УДК 579.222 577.152.531

L.I. Sapunova

Institute of Microbiology, Belarus National Academy of Sciences,
Kuprevich str., 2, Minsk, 220141, Belarus, tel.: 8 (017) 26 76 209,
e-mail: leonida@mbio.bas-net.by

XYLOSE ISOMERASE SYNTHESIS IN ACTINOBACTERIA *ARTHROBACTER UREAFACIENS* BIM B-6

The role of different carbon sources in biosynthesis of cell-bound xylose isomerase by Arthrobacter ureafaciens BIM B-6 was investigated. It was found that in this prokaryotic actinobacterium enzyme production is under control of induction and catabolite repression. High level of xylose isomerase production was recorded when bacterium was grown both on xylose media and on media with soy, citrus pulp and wheat bran.

K e y w o r d s: Arthrobacter ureafaciens, producer, xylose isomerase, biosynthesis

Xylose isomerase (D-xylose ketol isomerase, E.C. 5.3.1.5) is a key enzyme of xylose metabolism in prokaryotes and commodity in top demand at world biocatalyst market. Possessing non-strict substrate specificity, xylose isomerase, in addition to xylose isomerization into xylulose, catalyzes glucose-fructose conversion. The latter motivates wide commercial application of the enzyme for manufacturing glucose-fructose syrup from saccharified starchy feedstock.

Prerequisite for efficient management of genetic potential of strains producing biologically active agents, including xylose isomerase, is to reveal the factors affecting enzyme biosynthesis. As a rule, a critical role in generating enzymes involved in carbohydrate metabolism belongs to the source of carbon nutrition. In prokaryotes specific substrate and/or its structural analogues in most cases serve as the inducers of biosynthesis, in contrast to glucose repressing xylose isomerase production [1]. Among xylose-utilizing bacteria of genus *Arthrobacter* species showing inducible and constitutive type of xylose isomerase synthesis were detected [2-4]. Yet, detailed studies on mechanisms controlling enzyme formation in this group of gram-positive prokaryotes were not performed.

Earlier we have screened xylose isomerase-producing actinobacteria *Arthrobacter ureafaciens* BIM B-6 [5]. The aim of this investigation is to study the effect of different carbon sources on the growth of the culture and biosynthesis of xylose isomerase.

Materials and Methods

Actinobacteria *Arthrobacter ureafaciens* BIM B-6 deposited at National collection of non-pathogenic microorganisms (Institute of Microbiology, Belarus National Academy of Sciences) were chosen as the object of investigation.

Under the laboratory conditions the microbial culture was maintained on yeast-peptone agar of the following composition (%): peptone — 1.0; yeast extract — 0.5; glucose — 0.5; NaCl — 0.5; agar-agar — 1.5; initial pH 7.2-7.4.

Submerged cultivation of bacteria *A. ureafaciens* was carried out in 250 ml Erlenmeyer flasks containing 50 ml of nutrient medium on the shaker (180-200 rpm) at temperature 26-28 °C during 72 hours. The nutrient medium comprised (%): peptone — 1.0; yeast extract — 0.5; K_2HPO_4 — 0.3; $MgSO_4 \cdot 7H_2O$ — 0.1. The compounds of different chemical composition in concentration 1% (calculated as carbon) or, in case of polysaccharides, 1% (w/v) were used as carbon sources. Initial pH of the medium was adjusted to 6.8 with 0.1 M NaOH.

Water suspension of bacterial cells grown on peptone-yeast agar at 26-28 °C during 72 hours served as an inoculum in dose of 2 % (v/v).

Biomass accumulation was estimated photoelectrocolorimetrically at wavelength λ=540 nm and expressed in optical density units (OD_{540}) or in mg dry biomass per 1 ml of the medium (mg/ml). Dry biomass amount was determined from pre-plotted graph reflecting relationship between optical density of bacterial cell suspension (OD_{540}) and cell weight.

Specific growth rate of bacteria was calculated according to the following formula: $\mu = dx dt^{-1} x^{-1}$, where μ — specific growth rate (h^{-1}), x — biomass (OD_{540}), dx — biomass accumulation (OD_{540}) for the time interval dt (h).

