compete with glucose transport by

241   the facilitated diffusion system, and then allow the induction of the high-affinity transport,

242   still in the presence of glucose.

243   The accumulative monosaccharide transport systems usually have been described as

244   proton symports, driven by the proton motive force generated by the plasma membrane

245   H^+/ATPase, e. g. the H^+/xylose symport described in *E. coli* (17) and sugar transport in

246   different yeasts (3; 5; 10; 11). Surprisingly, in *D. hansenii*, no proton uptake could be detected

247   upon the addition of glucose or xylose to xylose-grown cell suspensions. Taking into

248   consideration that (i) *D. hansenii* is a halotolerant yeast (1; 16), (ii) a Na^+/glycerol symport

249   has been postulated in this yeast (12) and that (iii) this yeast has been described as regulating

250   K^+ and Na^+ intracellular contents as an even interchange, substituting one for the other and

251   generating ion potential from high intracellular sodium contents (13; 16), it is not unlikely

252   that glucose and xylose high-affinity transport systems are affected by a salt gradient over the

253   plasma membrane. Apparently, the presence of salt did not require time to induce the

254   reduction in V_{max} of radiolabeled sugar uptake that allows stoicheiometry determination. But,

255   on the other hand, a minimum salt concentration was required for proton uptake detection.

256   These results favoured the recognition of glucose and xylose high-affinity transport systems

257   as proton symports, possibly indirectly dependent on salt presence to determine sensible

258   variations on p.m.f. which can be critical for proton uptake detection.

259   Starvation led to the gradual induction of the high-affinity glucose-proton symport

260   whereas transfer of glucose-grown cells to xylose led to the gradual appearance of both high-

261   affinity glucose and xylose proton symports. From these results we concluded that the

262   glucose-proton symport was subject to glucose repression while the xylose-proton symport

263   needs induction by the substrate. This type of transport regulation is similar to what has been

264   published for glucose and xylose transport in *C. shehatae* (11) and *P stipitis* (5) as well as for

265   glucose transport in *C. utilis* (6). Furthermore, the results obtained from the transport studies

266   were consistent with the pattern observed for the consumption of mixed substrate and showed

267   that, in *D. hansenii*, in contrast to other more well studied pentose fermenting species (18),

268   xylose consumption was not prevented by the presence of other sugars, but just delayed. As

269   concluding remarks, we would like to stress that the results here obtained, reinforced that *D.*

270   *hansenii* could be a good candidate for the biodegradation of hemicellulose hydrolysates, and

271   therefore for further biochemical engineer with the scope of xylose consumption and xylitol

272   production improvement.

273   **ACKNOWLEDGEMENTS**

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332

332   **LEGENDS**

333   **Fig. 1**. Eadie-Hofstee plot and direct plot (insert) of initial uptake rates of labeled glucose in

334   glucose (E) and xylose-grown cells (J).

335   **Fig. 2.** Inhibition of low-affinity glucose transport in glucose-grown cells by addition of

336   xylose (B no xylose; E 300mM; C 400mM; P 500mM). *Insert:* Effect of xylose concentration

337   on K_m for glucose.

338   **Fig. 3**. (A) Effect of starvation of glucose-grown cells in mineral medium without carbon

339   source, on the formation of the high-affinity transport system for glucose: [U-^{14}C]glucose

340   (J,E) and [U-^{14}C]xylose (H). (B) Appearence of the high-affinity transport systems for

341   glucose and xylose: [U-^{14}C]glucose (J,E) and [U-^{14}C]xylose (H,C) upon transfer of glucose-

342   grown cells to medium with 2% xylose. White symbols indicate the incubations in the

343   presence of cycloheximide.

344   **Fig. 4.** Effect of incubation with 1M NaCl on V_{max} of the high-affinity transport system for

345   glucose (J) and xylose (E).

346   **Fig. 5.** V_{max} of glucose (J) and xylose (E) and proton uptake upon glucose (H) and xylose

347   addition (C) as a function of NaCl concentration in suspensions of xylose-grown cells. *Insert:*

348   ratio between V_{max} from proton uptake and radiolabeled glucose (J) or xylose (E) uptake as a

349   function of NaCl concentration in the assay.

350

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

Carbon source	μ_{max} (h^{-1})	$Y_{X/s}$ (g·g^{-1})	$\mu_{max}/Y_{X/s}$ (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 | [14C] substrate uptake parameters | | | |
| | GLUCOSE | | XYLOSE | |
	K_m (mM)	V_{max} (mmol.h^{-1} g^{-1} [d.wt.])	K_m (mM)	(mm
GLUCOSE	18.5 ± 2.3 (4)	8.6 ± 0.7 (4)	140.0 ± 17.0 (3)	
XYLOSE	0.2 ± 0.03 (4)	2.2 ± 0.4 (4)	0.8 ± 0.2 (4)	
	25.0 (2)	7.6 (2)	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*.

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Assays performed in 1M of:	Proton uptake accompanying by the addition of:					
	GLUCOSE		XYLOSE		GL	
	K_m (mM)	V_{max} (mmol.h^{-1}g^{-1}[d.wt.])	K_m (mM)	V_{max} (mmolh^{-1}g^{-1}[d.wt.])	K_m (mM)	
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.	

