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EXPERIMENTAL ARTICLES

Investigation of the Synthesis of Xylose/Glucose Isomerases in Six *Arthrobacter* sp. Strains

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Abstract—The substrate specificity of isomerases produced by six strains of *Arthrobacter* sp. was studied. The role of utilizable carbon sources in controlling enzyme biosynthesis was established. All of the strains studied were found to produce xylose isomerases efficiently, converting D-xylose into D-xylulose and D-glucose into D-fructose. All but *A. ureafaciens* B-6 strains showed low activity toward D-ribose, *Arthrobacter* sp. B-5 was slightly active toward L-arabinose, and *A. ureafaciens* B-6 and *Arthrobacter* sp. B-2239, toward L-rhamnose. In *Arthrobacter* sp. B-5, the synthesis of xylose/glucose isomerase was constitutive (i.e., it was not suppressed by readily metabolizable carbon sources. The synthesis of xylose/glucose isomerase induced by D-xylose in *Arthrobacter* sp. strains B-2239, B-2240, B-2241, and B-2242 and by D-xylose and xylitol in *A. ureafaciens* B-6 was suppressed by readily metabolizable carbon sources in a concentration-dependent manner. The data obtained suggest that D-xylose and/or its metabolites are involved in the regulation of xylose/glucose isomerase synthesis in the *Arthrobacter* sp. strains B-5, B-2239, B-2240, and B-2241.

Key words: bacteria, *Arthrobacter*, xylose/glucose isomerase, substrate specificity, biosynthesis

Microbial xylose isomerase (D-xylose ketol-isomerase, EC 5.3.1.5) has a broad substrate specificity and can convert D-xylose, D-glucose, and some other aldo sugars into respective keto sugars. Immobilized xylose/glucose isomerases are widely used for the production of glucose–fructose syrups [1].

In most microorganisms, the synthesis of xylose/glucose isomerase is induced by D-xylose or its structural analogues [2, 3], while only few microorganisms isolated from natural sources are capable of constitutive isomerase synthesis [4–6]. The majority of the known producers of xylose/glucose isomerase are mutant or genetically engineered strains with the constitutive synthesis of isomerase [7–9].

Earlier, we selected six *Arthrobacter* sp. strains with glucose isomerase activity [10]. The present work was aimed at studying the substrate specificity of the isomerases produced by these strains and elucidating the role of carbon sources in the regulation of enzyme synthesis.

MATERIALS AND METHODS

The bacteria *Arthrobacter ureafaciens* B-6, *Arthrobacter* sp. strains B-5, B-2239, B-2240, B-2241, and B-2242 with glucose isomerase activity were obtained from the Collection of Microorganisms of the Institute of Microbiology, National Academy of Sciences of Belarus.

The bacteria were cultivated in a submerged mode in shaken (180–200 rpm) 250-ml Erlenmeyer flasks at 28–30°C for 24–96 h. The growth medium contained (%) peptone, 1.0; yeast extract, 0.5; K_2HPO_4, 0.3; $MgSO_4 \cdot 7H_2O$, 0.1; and various carbon sources in amounts of 1% by carbon in the case of simple compounds or 1 wt % in the case of polysaccharides. The initial pH of the medium was pH 6.8. The medium was inoculated with aqueous suspensions of bacteria grown on a peptone–yeast extract agar at 28–30°C for 3 days. The initial density of cultures was $(0.5–1) \times 10^7$ cells/ml.

Bacterial growth was monitored by measuring culture turbidity at 540 nm (OD_{540}) or by estimating the concentration of biomass in mg dry wt per ml. The specific growth rate of bacteria was calculated by the formula $\mu = dxdt^{-1}x^{-1}$, where μ is the specific growth rate expressed in h^{-1}; x is biomass expressed in optical density units (OD_{540}); and dx is the biomass increase in time dt (time is expressed in h).

Isomerase activity was measured in a reaction mixture containing 0.2 ml of 1 M substrate solution, 0.5 ml of 0.2 M K,Na–phosphate buffer (pH 7.8), 0.1 ml of 0.1 M $MgSO_4 \cdot 7H_2O$, 20 mg of cells, and distilled water to a total volume of 2 ml. The mixture was incubated at 70°C for 1 h. The substrate specificity of isomerases was studied using D-glucose, D-xylose, D-mannose, L-arabinose, L-galactose, D-maltose, L-rhamnose, D-ribose, and 2-deoxy-D-glucose.