Cells of bacteria *A.ureafaciens* separated from the cultural liquid by centrifuging (8000 g, 15 min), were washed with distilled water and used to assay xylose isomerase activity.

Reaction mixture for quantitative evaluation of xylose isomerase contained: 0.2 ml of 1 M D-glucose solution; 0.5 ml of 0.2 M K,Na-phosphate buffer, pH 7.8; 0.1 ml of 0.1 M $MgSO_4 \cdot 7H_2O$; 0.5 ml of cell suspension and distilled water to 2 ml volume. Duration of isomerization reaction was 1 hour at 70 ^{0}C.

Fructose amount was determined by the cystein-carbazole method [6].

One unit of xylose isomerase activity was defined as the amount of enzyme transforming 1 μM glucose during 1 min under above-described conditions. Enzyme activity was expressed in U/mg dry biomass and in U/ml cultural liquid (productivity).

The presented results are the average values of data from 2-3 experiments performed in triplicate. In the course of statistical data processing confidence interval of arithmetical mean was calculated for probability level 0.05 [7-8]. The difference of 2 mean values was regarded reliable if their confidence intervals did not overlap. The obtained results were processed using Microsoft Windows package software.

Results and Discussion

Functioning of any living cell, like microbial one, is based on the balanced intracellular biochemical processes promoted by concerted catalytic activities of numerous different enzymes. It appears therefore that investigation of mechanisms regulating enzymatic reactions at metabolic, structural and genetic levels is an attractive challenge for contemporary biological science allowing to lay the theoretical basis for the controllable biotechnological systems governing synthesis of microbial products.

Conversion of pentoses dominated by xylose plays a key role in the cell metabolism. Xylose isomerization into xylulose mediated by xylose isomerase occurs at the initial metabolic stages in prokaryotic microorganisms.

Elucidation of mechanisms controlling production of xylose isomerase in actinobacteria *A. ureafaciens* will enable to intensify the process of enzyme biosynthesis and thereby to raise efficiency of derived enzyme preparation for fabrication of glucose-fructose syrup — the natural sweetener for diatetic and preventive-therapeutic products.

Polysaccharides, mono-, di-, aldo-, ketosugars, sugaralcohols, organic acids were supplemented as the carbon sources into the nutrient medium for culturing *A. ureafaciens* in studies on xylose isomerase biosynthesis. It may be seen from the data presented in Table 1 that bacteria generated the enzyme only if a specific substrate — xylose or its structural analogue xylitol was available in the cultural medium. It should be noted that natural polymer xylan (which may be hydrolyzed to xylose) did not stimulate xylose isomerase synthesis although it promoted the growth of *A. ureafaciens*.

Table 1

Effect of carbon sources on the growth of *A.ureafaciens* and xylose isomerase synthesis

Carbon source, 1%	Final pH	Biomass, mg/ml	Xylose isomerase	
			U/mg	U/ml
Apple pectin	7.5±0.3	8.1±0.15	0	0
Citric acid	6.9±0.2	5.3±0.11	0	0
Fructose	6.9±0.3	5.1±0.12	0	0
Glucose	7.2±0.2	7.9±0.16	0	0
Glycerol	7.4±0.2	4.9±0.15	0	0
Pyruvic acid	6.8±0.2	4.8±0.09	0	0
Starch	6.5±0.2	5.9±0.13	0	0
Sucrose	6.8±0.1	7.8±0.17	0	0
Xylan	7.3±0.1	7.1±0.14*	0	0
Xylitol	7.3±0.1	7.8±0.14	0.064±0.0018	0.499±0.008
Xylose	6.9±0.1	7.6±0.15	0.055±0.0016	0.418±0.012

*Note: * — biomass contains residual not utilized carbon source*

Xylose acting as an inducer of xylose isomerase production is a hardly digestible source of carbon nutrition for many microbial species, including the representa tives of the genus Arthrobacter [2, 9]. The experimental data summed up in Figure 1a indicate that xylose is a favourable source of carbon and energy for the growth of the tested strain *A. ureafaciens*. Bacterial growth started after 1-2 h lag-phase, with maximum specific growth rate of 0.114 h^{-1} recorded by 11 h of fermentation. This parameter for bacterial culture growing on the xylitol medium is equal to 0.146 h^{-1} upon 14 h (Figure 1b).