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367 Number of independent experiments is given in brackets.
368 n.d. Not determined
369 d.wt. dry weight.
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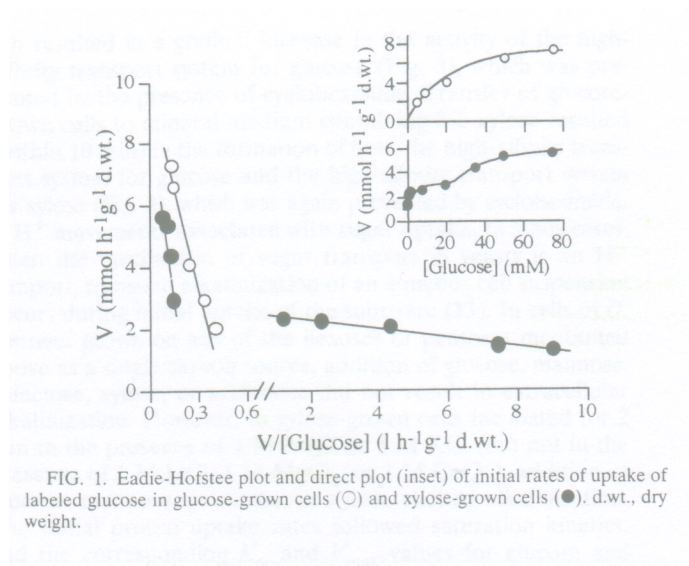
FIG. 1. Eadie-Hofstee plot and direct plot (inset) of initial rates of uptake of labeled glucose in glucose-grown cells (○) and xylose-grown cells (●). d.wt., dry weight.
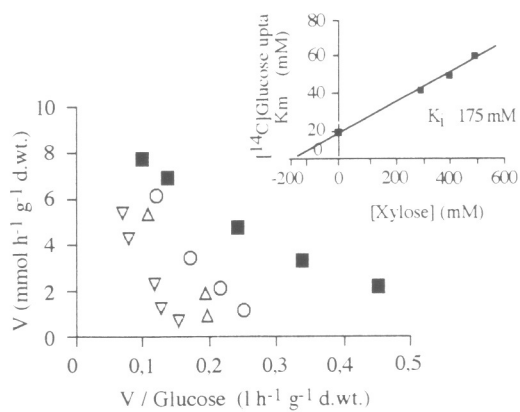
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FIG. 2. Inhibition of low-affinity glucose transport in glucose-grown cells by xylose. Symbols: ■, no xylose; ○, 300 mM xylose; △, 400 mM xylose; ▽, 500 mM xylose. (Inset) Effect of xylose concentration on K_m for glucose. d.wt., dry weight.
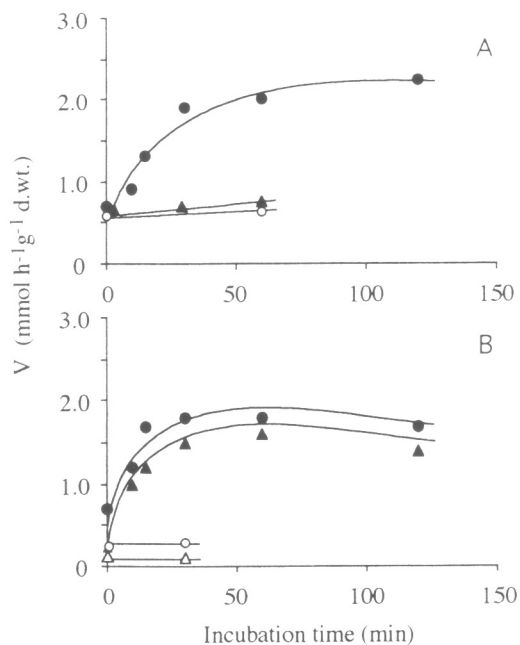
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FIG. 3. (A) Effect of starving glucose-grown cells in mineral medium without a carbon source on the formation of the high-affinity transport system for glucose. Symbols: ● and ○, [U-^{14}C]glucose; ▲, [U-^{14}C]xylose. (B) Appearance of the high-affinity 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.
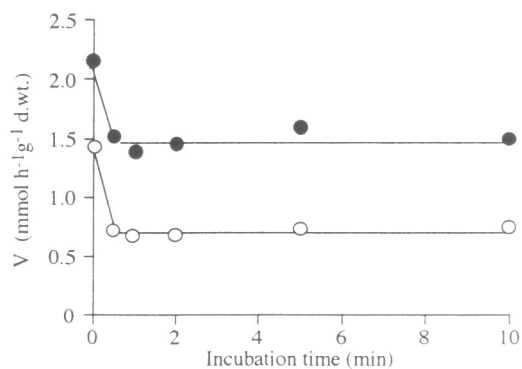
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FIG. 4. Effects of incubation with 1 M NaCl on the V_{max} values of the high-affinity transport systems for radiolabeled glucose (●) and xylose (○). d.wt., dry weight.
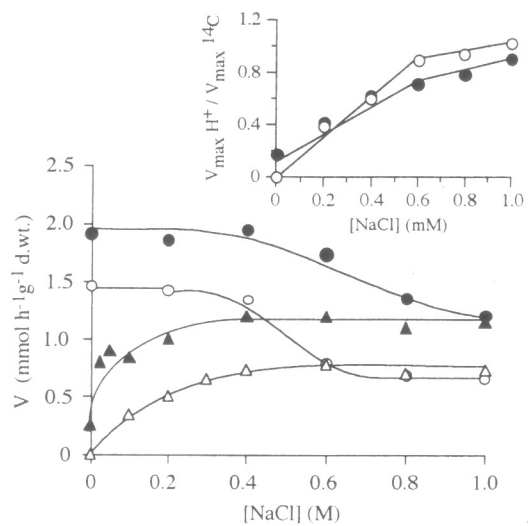
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FIG. 5. V_{max} values for [U-^{14}C]glucose (●) and [U-^{14}C]xylose (○) and proton uptake after glucose (▲) and xylose (△) were added as a function of NaCl concentration in suspensions of xylose-grown cells. (Inset) Ratio between V_{max} from proton 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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