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TRANSGALACTOSYLATION BY β-GALACTOSIDASE FROM NEW STRAIN *KLUYVEROMYCES LACTIS 3*

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ABSTRACT. ß-Galactosidase (EC.3.2.1.23 is an important enzyme industrially used for the hydrolysis of lactose from milk and milk whey for several applications.Lately, the importance of this enzyme was enhanced by its galaktosyltransferase activity, which is responsible for synthesis of transgalactosylated oligosaccharides that act as prebiotics, with several beneficial effects on consumers. ß-Galactosidase production by *K. lactis 3* was studied in shake flasks. The highest enzymatic activity was obtained at 10h of the fermentation. The optimum temperature for transferase activity was 50˚C. When incubated with a 30% lactose in 100 mM buffer, pH 6,0 the enzyme can synthesize up to 41% TOS. ß-Galactosidase from strain *Kl. Lactis 3* produces mainly oligosaccharides with DP 6 at 40°C and with DP 3 at 50°C

KEY WORDS. *K. Lactis,* ß-Galactosidase, galacto-oligosaccharides

INTRODUCTION

Oligosaccharides are increasingly being recognized as useful dietary tools for the modulation of the colonic microflora toward a healthy balance. Although many oligosaccharide preparations are used in functional foods in Japan, two general classes are widely used in Europe. These are fructans, such as inulin and fructooligosaccharides, and galacto-oligosaccharides. The latter are produced from lactose by glycosyl transfer catalyzed by ß-galactosidase and occur as complex mixtures with various glycosidic linkages. The linkage between the galactose units and the components in the final product depend on the enzymes and the conditions used in the reaction.

Transgalactooligosaccharides (TOS) are oligosaccharides produced by transgalactosylation of lactose using a ß-galactosidase (ß-Gal). The linkage between the galactose units and the components in the final product depend on the enzymes and the conditions used in the reaction. The production and characterization of these TOS have been described in various publications (Onishi, N., & T. Tanaka, 1997; Smart, J.B., 1991). Different linkages between galactose and the reducing glucose unit have been identified, namely, ß-D-Galp-(1-2)-D-Glcp, ß-D-Galp-(1-3)-D-Glcp and ß-D-Galp-(1-4)-D-Glcp. Also, branched Glcp residues occur, whereas oligogalactose fragments contain mainly 1-4 or 1-6 linkages(Smart, J.B., 1993; Zarate, S., & M.H. Lopez-Leiva, 1990). Recently, Fransen et al.(1998) showed that nonreducing galactooloigosaccharides were also formed during transgalactosylation of lactose. Given that the ß-Gal from different micro-organisms display differing rate constants for hydrolysis for specific glycosidic linkages and that synthesis of TOS is kinetically controlled, synthetic product mixtures made with different enzymes are likely to contain differing profiles of glycosidic linkages. There is, therefore, potential to see varying selectivities upon fermentation of these products.

The aim of this paper was to study transgalactosidase activity by ß-galactosidase from new strain *K. lactis* isolated from Bulgarian traditional dairy products.

MATERIAL AND METHODS

Microorganisms.

We obtained strain *Kluyveromyces lactis* 3 from SIBIO'93 LTD, Plovdiv, Bulgaria. Yeasts were maintained on YEPD(1% yeast extract, 2% bacto-peptone,2% glucose) agar slopes.

Media.

As basic media for enzyme synthesis, we used a medium containing 10g/l yeast extract; 20g/l bacto-peptone; 10g/l lactose; 3.0g/l KH2PO$_4$and 3.0g/l $MgSO_4$ x 7H_2O at the initial pH value 6.0.

Culture conditions

In shake-flask experiments, cultures were grown at 30°C using 250-ml erlenmeyer flasks, containing 50 ml medium. The flasks were permanently shaken at 200 rpm on a New Brunswick rotary shaker (New Brunswick Scientific, USA).

