0273-2289/96/6002—0107$06.75

An Epimerasic Activity on Galactose Induced by Xylose in *Kluyveromyces* sp.

MARÍA EDITH ELORZA DE ORELLANO,*
ANA MARÍA PONCE DE ASCHERI,
MARÍA ISABEL SANZ DE TOSETTI,
AND RODOLFO SEGOVIA

Laboratorio de Fermentaciones Industriales, Cátedra de Microbiologia Industrial, Facultad de Quimíca, Bioquimica y Farmacia, UNSL. 5700 San Luis, Argentina

Received May 1, 1995; Accepted June 12, 1995

ABSTRACT

Production of galactose epimerase by an *Kluyveromyces* sp. isolated from Kefir (dairy product) was investigated in batch culture. The microorganism was cultured in media with 1% galactose, 1% xylose, or 0.5% xylose plus 0.5% galactose, in Erlenmeyer flasks shaken at 200 rpm and maintained at 30°C. After 48 h, the biomass was harvested by centrifugation and permeated with 80% ethanol. Permeated cells were suspended in 0.1*M* sodium phosphate buffer pH 6.5. A part of this suspension was shaken for 17h at 140 rpm. The supernatant, free of cells, was separated. Partial characterization of *Kluyveromyces* sp. epimerase was carried out in the cellular suspension and the supernatant solution. Enzymatic activity, using galactose as substrate, was measured. The product of this reaction was measured by the use of glucose oxidase. The results indicated: (1) there was a strong effect of xylose on induction of epimerase activity; (2) the epimerase obtained was independent of the energetic cell activity; (3) the epimerase activity in whole cells was similar to the activity obtained from the supernatant; (4) epimerase showed a typical substrate-inhibition curve and dependence on magnesium; and (5) the best pH range was between 5.5 and 6.5 and the optimal temperature was 30°C.

Index Entries: Epimerase; galactose; *Kluyveromyces* sp; xylose.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

There are many cattle and goat farmers in the region (San Luis, Argentina) who produce milk in small factory farms. Generally, they release the whey from cheese manufacturing, which pollutes streams and soils and loses the economic potentiality of this effluent (*1*). An alternative in the exploitation of whey is lactose hydrolysis by using beta-galactosidase (EC 3.2.1.23). The glucose and galactose produced can be used in other profitable processes (*2*). However, if both of these products could be converted to a high-fructose syrup by means of the joint action of a galactose epimerase and a glucose isomerase, the whey could be used in regional confectionery.

The beta-galactosidase and glucose isomerase (EC 5.3.1.5: D-xylose ketol-isomerase) are available at low cost (Miles, Novo, Milar, Argentina). There is no information available regarding an epimerase that acts on galactose, except, of course, the epimerase that uses uridine diphosphate galactose as substrate (EC 5.1.3.2: UDPGlucose-4-epimerase) (*3*). Because of this, our research led to a search for an epimerasic activity that could be used in the bioconversion of galactose obtained by lactose hydrolysis of whey. Taking into account the model of glucose isomerase production (*4*), we intended to induce some epimerase involved in pentose metabolism (*5*) in cells of *Kluyveromyces* sp. isolated from Kefir with manifest activity of beta-galactosidase. This article reports the finding of an epimerasic activity on galactose induced by xylose in *Kluyveromyces* sp. A method for quantifying this activity is described, and the partial characterization of the enzymatic activity using galactose as substrate is also reported.

MATERIALS AND METHODS

Reagents

D-galactose containing less than 0.1% glucose was obtained from Merck (Darmstadt, Germany). The glucose oxidase peroxidase system was from Wiener (Buenos Aires, Argentina). Other reagents were of analytical grade.

Microorganism and Media

The microorganism chosen was *Kluyveromyces* sp. isolated in our laboratory from Kefir (dairy product). This organism produces beta galactosidase activity.

A storage medium, in nutrient agar, was kept at 4°C. The composition of the culture medium was as follows: 2% agar (Oxoid, England); 0.3% malt extract (Difco); 0.3% yeast extract (Difco); 0.5% bactopeptone (Difco) and 1% lactose (Merck). The pH was adjusted to 5.0.

