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EXPERIMENTAL ARTICLES

Catabolite Repression of Xylose Isomerase Synthesis in *Arthrobacter ureafaciens*

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Abstract—The effect of a specific substrate as well as other carbon sources on the biosynthesis of xylose isomerase in the actinobacterium *Arthrobacter ureafaciens* BIM B-6 has been studied. It was established that xylose and its structural analogue xylite induced the production of the enzyme by bacterial cells. The inducing effect peaked at a concentration of specific substrates of 0.025% (as carbon) and then remained unchanged irrespective of the substrate amount. It has been shown that the synthesis of xylose isomerase by *A. ureafaciens* is controlled by catabolite repression occurring at the transcription level and mediated by cyclic 3',5'-AMP.

Key words: Arthrobacter ureafaciens, actinobacteria, xylose isomerase, biosynthesis, induction, catabolite repression, cyclic 3',5'-AMP.

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Xylose isomerase (D-xylose ketol isomerase, EC 5.3.1.5) is the key enzyme in prokaryotic xylose metabolism, as well as one of the most important biocatalysts in the world market for enzymes. Xylose isomerase has broad substrate specificity; apart from isomerization of xylose to xylulose, it catalyzes conversion of glucose to fructose. Due to the latter capacity, xylose isomerase is widely used for commercial production of glucose–fructose syrups from saccharified starch-containing raw materials.

Elucidation of the regulatory mechanisms of the biosynthesis of bioactive compounds (including xylose isomerase) is a prerequisite for efficient use of the genetic properties of the strains which produce these compounds. As a rule, a nonspecific substrate and its structural analogues induce the production of xylose isomerase by prokaryotes, whereas glucose represses this process [1]. The organization and regulation of genes responsible for the synthesis of xylose isomerase and other enzymes of xylose metabolism was studied in *Escherichia coli* [2], *Salmonella typhimurium* [3], *Bacillus subtilis* [4, 5], *Bacillus megaterium* [6], and *Streptomyces rubiginosus* [7] as examples. Among the xylose-utilizing representatives of the genus *Arthrobacter*, species with both induced and constitutive synthesis of xylose isomerase synthesis were reported [8, 9]. However, no detailed investigations have been carried out into the mechanisms controlling enzyme production in this group of gram-positive prokaryotes.

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We have previously selected the actinobacterium *Arthrobacter ureafaciens*, which produces xylose isomerase during growth on xylose-containing media [10]. The goal of the present work was to study the role of the specific substrate and easily metabolized carbon sources in the production of xylose isomerase by *A. ureafaciens.*

MATERIALS AND METHODS

This study was conducted with the strain *Arthrobacter ureafaciens* BIM B-6 from the Belarusian Collection of Nonpathogenic Microorganisms (Institute of Microbiology, National Academy of Sciences of Belarus).

Bacteria were grown on an agarized medium (pH 7.2–7.4) containing the following (%): peptone, 1.0; yeast extract, 0.5; glucose, 0.5; NaCl, 0.5; and agar, 1.5.

To obtain exponential phase cells, submerged cultures of *A. ureafaciens* were grown at 28–30°C for 27 ± 3 h on a shaker (180–200 rpm) in 250-ml Erlenmeyer flasks with 50 ml of nutrient medium (pH 6.8) containing the following (%): peptone, 1.0; yeast extract, 0.5; K_2HPO_4, 0.3; and $MgSO_4 \cdot 7H_2O$, 0.1 [11].

The water suspension of *A. ureafaciens* cells (2 vol%, $OD_{540} = 0.2 \pm 0.01$) from the culture grown on the araized medium with peptone and yeast extract at 28–30°C for 3 days was used as inoculum.

After cultivation, bacterial cells were harvested by centrifugation (8000 *g*, 15 min) and washed with dis-

tilled water and then with 0.2 M potassium–sodium phosphate buffer (pH 7.8).

Experiments with the washed *A. ureafaciens* cells were carried out in 500-ml Erlenmeyer flasks containing 100 ml of the incubation media on a shaker (180–200 rpm) at 28–30°C for 4–6 h [11].