Analytical methods

Cell growth was monitored by measuring the optical density at 650 nm(OD650) during a fermentation experiment. Yeasts were harvested by centrifugation at 10 000 x g for 20 min at 4°C. The cells were washed twice, resuspended in 0,05 M phosphate buffer (pH 6,0), and subsequently disrupted by ultrasonic desintegrator. ß-Galactosidase activity was determined by hydrolysis of ONPG followed by measurement of absorbance at 420 nm. The total carbohydrate concentration was determined using the phenol-sulfuric acid method (DuBois et.al., 1956) and glucose as standard. Oligosaccharides were synthesized in 0,05 M phosphate buffer (pH 6,0) containing 5 – 30% lactose at 40, 50 and 60°C with

shaking. Samples were taken at hourly intervals, and the reaction was stopped by heating for 5 min at 100°C. Samples were diluted 1:6 and analyzed by TLC. The oligosaccharides produced were analyzed by HPLC using a C18 column and Hewlett-Packard 1050 series system. Oligosaccharides were detected by using an HP1047A refractometer.

RESULTS AND DISCUSSION

The effect of the initial concentration of lactose on the production of ß-galactosidase was studied. Enzyme production was conducted for 48h under various concentrations of lactose and glucose. The results are shown in Figure 1. The micro-organism was shown to produce maximum amount of enzyme at 10h of the fermentation process. In the case of enzyme production by the studied strain in glucose-containing media, no activity was not detected. Probably, ß-galactosidase from this strain is mainly inducible.

The synthesis of galacto-oligosaccharides was performed at varying concentrations of lactose and with an enzyme concentration of 1 U/ml. As illustrated in Figure 2 and 3, the maximum yield (47%) in total galacto-oligosaccharides was observed when 25% lactose was used.

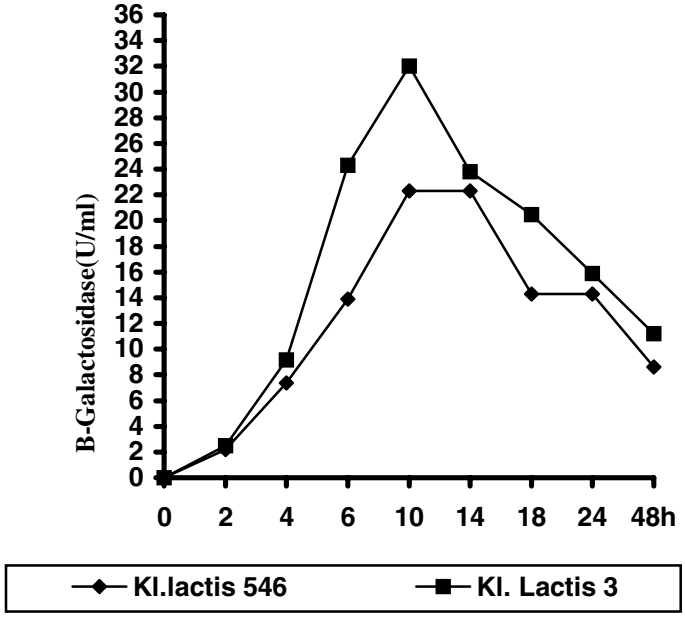

Figure 1. Typical profiles of □-Galactosidase production from *Kluyveromices lactis 546* and *Kluyveromices lactis 3* on synthetic medium with 5% lactose

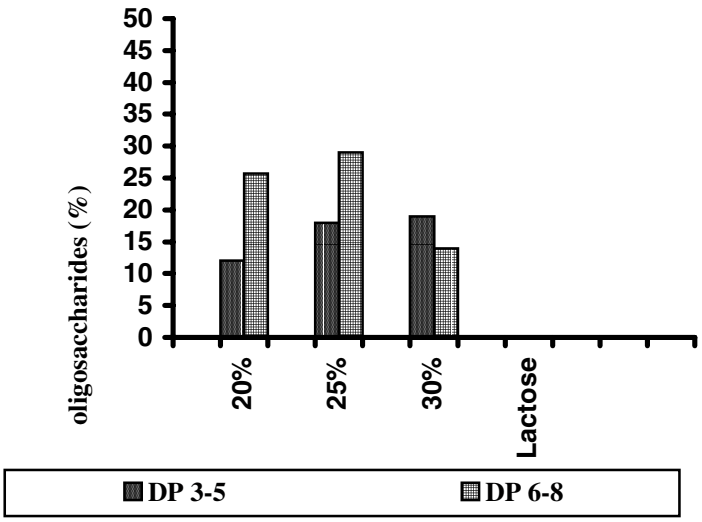

Figure 2. Oligosaccharide synthesis using ß-Galactosidase from *Kl. lactis* 3 at 40°C and initial concentration of lactose 20%, 25% and 30%

