Microbiology, Vol. 72, No. 4, 2003, pp. 414–417. Translated from Mikrobiologiya, Vol. 72, No. 4, 2003, pp. 466–469.

EXPERIMENTAL ARTICLES

The Activity of Xylose Reductase and Xylitol Dehydrogenase in Yeasts

E. N. Yablochkova*, O. I. Bolotnikova, N. P. Mikhailova*, N. N. Nemova**, and A. I. Ginak***

**St. Petersburg State Technological Institute (Technical University), Moskovskii pr. 26, St. Petersburg, 198013 Russia*

***Department of Molecular Biology and Biological and Organic Chemistry, Petrozavodsk State University, pr. Lenina 33, Petrozavodsk, 185640 Russia*

Received October 9, 2001

Abstract—The activity and the cofactor specificity of xylose reductase and xylitol dehydrogenase were studied in extracts of yeasts from the genera *Candida, Kluyveromyces, Pachysolen, Pichia,* and *Torulopsis* grown under microaerobic conditions. It was found that xylitol dehydrogenase in all of the yeast species studied is specific for NAD^+; xylose reductase in the xylitol-producing species *C. didensiae, C. intermediae, C. parapsilosis, C. silvanorum, C. tropicalis, Kl. fragilis, Kl. marxianus, P. guillermondii,* and *T. molishiama* is specific for NADPH; and xylose reductase in the ethanol-producing species *P. stipitis, C. shehatae,* and *Pa. tannophilus* is specific for both NADPH and NADH.

Key words: xylose-assimilating yeasts, xylose reductase, xylitol dehydrogenase.

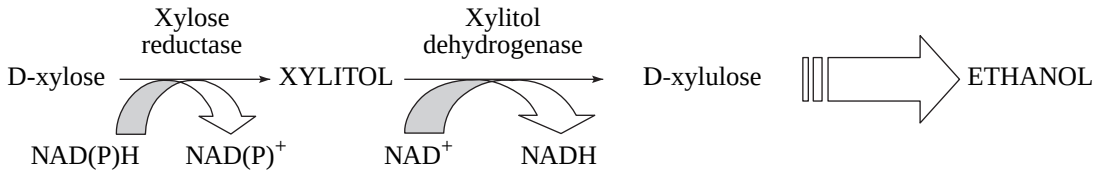
Under microaerobic conditions, yeasts are able to transform D-xylose-containing wastes with the formation of either xylitol or ethanol [1–3]. The production of these alcohols varies from strain to strain in even one species [4], suggesting the existence of a multilevel regulation of xylose catabolism in yeasts [2, 5, 6]. There are several metabolic stages that may affect the efficiency of D-xylose conversion into ethanol, the most considerable regulatory effect on the bioconversion being exerted by initial metabolic stages, during which D-xylose is first reduced to xylitol and then the latter is oxidized to D-xylulose [2, 3, 5, 6] (see figure). These redox reactions are catalyzed by xylose reductase (EC 1.1.1.21) and xylitol dehydrogenase (EC 1.1.1.9), respectively [7–9]. These enzymes are well studied in the ethanol-producing species *P. stipitis, C. shehatae,* and *Pa. tannophilus* [2, 3, 7–9], but not in xylitol-producing species. At the same time, a comparative analysis of these enzymes in these two groups of metabolically different xylose-assimilating yeasts could provide insight into the regulatory mechanisms involved in D-xylose conversion and contribute to the development of well-controllable biotechnologies of xylitol and ethanol production.

The aim of this work was to study the activity and the cofactor specificity of xylose reductase and xylitol dehydrogenase in yeasts with different abilities to produce xylitol and ethanol from D-xylose.

MATERIALS AND METHODS

The xylose-assimilating yeast strains used in this work, which are characterized by a relatively high efficiency of ethanol and xylitol production [4], are summarized in Table 1. The strains were maintained and cultivated in the media described by Ogorodnikova *et al.* [10]. The inocula were grown and the microaerobic fermentation of D-xylose was carried out as described earlier [11].