To study the effect of the carbon sources on xylose isomerase production, *A. ureafaciens* cells were incubated in the above-described buffer solution containing mono- and disaccharides, aldoses and ketoses, or sugar alcohols (0.004–0.5%).

In all experiments, the concentrations of carbohydrates were equalized according to their carbon content.

To study the repression of xylose isomerase synthesis, the cells were incubated in a buffer solution supplemented with the specific substrate (0.1%) for 1 h. After that, glucose or other carbon sources (0.1%), cyclic 3',5'-AMP (1×10^{-3} M), as well as translation (chloramphenicol) and transcription (actinomycin D and streptomycin) inhibitors (20 μg/ml) were added. The experiments aimed at the elucidation of the effect of glucose added in various concentrations and at various time points on xylose isomerase synthesis by *A. ureafaciens* were performed in a different manner. In the former case, the agent (0.025, 0.1, or 0.5%) was added to the medium simultaneously with bacteria and the inducer (0 h); in the latter case, it was added after 0, 1, 2, or 3 h of incubation.

During all the experiments, the biomass content (evaluated in terms of optical density at $\lambda = 540$ nm) in the incubation media was 0.2 ± 0.01 [11].

The quantitative assessment of biomass yield in the bacterial suspension was performed by photoelectric colorimetry at 540 nm. After that, the biomass was calculated using the calibration curve of the relationship between the optical density of the suspension and the dry biomass weight.

To determine xylose isomerase activity, as well as to monitor the quality and quantity of bacterial biomass, samples were taken either once after 4–6 h from the beginning of exposure in the model incubation media or every 0.5–1 h during the above-stated period of time.

The reaction mixture used for determination of xylose isomerase activity of *A. ureafaciens* contained 1 M xylose solution, 0.2 ml; 0.2 M K, Na phosphate buffer (pH 7.8), 0.5 ml; 0.1 M $MgSO_4 \cdot 7H_2O$, 0.1 ml; and cell suspension or extract, 0.5 ml. Distilled water was added to a volume of 2 ml. The duration of the isomerization reaction was 1 h at 70°C.

The amount of xylulose was determined by the cysteine–carbazole method [12].

A xylose isomerase activity unit corresponds to the enzyme quantity that, within 1 min, converts 1 μmol of xylose under the above conditions. The enzymatic activity was expressed in U/mg dry biomass.

To obtain the proteins produced by *A. ureafaciens*, the cells were mechanically homogenized with silica sand (1 : 1) and extracted with 0.2 M K–Na phosphate buffer (pH 7.8) at 28–30°C for 1 h [13]. Cell-free extracts were obtained by centrifugation of cell homogenates at 8000 *g* for 15 min.

Native protein electrophoresis was carried out according to Davis [14]. Electrophoresis duration was 1.5–2 h at 20 mA per one 130×130 mm plate gel using 0.025 M Tris glycine electrode buffer (pH 8.5).

After electrophoresis, the gels were washed with distilled water and incubated at 70°C for 10–20 min in the reaction mixture containing distilled water, 0.2 M K–Na phosphate buffer (pH 7.8), 0.1 M $MgSO_4 \cdot 7H_2O$ solution, and 1 M xylose solution (12 : 5 : 2 :1). The gels were washed with distilled water and developed with a 0.1% 2,3,5-triphenyl tetrazolium chloride solution in 1 M NaOH in the dark at 30°C for 1 min. Xylose isomerase can thus be identified as a pink-red formazan band produced by the oxidation of colorless 2,3,5-triphenyl tetrazolium chloride by xylulose, the product of xylose isomerization [15, 16].

The protein concentration was determined by the Bradford method [17]; pH was measured potentiometrically.

The results presented in the paper are averages of two or three measurements, each of which was performed in triplicate. The results were statistically examined. For a simple average, a confidence interval was determined setting the probability level at 0.05 [18, 19]. The difference between two simple averages was considered reliable if their confidence intervals did not overlap. The results were statistically examined with the Microsoft Windows software package.