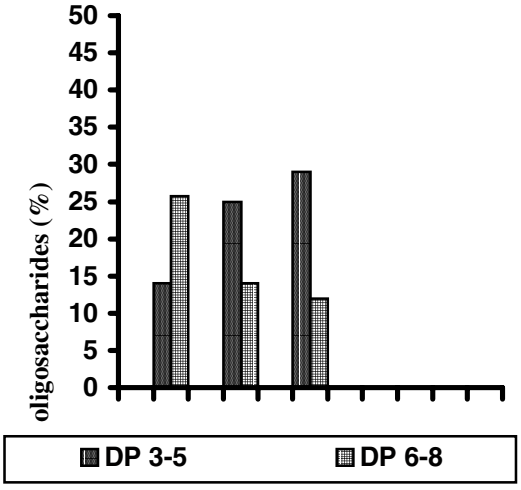

Figure 3. Oligosaccharide synthesis using β-Galactosidase from *Kl. lactis* 3at 50˚C and initial concentration of lactose 20%, 25% and 30%

As shown on HPLC chromatograms of transgalactosylated reaction products synthesized with ß-Galactosidase from studied strain displayed two mainly peaks for DP 3 and DP 6(Figure 4). The oligosaccharides synthesized by the studied enzyme show the same retention time as those obtained with the Amano ß-Galactosidase. Among the TOS, only tri and tetrasaccharides were synthesized and longer chain oligosaccharides than DP 6 were not found at any time during the course of the

reaction. This observation confirms the results described by Smart et al.(1993), according to which high lactose concentrations favor the synthesis of short chain oligosaccharides. It was observed that complete inhibition of oligosaccharide synthesis occurred when the monosaccharides content of the reaction medium reached the total concentration of TOS.

As shown on Figure 2 and 3 ß-Galactosidase from strain *Kl. Lactis 3* produces mainly oligosaccharides with DP 6 at 40°C and with DP 3 at 50°C. The results demonstrate that the ß-Galactosidase from strain 3 efficiently synthesized oligosaccharides with DP 6 overall yield of 29% using 25% initial lactose concentration at 40°C and only 14% using 25% initial lactose concentration at 50°C. The yield of the TOS with DP 3 using 30% initial lactose concentration is higher by increasing of the reaction temperature(50°C). The values reported here are slightly higher than those reported by Dumotrtier et al.(1994).

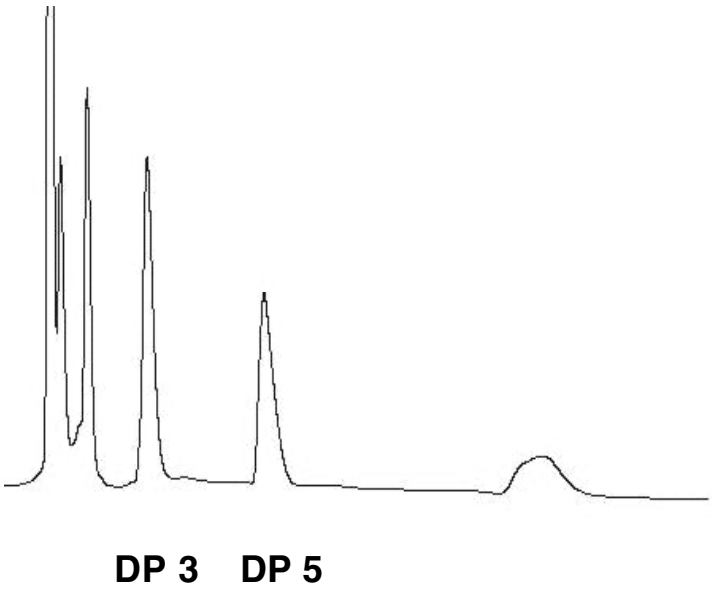

Figure 4. Chromatogram of the oligosaccharides synthesized in the presence of 25% lactose by ß-Galactosidases from *Kl. Lactis 3*

CONCLUSIONS:

We describe the production of ß-Galactosidases from new strains *Kluyveromices lactis 546* and *Kluyveromices lactis 3*, isolated from bulgarian traditional dairy foods.

The studied ß-Galactosidase from *K. lactis 3* is very efficient enzyme for the synthesis of TOS because of its high product yields and transfer rates. The enzyme from the strain No. 3 showed higher effectivity during synthesis for the low molecule TOS.

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