Inocula and Proliferation Media

Liquid cultures were prepared in three different media. Medium No. 1: 0.3% malt extract; 0.3% yeast extract; 0.5% bactopeptone; and 1% Xylose. Medium No. 2: 0.3% malt extract; 0.3% yeast extract; 0.5% bactopeptone; 0.5% Galactose, and 0.5% Xylose. Medium No. 3: 0.3% malt extract; 0.3% yeast extract; 0.5% bactopeptone; and 1% Galactose. The pH was adjusted to 5.0 with HCl 3*N* in the three media.

Culture Conditions

Fermentations were carried out in 1000 mL Erlenmeyer flasks with stainless steel baffles (UNSL, Argentina), containing 200 mL medium. Unless otherwise stated, cultures were carried out at 30°C for 48 h with orbital shaking (200 rpm). Cells were harvested by centrifugation at 6600 × g at 4°C for 20 min in a Sorvall RC-5B Refrigerated Superspeed Centrifuge GSA rotor. The cell pellet was washed once with 2 vol of 0.1*M* sodium phosphate buffer, pH 6.5.

Obtention of Enzyme Extract

Cell Permeabilization

The yeast cake was treated with 80% ethanol (v/v) (*6*). Treatment time was 90 min, temperature was 30°C, and agitation was 200 rpm. Cells were harvested by centrifugation and resuspended in 0.1*M* sodium phosphate buffer, pH 6.5. Biomass to buffer ratio was 1:10.

Obtaining Free Cells Extract

A portion of the suspension was shaken for 17 h at 140 rpm. The supernatant was then separated by centrifuging.

Determination of Enzymatic Activity

The enzymatic activity in the supernatant and the cell suspension was determined. Enzymatic assays were performed in triplicate. Each experiment was repeated at least three times. The results presented are from a typical experiment. The method developed to measure the activity was as follows: 1 mL galactose (1 g/L), 0.5 mL $MgCl_2$, and 0.5 mL enzymatic extract (supernatant or cell suspension) were put in a reaction tube; the reaction mixture was incubated at 37°C for 30 min; the reaction was stopped by immersing the tubes in boiling bath for 10 min. The blanks were prepared in a similar way, but the inactivation of enzymatic extract was completed before adding the substrate. The quantity of glucose produced from galactose was determined after centrifuging, using the glucose-oxidase-peroxidase system. Enzyme units (IU) were defined as μmol of glucose produced per min at 37°C. The specific activity was expressed as IU/g of dry weight of biomass.

Table 1
Effect of Xylose Presence on Epimerase Production by *Kluyveromyces* sp.

C Source	Specific activity (IU/g cell)		
	Nonpermeabilized cells	Permeabilized cells	Free cell extract[a]
1% Xylose	0.0	1112	—
0.5% Galactose +0.5% Xylose	0.0	1037	—
0.5% Galactose +0.5% Xylose[b]	—	6112	5000
1% Galactose	0.0	0.0	—

[a]1.50 mg/mL protein (Lowry method).
[b]Xylose was added 12 h before harvesting cells

RESULTS AND DISCUSSION

Induction by Xylose

Induction of galactose epimerase was researched in cultures in Erlenmeyer flasks.

Preliminary assays of epimerase activity induction by xylose were carried out by growing *Kluyveromyces* sp. in media described in Materials and Methods. Results showed a weak epimerase activity only in media with xylose (Table 1). But, when xylose (0.5% final concentration) was added to 36 h-old cells grown on 0.5% galactose, and they were harvested after 12 h, galactose epimerase activity was dramatically increased (Table 1).

Biomass growth on 1% xylose and epimerase production are shown in Fig. 1. A diauxic behavior and a maximal production of epimerase can be seen at 12 h, when perhaps xylose uptake begins. Before that, growth is possibly sustained by other components of the medium. Inoculum was made in a 1% xylose medium, which would explain the activity observed at the beginning.

Time-course of epimerase production would explain why the cells harvested at 48 h had a weak epimerase activity, although they grew in the presence of xylose. Effectiveness of induction by xylose 12 h before harvesting cells could thus be explained, too.

Results would lead one to believe that the enzyme could be an epimerase that participates in xylose metabolism. This activity is also present in the crude extract free of cells (Table 1), so it could be stated that it is a soluble enzyme that would not need the energetic system of the cell to act as uridine diphosphate galactose (5.1.3.2) does (3).