RESULTS AND DISCUSSION

Short-term, so-called acute experiments designed to maintain the required physicochemical conditions represent one of the methodological techniques widely used in the studies of the mechanisms that control the production of extra- and intracellular enzymes in microbial cells. In such a setup, microbial cells, including the *A. ureafaciens* cells used in our studies, remain viable, and their numbers do not vary during the experiments [11, 20–23].

The data on the effect of various carbon sources on the production of xylose isomerase by *A. ureafaciens* cells presented in the table demonstrate that the enzymatic activity was detected in bacterial cells only when they were incubated in the media containing xylose or xylite, its structural analogue. The addition of even small amounts (0.004%) of these specific substrates to the incubation medium induced xylose isomerase synthesis in *A. ureafaciens* cells. The maximal inducing effect exerted by xylose or xylite on the enzyme production by bacterial cells was detected when their concentration in the medium was 0.025%; higher substrate

Effect of carbon sources on xylose isomerase synthesis by *A. ureafaciens*

Carbon source	Carbon source concentration, % C	Xylose isomerase, U/mg
K, Na phosphate buffer (pH 7.8)	0	0
Xylose	0.004	0.030 ± 0.001
	0.01	0.046 ± 0.001
	0.025	0.060 ± 0.002
	0.1	0.060 ± 0.002
	0.25	0.061 ± 0.001
	0.5	0.061 ± 0.001
Xylite	0.004	0.044 ± 0.001
	0.01	0.054 ± 0.001
	0.025	0.062 ± 0.002
	0.1	0.063 ± 0.001
	0.25	0.062 ± 0.002
	0.5	0.062 ± 0.002
Glucose	0.1	0
Fructose	0.1	0
Rhamnose	0.1	0
Arabinose	0.1	0
Glycerol	0.1	0
Sucrose	0.1	0
Citric acid	0.1	0

concentrations did not result in its further increase under experimental conditions. Similar results were obtained by Takasaki (1974), who studied the inducing effect of xylose and xylobiose on xylose isomerase biosynthesis by the non-growing culture of *Streptomyces* sp. YT-no. 5 [20].

It was established that the inducing effect of the specific substrates on xylose isomerase production by *A. ureafaciens* can be discovered no later than 30 min after their addition to the incubations medium. This effect was intensified over a period of at least four hours (Fig. 1a). Electrophoresis of the native intracellular proteins of *A. ureafaciens* and identification of xylose isomerase in the gel plates also indicated that the de novo synthesis of this enzymatic protein was induced only by xylose or xylite (Fig. 1b).

Figure 2 shows the results of our study on the effect of glucose and other easily metabolized carbon sources (0.1%) on xylose isomerase synthesis by the *A. ureafaciens* cells preincubated in the presence of xylose or xylite for 1 h. Glucose, rhamnose, and glycerol inhibited the production of xylose isomerase by bacterial cells almost completely after 1–2 h. Under the experimental conditions, the repression by disaccharides lactose and sucrose 2 h after their addition to the incubation medium was only 60%.

In *A. ureafaciens* cells, even the smallest concentrations of glucose (0.025%) inhibited xylose isomerase synthesis by more than 90%. An increase in the repressor concentration in the medium up to 0.25% completely inhibited the enzyme production by bacterial cells, irrespective of the time of addition of the repressor to the xylose-containing incubation medium (Fig. 3).

To determine the level of realization of catabolite repression of xylose isomerase synthesis, the enzymatic activity of the *A. ureafaciens* cells incubated in the presence of glucose and the protein synthesis inhibitors was compared. The data shown in Fig. 4 indicate that the addition of chloramphenicol (which inhibits the translation stage of protein biosynthesis in prokaryotes) to the xylose-containing medium resulted in a small increase in the enzymatic activity of *A. ureafaciens* cells, probably due to the incomplete blockage of translation. The effect of glucose on the enzyme production by bacterial cells was similar to that of the transcription suppressors actinomycin D and streptomycin. Taking into account the results obtained, it can be suggested that the repression of xylose isomerase synthesis in *A. ureafaciens* by glucose occurs at the transcription level.

The regulatory function of cyclic 3',5'-AMP in the course of catabolite repression of the synthesis of xylose catabolism enzymes in some prokaryotic cells is well known. The derepression of enzymatic protein synthesis under the influence of an exogenic nucleotide [24, 25] may be considered a circumstantial evidence of this fact.