Xylose isomerase activity was detected at early exponential phase of *A. ureafaciens* growth, biosynthetic process progressed during subsequent 2 days, reaching the peak at stationary phase by the 3^d day of bacterium cultivation (figure 2). The level of enzyme production by *A. ureafaciens* on the media with xylose and xylitol under non-optimized conditions constituted 0.064 U/mg and 0.073 U/mg and did not decline throughout the whole fermentation period.

It may be stated that xylose inducing effect was directly correlated with its concentration in the medium, and enzyme production by bacteria *A. ureafaciens* attained top value at specific substrate concentration 1.25 % (Figure 3).

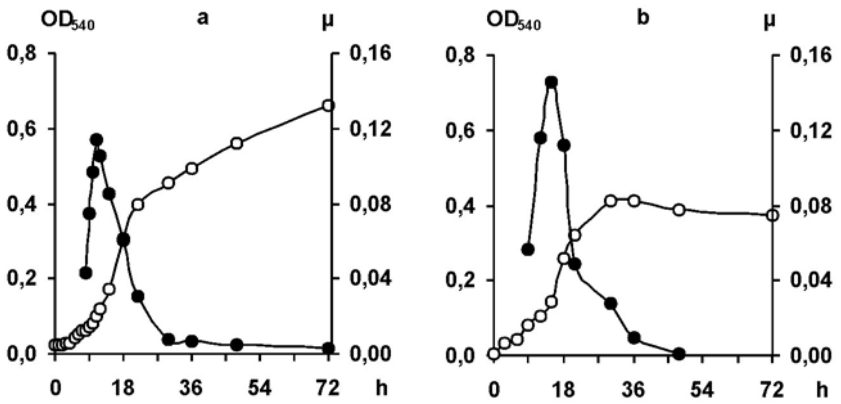

Figure 1. Specific growth rate (μ, h^{-1},●) and biomass accumulation (OD_{540}, ○) by *A. ureafaciens* on the media with xylose (a) and xylitol (b)

Рис. 1. Сравнительные характеристики скорости роста и накопления биомассы($ОД_{540}$, ○) *A. ureafaciens* на средах, содержащих (а) ксилозу и (в) ксилит

It is evident that application of xylose for commercial manufacturing of xylose isomerase is not profitable. To define production media for growing strain-producers, xylose is partially replaced by cheaper carbon sources or substituted by xylan-containing plant substrates (wheat bran, corn cobs, grain husk, fruit pulp, cotton seeds, rice bran) and their hydrolyzates [1, 4, 10-13]. According to our findings, chopped plentiful and cheap wastes of vegetable origin, like soya, beet and citrus cake, along with wheat bran, oat and soya meals exerted similar and, in some cases, even stronger beneficial effect in comparison with xylose on xylose isomerase synthesis by *A. ureafaciens* (Table 2).

Table 2

Effect of xylan-containing plant substrates on the growth of *A. ureafaciens* and xylose isomerase synthesis

Carbon source, 1%	Final pH	Biomass, mg/ml	Xylose isomerase	
			U/mg	U/ml
Xylose	6.9±0.1	7.6±0.15	0.055±0.002	0.418±0.011
Wheat bran	8.3±0.3	8.4±0.16*	0.063±0.003	0.529±0.011
Oat meal	7.9±0.2	7.4±0.15*	0.053±0.004	0.392±0.010
Beet cake	8.0±0.4	7.6±0.12*	0.059±0.002	0.448±0.012
Soya cake	8.4±0.2	8.3±0.16*	0.060±0.005	0.498±0.014
Citrus cake	80±0.2	8.0±0.14*	0.054±0.002	0.432±0.010
Soya meal	8.2±0.1	7.3±0.18*	0.058±0.001	0.423±0.013