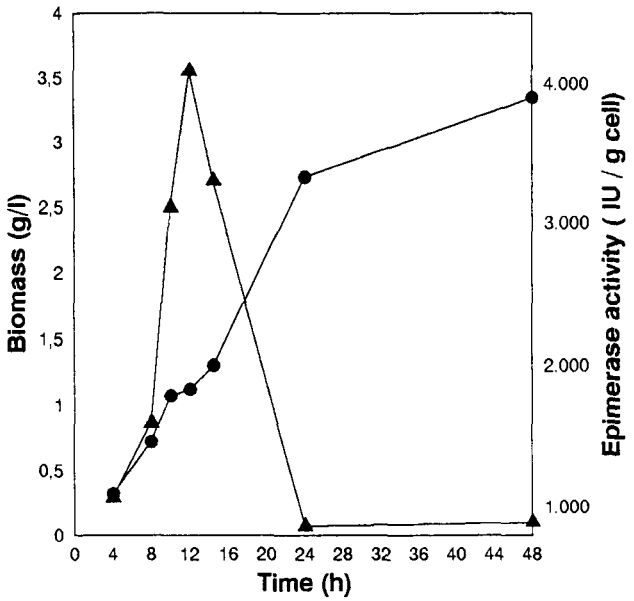

Fig. 1. Biomass growth (●) and galactose epimerase production by *Kluyveromyces* sp. in shaken flask culture (▲). Medium with 1% D-xylose.

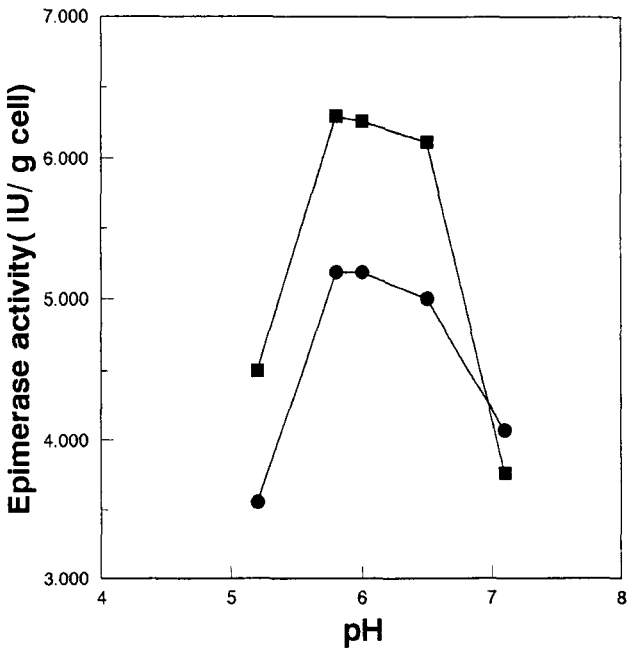

Fig. 2. Effect of pH on epimerase activity. (■) whole cells; (●) free cell extract.

Influence of pH on Enzymatic Activity

The activity measured in free cell extract and whole cells showed the same pattern at different pH. The highest activity was reached between pH 5.5 and 6.5 (Fig. 2).

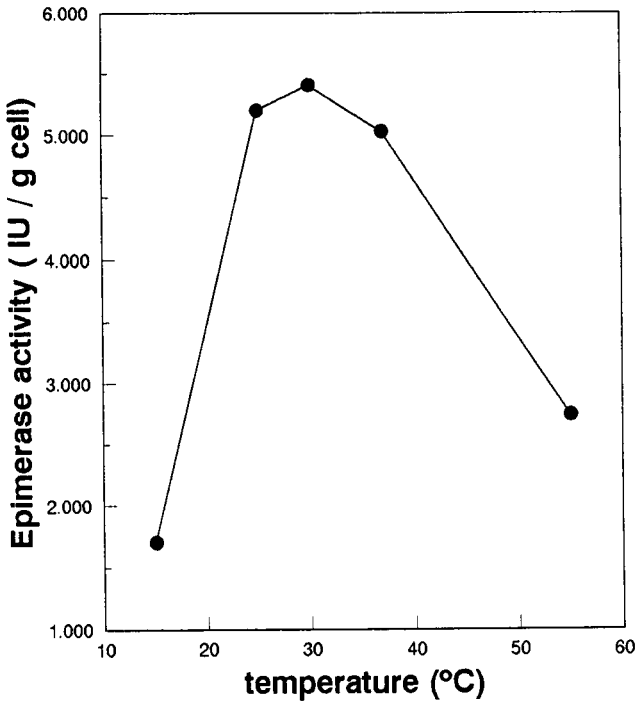

Fig. 3. Effect of temperature on epimerase activity in free cell extract.