The keto sugars produced were determined quantitatively by the cysteine–carbazole method [11] and qualitatively by ascending thin-layer chromatography on Silufol UV 254 plates developed two times in a

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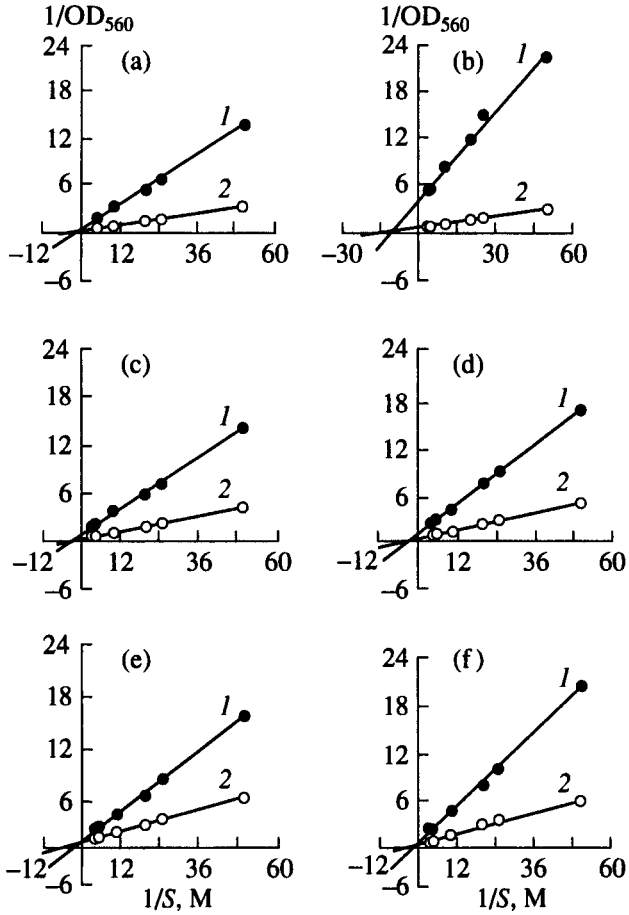

Fig. 1. Lineweaver–Burk double reciprocal plot of the rates of isomerization of (*1*) D-glucose and (*2*) D-xylose by the xylose/glucose isomerases of (a) *Arthrobacter* sp. B-5, (b) *A. ureafaciens* B-6, (c) *Arthrobacter* sp. B-2239, (d) *Arthrobacter* sp. B-2240, (e) *Arthrobacter* sp. B-2241, and (f) *Arthrobacter* sp. B-2242.

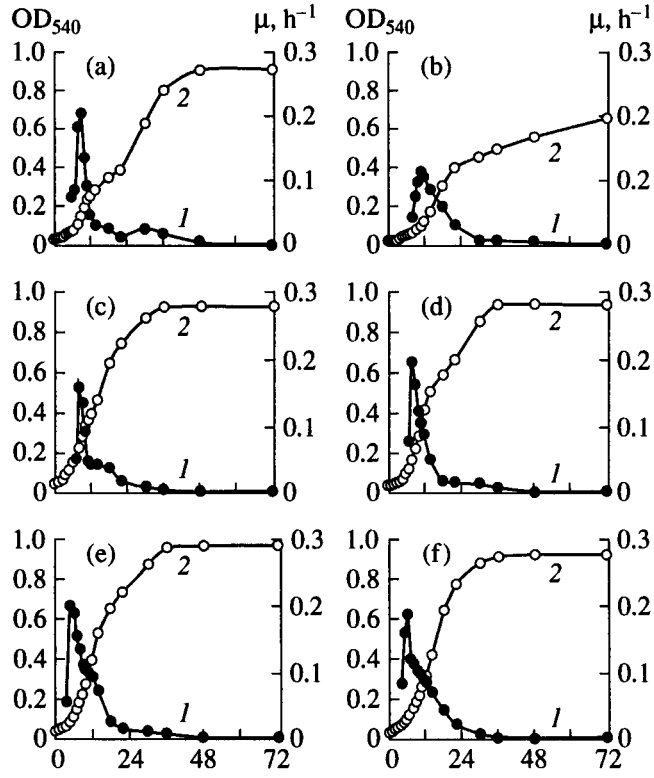

Fig. 2. (*1*) Specific growth rate (μ) and (*2*) biomass (OD_{540}) accumulated in media with D-xylose by (a) *Arthrobacter* sp. B-5, (b) *A. ureafaciens* B-6, (c) *Arthrobacter* sp. B-2239, (d) *Arthrobacter* sp. B-2240, (e) *Arthrobacter* sp. B-2241, and (f) *Arthrobacter* sp. B-2242.

chloroform–acetic acid–water (6 : 7 : 1) mixture. To visualize spots, the developed plates were dried, sprayed with a 0.2% solution of β-naphthoresorcinol in 96% ethanol containing 0.1 vol % phosphoric acid, and heated at 105°C for 5 min.