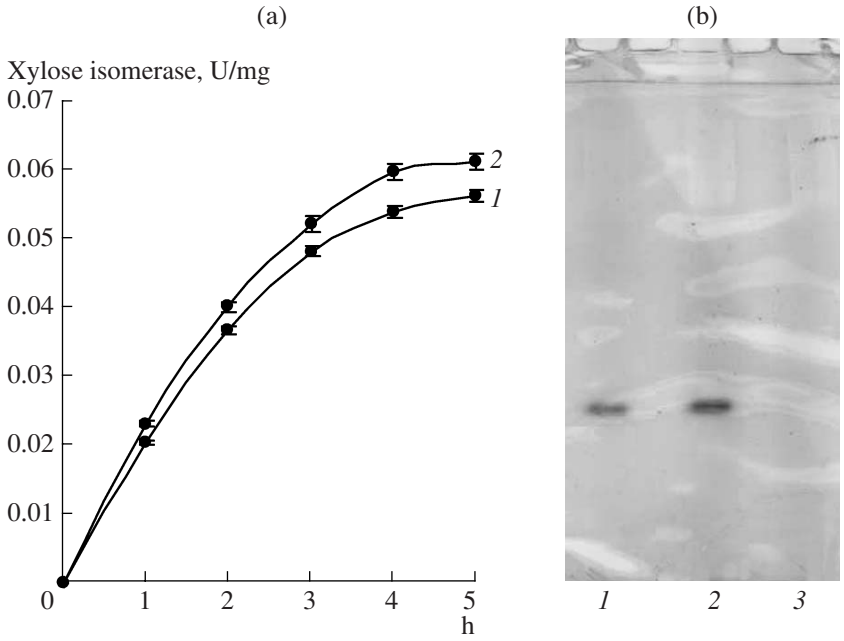

Fig. 1. Effect of xylose (*1*), xylite (*2*), and glucose (*3*) on the synthesis of xylose isomerase by *A. ureafaciens*: (a) process dynamics and (b) zymogram of the native intracellular proteins.