*Note: * – biomass contains residual not utilized carbon source*

The complete absence of xylose isomerase activity in *A. ureafaciens* cells grown on media lacking the specific substrate points to the inducible type of xylose isomerase synthesis and to possible role of catabolite repression as a regulating factor. It provoked

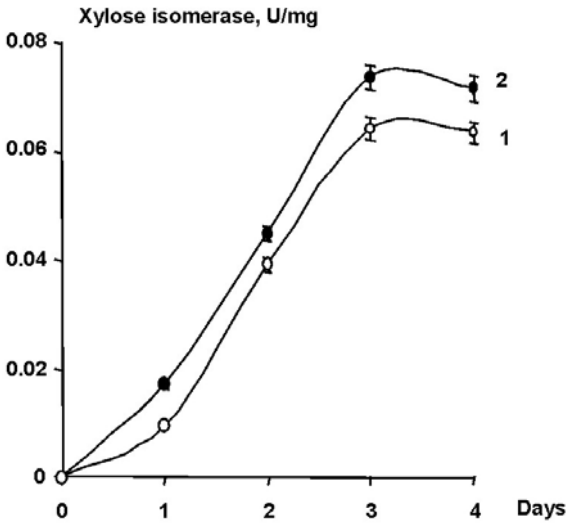

Figure 2. Dynamics of xylose isomerase synthesis by *A. ureafaciens* **on the media with xylose (1) and xylitol (2)**

Рис. 2. Динамика синтеза ксилозоизомеразы *A. ureafaciens* **на средах, содержащих (1) ксилозу и (2) ксилит**

studies on impact of different carbon sources supplied into xylose medium on enzyme production by the examined bacterial strain. The data presented in Table 3 provide the evidence that enzyme synthesis in growing culture *A. ureafaciens* was inhibited by all tested compounds, though minimal repressing effect was shown by disaccharides lactose and sucrose, maximum repressing effect — by glucose and fructose.

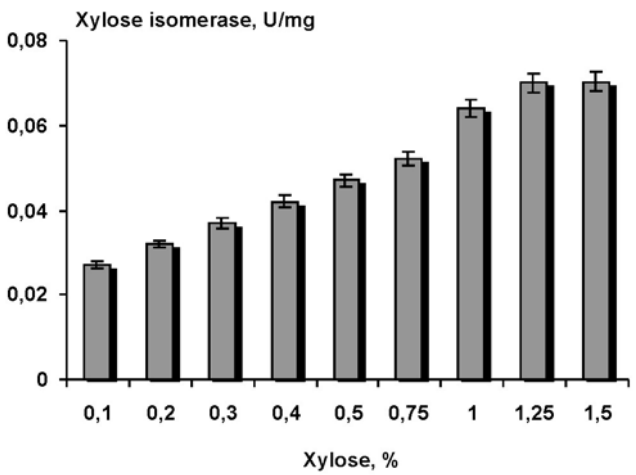

Figure 3. Correlation of xylose isomerase synthesis by *A. ureafaciens* **with xylose concentration**

Рис. 3. Зависимость синтеза ксилозоизомеразы актинобактериями *A. ureafaciens* **от концентрации ксилозы**

Table 3

Effect of different carbon sources on growth of *A. ureafaciens* and xylose isomerase synthesis on xylose media

Carbon source, 1%	Final pH	Biomass, mg/ml	Xylose isomerase	
			U/mg	U/ml
Xylose*:	6.6±0.1	2.2±0.12	0.010±0.0003	0.022±0.0007
+ xylose**	6.8±0.2	3.3±0.10	0.058±0.0017	0.191±0.0054
+ xylitol	7.0±0.1	3.3±0.08	0.061±0.0015	0.201±0.0050
+ glucose	6.9±0.2	3.8±0.09	0.016±0.0005	0.061±0.0013
+ fructose	6.9±0.2	3.2±0.10	0.019±0.0007	0.061±0.0015
+ sucrose	6.7±0.1	3.0±0.11	0.031±0.0009	0.093±0.0023
+ lactose	6.9±0.1	2.9±0.09	0.030±0.0008	0.087±0.0027

Notes:

** - bacteria were cultured on the media with 0.5% xylose during 24 h;*

***- after sampling and supplementing 0.5% of respective carbon sources bacteria were additionally grown for 48 h*

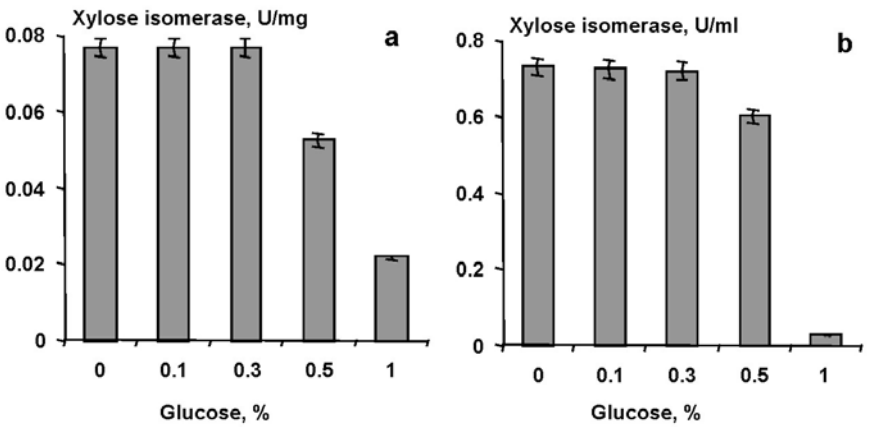

Figure 4. Correlation of xylose isomerase (a — U/mg, b — U/ml) synthesis by *A. ureafaciens* with concentration of glucose supplied into xylose medium

Рис. 4. Взаимосвязь синтеза ксилозоизомеразы (а) — U/mg; (в) — U/ml актинобактериями *A. ureafaciens* и концентрации глюкозы при выращивании бактерий на средах с ксилозой

It should be noted that under experimental conditions glucose repressing effect depended on its concentration. It may be seen from Figure 4 that production of xylose isomerase by *A. ureafaciens* was not suppressed in presence of 0.1-0.3 % levels of catabolic repressor in the cultural medium. When concentration of glucose fed into the nutrient medium simultaneously with bacterial inoculation increased to 0.5 and 1.0 %, enzyme production decreased by 32 % and 72 % as compared to the control.

Summing up, synthesis of xylose isomerase by *A.* ureafacien, induced by specific substrate xylose and its structural analog xylitol, is repressed by glucose and other readily digestible carbohydrates. High level of xylose isomerase production under non-optimized conditions and possibility of using diverse xylose-containing plant materials and derived processing wastes as components of nutrient media for culturing *A.ureafaciens* motivate the choice of this microbial strain as potential industrial xylose isomerase producer.