One unit (U) of isomerase activity was defined as the amount of enzyme converting 1 μmol of substrate in min under the conditions described above. Isomerase activity was expressed in U/mg dry biomass or in optical density units (OD_{560}). The productivity of enzyme synthesis by strains was expressed in U/ml medium.

The Michaelis constant (K_m) was determined from the Lineweaver–Burk double reciprocal plot.

The pH of the medium was measured potentiometrically.

The data presented in this paper are the means of 3–5 independent experiments performed in triplicate.

RESULTS AND DISCUSSION

Microbial xylose/glucose isomerases have strong affinity for D-xylose and D-glucose [12–14], but they often possess some affinity for D-ribose, D-rhamnose, D-galactose, L-arabinose, and 2-deoxy-D-glucose [15, 16].

When grown in media with D-xylose, all the studied *Arthrobacter* sp. strains produced isomerases capable of converting D-xylose into D-xylulose and D-glucose into D-fructose. The isomerases of all but *A. ureafaciens* B-6 strains also showed low activity toward D-ribose, while the isomerase of *Arthrobacter* sp. B-5 was slightly active toward L-arabinose, and the isomerases of *A. ureafaciens* B-6 and *Arthrobacter* sp. B-2239, toward L-rhamnose.

The effect of various substrate concentrations on the rate of isomerization of D-xylose and D-glucose is shown in Fig. 1 in Lineweaver–Burk coordinates. The lower values of the Michaelis constants (K_m) of isomerases from all of the strains studied with respect to D-xylose indicated a higher enzyme affinity for this substrate. This allowed us to classify these isomerases as relatively nonspecific xylose isomerases (D-xylose ketolisomerase, EC 5.3.1.5) having higher or lower, depending on the particular strain, affinity to D-glucose. The xylose/glucose isomerase of *A. ureafaciens*

Table 1. Effect of carbon sources on the synthesis of xylose/glucose isomerase by bacteria of the genus *Arthrobacter*

Carbon source, 1%	Xylose/glucose isomerase activity, U/mg					
	Arthrobacter sp. B-5	*A. ureafaciens* B-6	*Arthrobacter* sp. B-2239	*Arthrobacter* sp. B-2240	*Arthrobacter* sp. B-2241	*Arthrobacter* sp. B-2242
Xylose	0.0188	0.0308	0.0104	0.0064	0.0020	0.0036
Xylan	0.0288	0	0	0	0	0
Xylitol	0.0284	0.0316	0	0	0	0
Apple pectin	0.0252	0	0	0	0	0
Glycerol	0.0068	0	0	0	0	0
Starch	0.0076	0	0	0	0	0
Citric acid	0.0196	0	0	0	0	0
Sucrose	0.0304	0	0	0	0	0
Fructose	0.0052	0	0	0	0	0
Glucose	0.0216	0	0	0	0	0

Table 2. Effect of supplementary carbon sources on the synthesis of xylose/glucose isomerase by bacteria of the genus *Arthrobacter*

Supplementary carbon source	*Arthrobacter* sp. B-5		*A. ureafaciens* B-6		*Arthrobacter* sp. B-2239		*Arthrobacter* sp. B-2240		*Arthrobacter* sp. B-2241		*Arthrobacter* sp. B-2242	
	Xylose/glucose isomerase activity											
	U/mg	U/ml	U/mg	U/ml	U/mg	U/ml	U/mg	U/ml	U/mg	U/ml	U/mg	U/ml
None	0.0112	0.101	0.0108	0.073	0.0080	0.098	0.0120	0.120	0.0080	0.076	0.0072	0.068
Xylose	0.0156	0.285	0.0192	0.186	0.0104	0.204	0.0124	0.326	0.0084	0.193	0.0108	0.240
Glucose	0.0164	0.324	0.0052	0.042	0.0044	0.111	0.0044	0.136	0.0056	0.150	0.0036	0.109
Fructose	0.0124	0.223	0.0096	0.098	0.0056	0.132	0.0064	0.116	0.0052	0.114	0.0048	0.116
Xylitol	0.1640	0.288	0.0204	0.201	0.0052	0.147	0.0072	0.197	0.0044	0.097	0.0048	0.140
Sucrose	0.0180	0.350	0.0036	0.037	0.0024	0.068	0.0040	0.108	0.0048	0.116	0.0036	0.113
Lactose	0.0168	0.307	0.0100	0.102	0.0024	0.061	0.0024	0.061	0.0012	0.028	0.0032	0.085

Note: Bacteria were cultivated in media with 0.5% D-xylose for 24 h and then the respective supplementary carbon source was added to the medium at a concentration of 0.5%.