Determination of Optimum Temperature

The effect of temperature on epimerasic activity is shown in Fig. 3. It can be observed that the enzyme possesses the maximal activity at about 30°C, decaying at higher temperatures (Fig. 3).

Influence of the Concentration of Substrate and Magnesium

Using galactose as substrate, it seems enzyme behavior showed a typical inhibition curve by substrate (*9*). This behavior was remarkable in free cell extract and could also be due to the presence of some cofactor. Furthermore, assays made in the absence of magnesium showed that the activity depends on it (Fig. 4).

CONCLUSIONS

The feasibility of epimerizing galactose by an epimerase induced by xylose, independent of the energetic cell system, is the beginning of a series of investigations on the production of the mentioned enzyme, by using *Kluyveromyces* sp. or other microorganism, in which this activity should easily be induced.

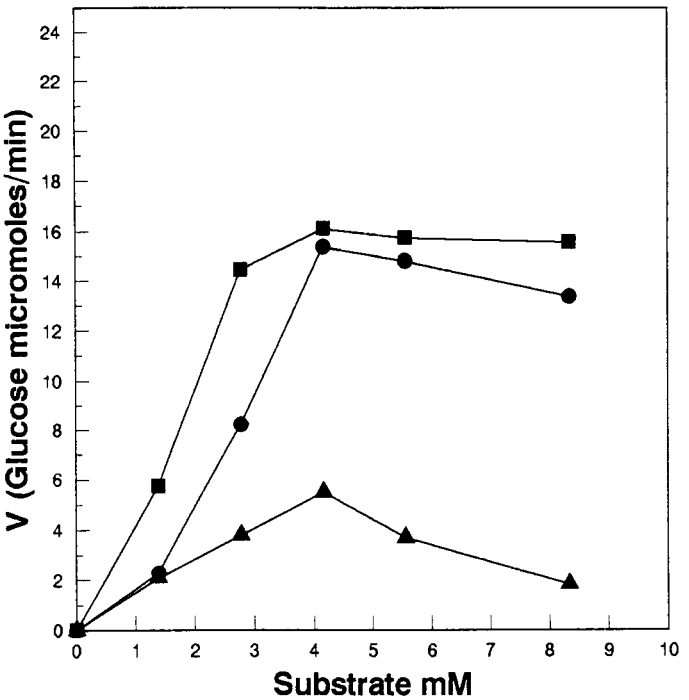

Fig. 4. Influence of the concentration of substrate on reaction rate. (■) whole cells; (●) free cell extract; (▲) free cell extract in absence of magnesium.

ACKNOWLEDGMENTS

The authors would like to thank the National University of San Luis (UNSL, Argentina) for financial support, the Government of San Luis, Mr. Juan C. Soloa for technical guidance, and Prof. Marta Vivas for linguistic advice.

REFERENCES

1. "Programa de Desarrollo Caprino" (Government Provincia de San Luis). Private Communication.
2. Illanes, A., Ruiz, A., Zuñiga, M. E., Aguirre, C., O'Reilly, S., and Curoto, E. (1990), *Bioprocess Eng.* **5,** 257-262.
3. Mayes, P. A. (1992), in *Bioquimica de Harper,* Murray, D., Mayes, P., Rowell, V., Granner, Ed. El Manual Moderno, S.A de C.V. Mexico.
4. Verhoff, F. H., Boguslawski, G., Lantero, O. J., Schlager, S. T., and Jao, Y. C. (1985), in *Comprehensive Biotechnology,* vol. 3, Murray Moo-Young, ed., Pergamon, pp. 837-859.
5. Hahn-Hagerdal, B., Jeppsson, H., Skoog, K., and Prior, B. A. (1994), *Enzyme Microb. Technol.* **16,** 933-943.
6. Siso, M. I. G., Cerdan, E., Picos, M. A. F., Ramil, E., Belmonte, E. R., and Torres, A. R. (1992), *Biotechnol. Techniques* **6,** 289-292.
7. Hasal, P., Cejkova, A., and Vojtisek, V. (1992), *Folia Microbiol.* **37,** 365-371.
8. Fenton, D. M. (1982), *Enzyme Microb. Technol.* **4,** 229-232.
9. Dixon, M. and Webb, E. C. (1964), *Enzymes,* 2nd ed., Academic, New York, p. 78.