REFERENCES

1. Bhosale S.H., Rao M.B., Deshpande V.V. Molecular and industrial aspects of glucose isomerase // Microbiol. Rev. — 1996. — 60, № 2. — P. 280 — 300.
2. Ананьин В.М., Головлев Е.Л. Активность ксилозоизомеразы в процессе роста Arthrobacter sp. // Прикладная биохимия и микробиология. — 1981. — 17, № 6. — С. 826 — 831.
3. Лобанок А.Г., Сапунова Л.И., Казакевич И.О., Парахня Е.В. Независимый от специфического субстрата синтез ксилозо(глю-козо)изомеразы бактериями Arthrobacter species // Доклады НАН Беларуси. — 2000. — 4, № 1. — С. 69 — 71.
4. Sapunova L.I., Lobanok A.G., Kazakevich I.O., Parakhnya E.V. Effect of medium composition on constitutive production of glucose isomerase in Arthrobacter sp. // Bul. Polish Acad. Sci.: Biol. — 2002. — 50, № 4. — P. 213 — 222.
5. Lobanok A.G., Sapunova L.I., Dikhtievski Ya.O., Kazakevich I.O. Screening of glucose isomerase-producing microorganisms // World J. Microbiol. Biotechnol. — 1998. — 14, № 2. — P. 259 — 262.
6. Dische Z., Borenfreund E. A new spectrophotometric method for the detection and determination of keto sugars and trioses // J. Biol. Chem. — 1951. — 192, № 2. — P. 583 — 587.
7. Рокицкий П.Ф. Биологическая статистика.— Минск: Высшая школа, 1973. — 320 с.
8. Тюрин Ю.Н., Макаров А.А. Статистический анализ данных на компьютере.— М.: ИНФА-М, 1998. — 544 с.
9. Joo G.J., Rhee I.K. Production of glucose isomerase from xylB mutant Streptomyces chibaensis J-59 // Sanop Misaengmul Hakhoechi. — 1997. — 25, № 1. — P. 75 — 81.
10. Wang F., Whitaker R.D., Batt C.A. Production of glucose isomerase in a recombinant strain of Streptomyces lividans // Appl. Microbiol. Biotechnol. — 1998. — 50, № 1. — P. 65 — 70.
11. Joo G.-J., Kwon G.-S., Rhee I.-K. Isolation and identification of a Streptomyces chibaensis J-59 producing thermostable glucose isomerase // Sanop Misaengmul Hakhoech. — 1997. — 25, № 1. P. 15 — 22.
12. Teeradakorn S., Kishimoto M., Seki T., Pinphanichakarn P., Yoshida T. Process development and simulation of glucose isomerase production from birchwood xylan by a Streptomyces fusant // Biochem. Eng. J. — 1998. — 1, № 2. — P. 159 — 168.
13. Христов Л., Стойчев М., Григорова Й., Джежева Г., Драганова Р., Видимски Е. Биосинтеза на гликозоизомераза от Streptomyces thermovulgaris щам 127 с използуване на обработен предхидролизат // Acta Microbiol. Bulg. — 1990. — 26. — P. 53 — 59.

УДК 579.222 577.152.531

Л.І. Сапунова

Інститут мікробіології НАН Білорусі, вул. Купревіча, 2, Мінськ, 220141, Білорусь, tel.: 8 (017) 26 76 209, e-mail: leonida@mbio.bas-net.by

СИНТЕЗ КСИЛОЗОІЗОМЕРАЗИ АКТИНОБАКТЕРІЯМИ *ARTHROBACTER UREAFACIENS* БІМ В-6

Реферат

Вивчено вплив джерел вуглецю різного хімічного складу на утворення клітиннозв'язаної ксилозоізомерази у *Arthrobacter ureafaciens* БІМ В-6. Встановлено індукований підвержений катаболітній репрессії характер біосинтезу фермента бактеріями. Високий рівень утворення ксилозоізомерази при вирощуванні бактерій на середовищах з ксилозою або ксилозовміщуючи-

ми відходами переробки рослинної сировини — соевим та цитрусовим жомом, пшеничними висівками — обумовлюють вибір *Arthrobacter ureafaciens* БІМ В-6 як потенційного продуцента фермента.

К л ю ч о в і с л о в а: *Arthrobacter ureafaciens*, продуцент, ксилозоізомераза, біосинтез.

УДК 579.222 577.152.531

Л.И. Сапунова

Институт микробиологии НАН Беларуси, ул. Купревича, 2, Минск, 220141, Беларусь, tel.: 8 (017) 26 76 209, e-mail: leonida@mbio.bas-net.by

СИНТЕЗ КСИЛОЗОИЗОМЕРАЗЫ АКТИНОБАКТЕРИЯМИ *ARTHROBACTER UREAFACIENS* БИМ В-6

Реферат

Исследовано влияние источников углерода различного химического строения на образование клеточносвязанной ксилозоизомеразы у *Arthrobacter ureafaciens* БИМ В-6. Установлен индуцированный подверженный катаболитной репрессии характер биосинтеза фермента бактериями. Высокий уровень образования ксилозоизомеразы при выращивании бактерий на средах с ксилозой или ксилозосодержащими отходами переработки растительного сырья — соевым и цитрусовым жомом, пшеничными отрубями — обусловливают выбор *Arthrobacter ureafaciens* БИМ В-6 в качестве потенциального продуцента фермента.

К л ю ч е в ы е с л о в а: *Arthrobacter ureafaciens*, продуцент, ксилозоизомераза, биосинтез.