B-6 (K_m = 0.091 M) showed maximal affinity for D-glucose, whereas the enzymes of *Arthrobacter* sp. B-5 (K_m = 0.581 M) and *Arthrobacter* sp. B-2242 (K_m = 0.516 M) had the lowest affinity for D-glucose.

All of the bacteria studied, except *Arthrobacter* sp. B-5, produced xylose/glucose isomerases only in the presence of D-xylose in the growth medium; *A. ureafaciens* B-6 also required xylitol (Table 1). *Arthrobacter* sp. B-5 was the only strain that possessed high isomerase activity on all carbon sources tested, which implies that this bacterial strain synthesizes xylose/glucose isomerase constitutively.

The ability to produce xylose/glucose isomerases on various growth substrates was also shown for *Aerobacter aerogenes* HN-56, *Arthrobacter* sp. ATCC 13717, and *Escherichia intermedia* HN-500 [4–6]. Although some readily metabolizable carbon sources could induce isomerase production in *Arthrobacter* sp. 376A, the presence of D-xylose in the growth medium stimulated the synthesis of this enzyme [12]. *Bacillus coagulans* HN-68 grown in a medium without D-xylose exhibited no xylose/glucose isomerase activity [17].

For many microorganisms, D-xylose is a not easily utilizable carbon source [12, 18–20]. In view of this, readily metabolizable carbon sources are typically introduced into growth media to enable the microorganisms to efficiently produce xylose/glucose isomerases [2, 3, 6].

As follows from the data presented in Fig. 2, all of the *Arthrobacter* sp. strains under study showed high specific growth rates during growth on D-xylose but differed with respect to the time in which the maximum specific growth rate was reached. Thus, strains B-2239, B-2241, and B-2242 utilized D-xylose almost without a lag period, showing a maximal specific growth rate by the 5th–6th hour of cultivation. *Arthrobacter* sp. B-5,

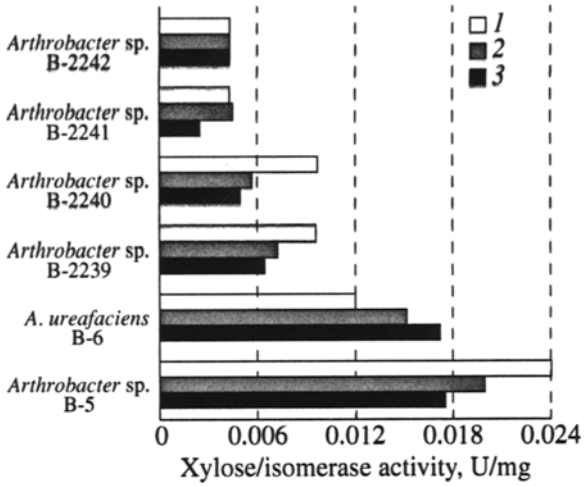

Fig. 3. Synthesis of xylose/glucose isomerases by bacteria of the genus *Arthrobacter* at different concentrations of D-xylose in the medium (%): (*1*) 0.5, (*2*) 1.0, and (*3*) 1.5.

A. ureafaciens B-6, and *Arthrobacter* sp. B-2240 had a lag period of 1–2 h and showed a maximal specific growth rate (0.114–0.205 h^{-1}) by the 8th–11th hour of cultivation. In the case of *Arthrobacter* sp. 36A, this parameter was the lowest (0.026 h^{-1}) [12].

The studied synthesis of xylose/glucose isomerase in the bacteria depended on the concentration of D-xylose in the growth medium (Fig. 3). Increasing D-xylose concentration to 1.5% did not affect enzyme synthesis in *Arthrobacter* sp. B-2242, enhanced it in *A. ureafaciens* B-6, and inhibited it in *Arthrobacter* sp. strains B-5, B-2239, and B-2241. The reasons for the inhibitory effect of high concentrations of D-xylose on xylose/glucose isomerase can be the subject of a separate study. Now, we can only suggest the involvement of D-xylose metabolites in the regulation of the enzyme biosynthesis.