To prepare cell-free extracts, yeast cells were harvested by centrifugation at 5000 *g* for 5 min, washed with a 10 mM potassium phosphate buffer (pH 6.5) containing 1 mM 2-mercaptoethanol, and disrupted in a homogenizer as described by Zverlov *et al*. [12].



The initial stages of D-xylose catabolism in yeasts.

0026-2617/03/7204-0414$25.00

Table 1. The total specific activities of xylose reductase and xylitol dehydrogenase in yeasts with different abilities to produce xylitol and ethanol

Group	Yeast species and strain	Product yield, g/g xylose		Total specific activity, μmol/(min mg protein)	
		xylitol	ethanol	xylose reductase	xylitol dehydrogenase
I	*C. didensiae*, F-3	0.31–0.65	0.01–0.05	0.66	0.40
	C. intermediae, TabII (85)			4.90	3.83
	C. parapsilosis, 42			0.94	0.01
	C. silvanoru, VGI-II			5.36	4.32
	C. tropicalis, Y-456			6.57	1.16
	Kl. marxsianus, Y-488			0.16	0.02
	P. guilliermondii, Y-1017			0.63	0.18
	T. molishiama, 55			0.11	0.01
II	*Pa. tannophilus*, Y-1532, Y-1533, Y-1634	0.24–0.26	0.25–0.28	3.45–3.58	4.16–526
III	*C. shehatae*, Y-1632	0.03–0.09	0.39–0.40	8.33	13.95
	P. stipitis, Y-2160			15.21	8.64

Note: For each group of yeasts, the product yield ranges are given (data are taken from Ref. [4]). Similarly, for *Pa. tannophilus* strains, the enzymatic activity ranges are given. The standard error of measurements is within 5%.

The activities of xylose reductase and xylitol dehydrogenase in cell-free extracts were determined at room temperature from the rates of NAD(P)H oxidation and $NAD(P)^+$ reduction, respectively, which were measured spectrophotometrically at 340 nm [12]. One unit of activity was defined as the amount of enzyme oxidizing 1 μmol NAD(P)H or reducing 1 μmol $NAD(P)^+$ per min.

Protein concentration was determined by the method of Lowry *et al.* [13] using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Xylose reductase is an oxidoreductase that catalyzes the reduction of D-xylose into xylitol with NAD(P)H as the donor of hydrogen atoms. The xylitol formed in this reaction is oxidized by another oxidoreductase (xylitol dehydrogenase) with $NAD(P)^+$ as the acceptor of hydrogen atoms (figure). In these reactions, the rates of NAD(P)H oxidation and $NAD(P)^+$ reduction show the activity of the respective oxidoreductases [12].

Most yeasts produce xylitol and ethanol from D-xylose primarily under microaerobic conditions [4]. For this reason, cells for the analysis of the oxidoreductases were grown under a limited aeration of the cultivation medium. In terms of the efficiency of xylitol and ethanol formation, the strains under study were arbitrarily divided into three groups, I, II, and III [4] (Table 1).

The total xylose reductase and xylitol dehydrogenase activities. The total specific activity of xylose reductase was defined as its specific activity in the presence of both possible donors of hydrogen atoms, NADH and NADPH. Analogously, the total specific activity of xylitol dehydrogenase was defined as its specific activity in the presence of both possible acceptors of hydrogen atoms, NAD^+ and $NADP^+$. The respective data are presented in Table 1, from which it can be seen that the total specific activity of xylose reductase in the yeasts of group I varied from 0.11 to 6.57 μmol/(min mg protein), while that of xylitol dehydrogenase from 0.01 to 4.32 μmol/(min mg protein). The most active oxidoreductases (especially xylose reductase) were detected in the xylitol-producing yeasts *C. tropicalis*, *C. silvanorum*, and *C. intermediae.*

The three *Pa. tannophilus* strains of group II differed insignificantly in the total specific activities of the oxidoreductases, xylitol dehydrogenase activities being slightly higher than xylose reductase activities (Table 1).

The highest total specific activities of the oxidoreductases were detected in the yeasts of group III, the most active xylitol dehydrogenase (13.95 μmol/(min mg protein)) and xylose reductase (15.21 μmol/(min mg protein)) being in *C. shehatae* in *P. stipitis*, respectively (Table 1).