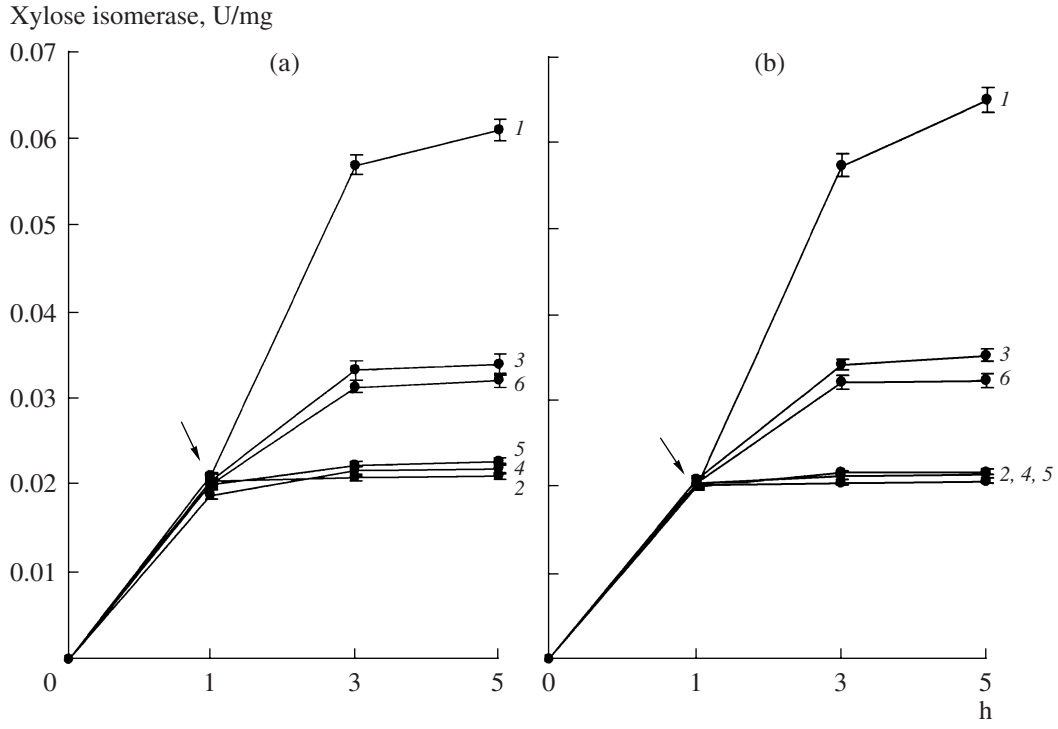

Fig. 2. Repression of xylose isomerase synthesis by *A. ureafaciens* in the incubation media with (a) xylose and (b) xylite: xylose (control) (*1*); glucose (*2*); lactose (*3*); glycerol (*4*); rhamnose (*5*); and sucrose (*6*); ⟶ designates the moment of repressor addition.

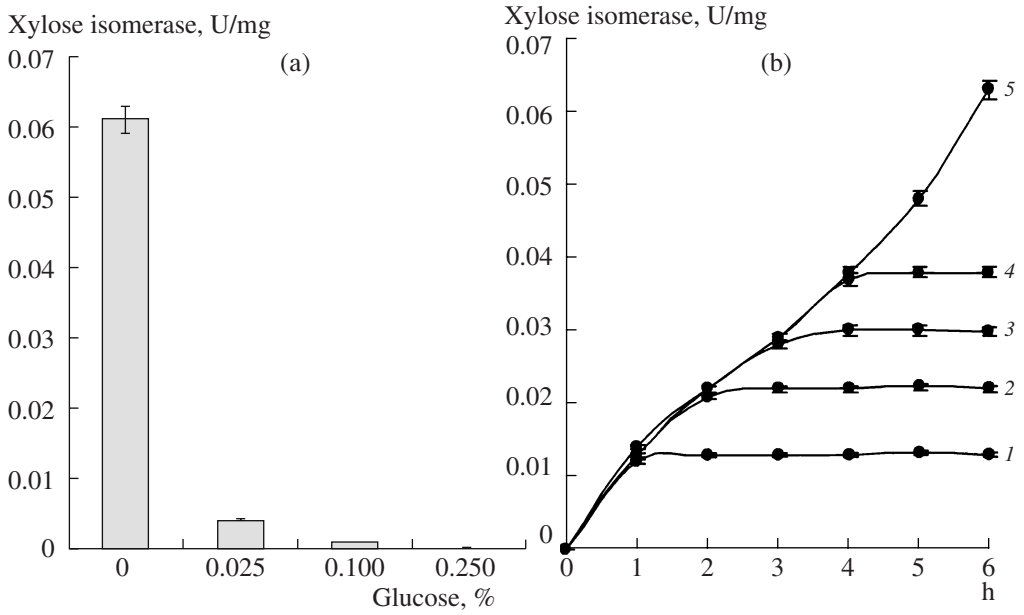

Fig. 3. Xylose isomerase biosynthesis by *A. ureafaciens* as dependent on (a) glucose concentrations and (b) time of glucose addition; glucose was added after 0, 1, 2, and 3 h of incubation (*1–4*) in xylose-containing medium (*5*).

Inhibition of xylose isomerase synthesis in the studied bacterium *A. ureafaciens* was relieved by 90% under the influence of the above-mentioned agent (1×10^{-3} M) added to incubation medium which contained the specific substrate and the catabolic repressor. The obtained data were confirmed by the results of comparative electrophoretic analysis of the intracellular proteins produced by bacteria in the presence of inducing, repressing, and derepressing agents (xylose, glucose, and cyclic 3',5'-AMP, respectively) (Fig. 5).