The addition of any of the carbon sources tested to the D-xylose-containing growth medium inhibited the synthesis of xylose/glucose isomerase by *Arthrobacter* sp. strains B-2239, B-2240, B-2241, and B-2242, although the same carbon sources added at the same concentrations failed to suppress the enzyme activity in cell-free extracts (Table 2). Xylitol was the only carbon source that did not suppress enzyme synthesis in *A. ureafaciens* B-6. On the other hand, only D-fructose, the product of isomerization of D-glucose, inhibited xylose/glucose isomerase synthesis in *Arthrobacter* sp. B-5.

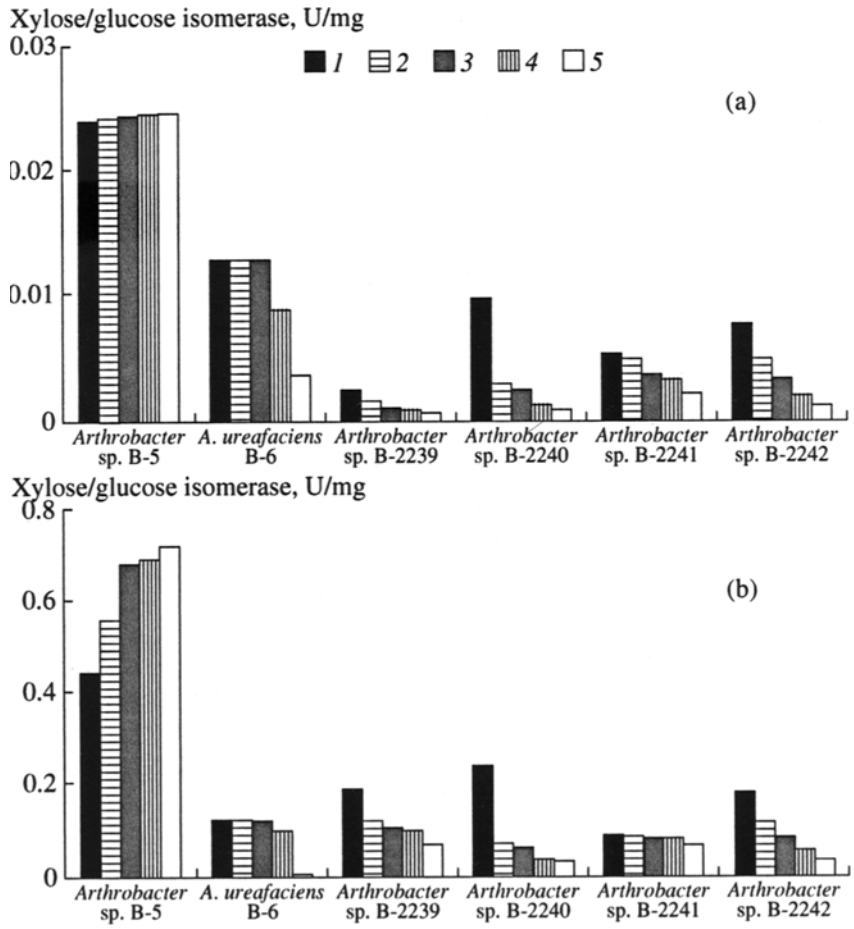

Fig. 4. Effect of different concentrations of D-glucose added to the medium with 0.5% D-xylose on the (a) intensity and (b) productivity of xylose/glucose isomerase synthesis by bacteria of the genus *Arthrobacter*. Glucose was added at concentrations (%): (*1*) 0, (*2*) 0.1, (*3*) 0.3, (*4*) 0.5, and (*5*) 1.0.

The results of investigation of the effect of glucose on enzyme synthesis in the bacteria studied are presented in Fig. 4. It can be seen that the addition of this readily metabolizable carbon source to the medium containing 0.5% xylose substantially increased the productivity of enzyme synthesis in *Arthrobacter* sp. B-5. In *A. ureafaciens* B-6, glucose present in the medium at a concentration of 0.1–0.3% did not affect enzyme synthesis but substantially inhibited it when present at a concentration of 0.5–1%. The inhibitory effect of glucose on enzyme production in *Arthrobacter* sp. strains B-2239, B-2240, B-2241, and B-2242 depended on its concentration and was particularly pronounced in strains B-2240 and B-2242.

Thus, the data obtained in the present work suggest that prokaryotes possess complex systems of regulation of the constitutive and inducible syntheses of xylose/glucose isomerase. Further investigations should throw more light on the regulation mechanisms of the xylose/glucose isomerase activity in bacteria.

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