Thus, xylose-assimilating yeasts considerably differ in the total specific activity of enzymes catalyzing the conversion of D-xylose into D-xylulose. The highest total specific activities of xylose reductase and xylitol dehydrogenase were detected in ethanol-producing species; the lowest, in xylitol-producing species.

Specific activities of xylose reductase and xylitol dehydrogenase with particular cofactors. The specific activity of xylose reductase was measured in the presence of either NADH or NADPH. Correspondingly, the specific activity of xylitol dehydrogenase was measured in the presence of either NAD^+ or $NADP^+$.

Table 2. The specific activities of xylose reductase and xylitol dehydrogenase in yeasts

Group	Yeast species and strain	Specific activity, μmol/(min mg protein)			
		xylose reductase		xylitol dehydrogenase	
		NADPH	NADH	NAD^+	$NADP^+$
I	*C. didensiae*, F-3	0.63	0.03	0.40	<0.01
	C. intermediae, TabII (85)	4.74	0.16	3.62	0.21
	C. parapsilosis, 42	0.90	0.04	0.01	<0.01
	C. silvanoru, VGI-II	5.10	0.26	4.11	0.21
	C. tropicalis, Y-456	6.43	0.14	1.10	0.06
	Kl. marxsianus, Y-488	0.16	<0.01	0.04	<0.01
	P. guilliermondii, Y-1017	0.61	0.02	0.18	<0.01
	T. molishiama, 55	0.11	<0.01	0.01	<0.01
II	*Pa. tannophilus*, Y-1532, Y-1533, Y-1634	2.00–2.10	1.39–1.56	4.04–5.26	0.01–0.12
III	*C. shehatae*, Y-1632	3.40	4.93	13.53	0.42
	P. stipitis, Y-2160	4.84	10.37	8.37	0.27

Note: For *Pa. tannophilus* strains, the enzymatic activity ranges are given. The standard error of measurements does not exceed 5%.

The results presented in Table 2 show that the specific activities of NADPH-dependent xylose reductase in the yeasts of group I varied from 0.11 to 6.43 μmol/(min mg protein) and comprised 95–100% of the respective total specific activities. At the same time, xylitol dehydrogenases in the yeasts of this group turned out to be predominantly NAD^+-dependent.

The xylose reductases of *Pa. tannophilus* strains (group II) were specific for both cofactors, NADPH and NADH, although their activities with NADPH (2–2.1 μmol/(min mg protein) or 56–60% of the total specific activity) were higher than with NADH (Table 2). In contrast, the xylose reductases of ethanol-producing strains (group III) were more active with NADH than with NADPH.

The xylitol dehydrogenases of *Pa. tannophilus* strains (group II) and *C. shehatae* and *P. stipitis* (group III) were predominantly NAD^+-specific. The xylitol dehydrogenase activities of *Pa. tannophilus* strains varied from 4.04 to 5.26 μmol/(min mg protein). The NAD^+-specific xylitol dehydrogenase activities of *C. shehatae* and *P. stipitis* were very high (13.53 and 8.37 μmol/(min mg protein), respectively) and comprised 97–99% of their total specific activities.

Thus, all of the yeast strains under study had NAD^+-specific xylitol dehydrogenase, irrespective of the fermentation product produced from D-xylose (xylitol or ethanol). At the same time, the xylose reductase of xylitol-producing strains was specific for NADPH, whereas the xylose reductase of ethanol-producing strains was specific for both NADPH and NADH.

The type of the cofactor dependence of xylose reductase may affect the proportion between the amounts of the xylitol and D-xylulose produced. This suggestion is confirmed by the fact that the *Pa. tannophilus* strains, whose xylose reductases are almost equally specific for NADH and NADPH, produce xylitol and ethanol in comparable amounts, while *C. shehatae* and *P. stipitis*, whose xylose reductases are more specific for NADH than for NADPH, produce predominantly ethanol. Therefore, the intense formation of D-xylulose from D-xylose is a necessary condition for the production of ethanol (figure). The blocking of xylulose formation because of the predominant dependence of xylose reductase on NADPH leads to the production of xylitol as the major product of D-xylose fermentation. These data are in agreement with the data published by other authors [7–9, 12].