Thus, in *A. ureafaciens* cells, xylose isomerase synthesis induced by the specific substrate xylose and its structural analog xylite is repressed at the transcription level by glucose and other carbohydrates and mediated

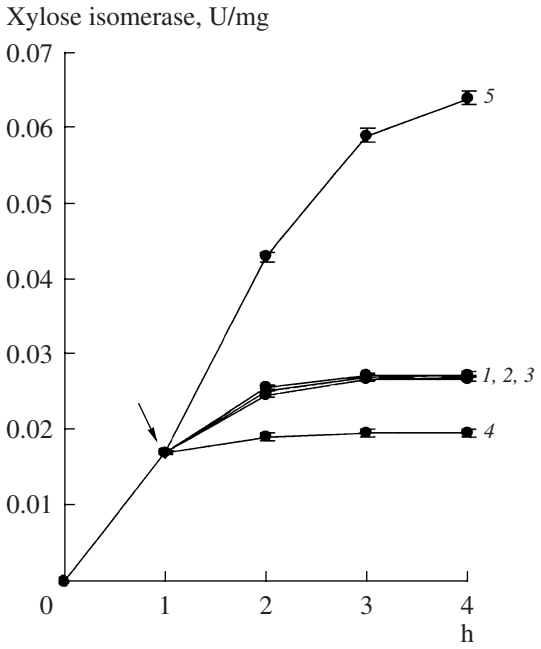

Fig. 4. Effect of glucose and transcription and translation inhibitors on the production of xylose isomerase by *A. ureafaciens*: xylose + glucose (*1*); xylose + streptomycin (*2*); xylose + actinomycin D (*3*); xylose + chloramphenicol (*4*); and xylose (control) (*5*); ⟶ designates the moment of the agent addition.

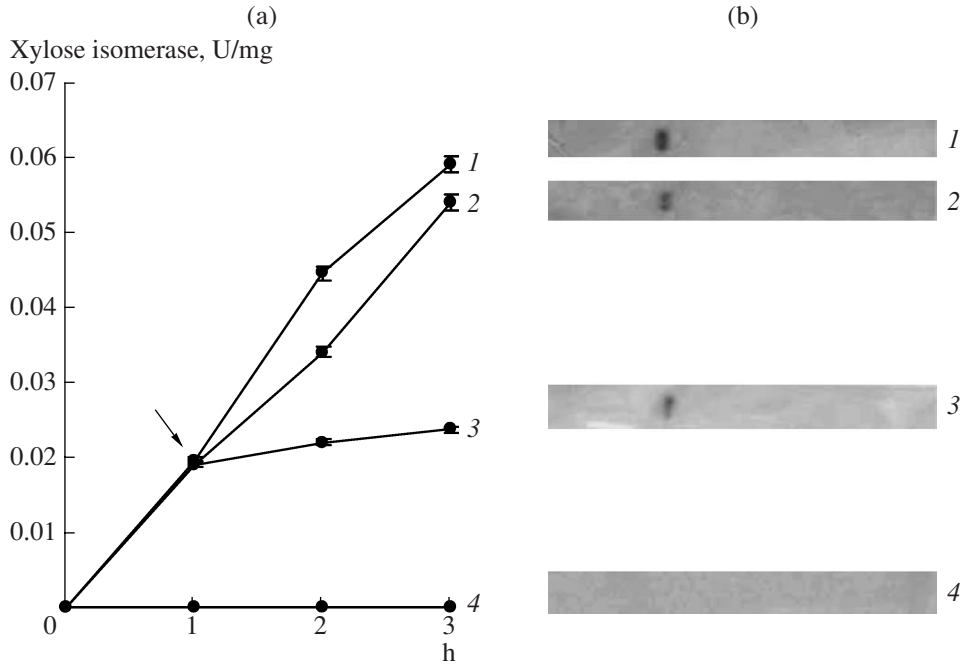

Fig.5. Derepression of xylose isomerase synthesis in *A. ureafaciens* cells by c-3',5'-AMP: (a) process dynamics; (b) zymogram of the native intracellular proteins; (*1*) xylose (control); (*2*) xylose + glucose + c-3',5'-AMP; (*3*) xylose + glucose; (*4*) glucose; ⟶ designates the moment of agent addition.

by cyclic 3',5'-AMP. Further molecular genetic studies of *A. ureafaciens* will allow us to interpret the structure and understand the principles of organization and regulation of the genes encoding the enzymatic proteins of xylose metabolism in the representatives of the genus *Arthrobacter.*

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