It can be anticipated that yeast strains with a high activity of NADPH-dependent xylose reductase must be potential producers of xylitol from D-xylose. The increased activity of NADH-dependent xylose reductase will shift the equilibrium of the initial reactions of D-xylose metabolism toward the formation of D-xylulose and, in the final analysis, ethanol (figure). *Pa. tannophilus* is distinguished from other xylose-assimilating yeasts in that its xylose reductase is specific for both NADH and NADPH, which makes this species a convenient tool for elucidating and studying the rate-limiting steps of xylose biotransformation. Knowledge of the regulatory mechanisms involved in D-xylose catabolism should make possible the cell engineering of xylose-assimilating yeasts with a high yield of required products.

REFERENCES

1. Dart, R.K. and Bets, W.F., *Uses and Potential of Lignocellulose*, London, 1991.

2. Schneider, H., Conversion of Pentoses to Ethanol by Yeast and Fungi, *Crit. Rev. Biotech.*, 1989, no. 9, pp. 1–40.
3. Ojamo, H., Yeast Xylose Metabolism and Xylitol Production, *VTT Biotech. Food Research*, Finland, 1994, no. 176.
4. Yablochkova, E.N., Bolotnikova, O.I., Mikhailova, N.P., Nemova, N.N., and Ginak, A.I., Transformation of D-Xylose and D-Glucose by Xylose-assimilating Yeasts, *Mikrobiologiya* (in press).
5. Prior, B.A., Kilian, S.G., and du Preez, J.C., Fermentation of D-Xylose by the Yeasts *Candida shehatae* and *Pichia stipitis*, *Process Biochem.*, 1989, vol. 24, no. 1, pp. 21–32.
6. Slininger, P.J., Bolen, P.L., and Kurtzman, C.P., *Pachysolen tannophilus*: Properties and Process Considerations for Ethanol Production from D-Xylose, *Enzyme Microb. Technol.*, 1987, vol. 9, no. 1, pp. 5–10.
7. Bolen, P.L. and Detroy, R.W., Induction of NADPH-linked D-Xylose Reductase and NAD^+-linked Xylitol Dehydrogenase in *Pachysolen tannophilus* by D-Xylose, L-Arabinose, or D-Galactose, *Biotechnol. Bioeng.*, 1985, vol. 27, pp. 302–306.
8. Bicho, P.A., Runnals, P.L., Cunningham, J.D., and Lee, H., Induction of Xylose Reductase and Xylitol Dehydrogenase Activities in *Pachysolen tannophilus* and *Pichia stipitis* on Mixed Sugars, *Appl. Environ. Microbiol.*, 1986, vol. 54, pp. 50–57.
9. Girio, F.M., Peito, M.A., and Amaral-Collaco, M.T., Enzymatic and Physiological Study of D-Xylose Metabolism by *Candida shehatae*, *Appl. Microbiol. Biotechnol.*, 1989, vol. 32, pp. 199–204.
10. Ogorodnikova, T.E., Mikhailova, N.P., Borokhova, O.E., Yablochkova, E.N., Andreevskii, V.V., and Shapovalov, O.I., The Growth Parameters of the Xylose-assimilating Yeasts *Pachysolen tannophilus* and *Candida shehatae*, *Mikrobiologiya*, 1995, vol. 64, no. 1, pp. 13–17.
11. Borokhova, O.E., Ogorodnikova, T.E., Mikhailova, N.P., and Shapovalov, O.I., Microbial Conversion of Hemicellulose into Ethanol: 3. The Fermentation of Wood Hemicellulose Sugars into Ethanol by *Pachysolen tannophilus*, *Gidrol. Lesokhim. Prom.*, 1993, no. 3, pp. 3–5.
12. Zverlov, V.V., Yur'ev, M.Z., and Mogutov, I.A., The Study of Xylose Metabolism Enzymes in Yeasts, *Biotechnologiya*, 1990, no. 5, pp. 20–21.
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.G., Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem.*, 1951, vol. 193, no. 1, pp. 265–275.