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THE EFFECT OF SELECTED INDUCTORS ON BIOSYNTHESIS AND PROPERTIES OF β–GALACTOSIDASE

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Two strains of yeast *Candida sphaerica* WKIIW3b and *Kluyveromyces fragilis* 28 and hypha fungi *Penicillium canescens* were grown on culture media where the source of carbon was permeate after ultrafiltration of milk, soybean flour or wheat bran applied individually or in various combinations. In the obtained preparations of β-galactosidase the activity of hydrolysis with ONPG and lactose was determined and the influence of some of the cations and EDTA on the activity of β-galactosidase both with ONPG and with lactose as a substrate was studied.

The examination of the properties of transgalactosilation of β-galactosidase preparations synthesised by fungi on media with various sources of carbon involved the application of these preparations for bioconversion of lactose in milk permeate where the concentration of such varied from 17.4 to 28.8%. Saccharides in the obtained products were determined with TLC method.

INTRODUCTION

β-Galactosidase is a common enzyme in nature. The source of β-galactosidase can be plant (apples, dog rose, kiwi, carrot) and animal tissues (mammal small intestine) as well as some microorganisms.

For industrial purposes β-galactosidase is obtained from microorganisms. Most of them synthesise β-galactosidase intracellularly on media with lactose. These are bacteria [Ramana & Dutta, 1981; Chang & Mahoney, 1989; Hung & Lee, 1998; Nakao *et al.*, 1994], yeast [Mahoney & Whitaker, 1978; Friedurek & Szczodrak, 1994] as well as hypha fungi [Kowalewska *et al.*, 1986]. Some hypha fungi such as *Aspergillus oryzae* [Ogushi *et al.*, 1980], *Penicillium canescens* [Sawicka-Żukowska *et al.*, 2001], and *Aspergillus fonsecaeus* [Gonzales & Monsan, 1991] release β-galactosidase out of the cell. The source of carbon for hypha fungi which synthesise β-galactosidase extracellularly was wheat bran or soybean flour or a culture medium mixed with these sources of carbon.

Thus, the synthesis of β-galactosidase by microorganisms depends on the concentration in the medium of not only lactose but also other β-D-galactosides *e.g.* raffinoses or stachioses present in soybean flour. Moreover, lactose is not the only substrate hydrolysed by β-galactosidase. Other oligosaccharides which are present in milk can also be hydrolysed by β-galactosidase [Amarita *et al.*, 1995].

Beside hydrolytic properties, β-galactosidase shows transgalactosilation properties [Huber *et al.*, 1976; Nakao *et al.*, 1994; Kowalewska-Piontas & Bednarski, 2001]. Both hydrolytic and transgalactosilation properties depend on the source of β-galactosidase.

The aim of these studies was to compare the properties of lactose hydrolysis and transgalactosilation of β-galactosidase preparations obtained after the growth of yeast *Candida sphaerica* WKIIW3b or *Kluyveromyces fragilis* 28 or hypha fungi *Penicillium canescens* multiplied on media containing oligosaccharides or lactose.

MATERIAL AND METHODS

A yeast strain *Candida sphaerica* WKIIW3b was received from the culture collection at the Department of Biotechnology and Food Microbiology of the Agricultural University in Wrocław. The strain *Kluyveromyces fragilis* 28 comes from a collection of the Department of Food Microbiology, UWM in Olsztyn. *Penicillium canescens* was purchased in the Institute of Microbiology and Virology of the Ukrainian Academy of Sciences in Kijów.

The source of carbon was permeate after ultrafiltration of milk received from the Dairy Industry in Wolsztyn with the following composition: 97% of dry matter, 5.3% of ash; 3.5% of protein, and 86% of lactose; soybean flour and wheat bran were purchased on the market.

The examined strains of fungi were grown on media where the source of carbon was: No. 1. milk permeate 5%; No. 2. soybean flour 2%, wheat bran 2%; No. 3. milk permeate 2.5%, soybean flour 2%; No. 4. milk permeate 2.5%, wheat bran 2%; No. 5. soybean flour 4%; No. 6. wheat bran 4%.

All the media, except for the source of carbon, were provided with 0.1% of yeast extract; 0.2% of K_2HPO_4; 0.1% of NH_4HPO_4 and 0.01% of $MgSO_4 \times 7H_2O$. The acidity of the culture medium for the growth of *P. canescens* amounted to pH 4.2 and for the yeast – to pH 5.5.

The yeast strains were grown on agar slants with whey [Burbianka & Pliszka, 1983]. *Penicillium canescens* was grown on slants of the following composition: lactose 3%;

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$NaNO_3$ – 0.2%; KH_2PO_4 – 0.1%, KCl – 0.05%; $MgSO_4$ – 0.05%; $FeSO_4$ – 0.001%; agar 2%; pH 5.5–6.0.

After the initial growth of yeast (2 days) and hypha fungus (7 days) on solid base at a temperature of 30°C, biomass was washed off with physiologic saline and transferred to 100 cm^3 of liquid culture medium. The multiplication of fungi was continued in a shaker at a temperature of 30°C. A 24-h growth of yeast and 48-h growth of hypha fungus performed twice constituted inoculum which was added to the medium in the amount of 5%.

After 2 days, the culture of yeast was centrifuged at 1800 g/10 min and supernatant was poured out. After washing with distilled water and repeated centrifugation, the deposit of yeast biomass was mounted in phosphate buffer of pH 6.8, resulting in constant volume (*K. fragilis* 32 cm^3, *C. sphaerica* 40 cm^3), frozen and disintegrated in a Biotex X25 mechanical disintegrator.

After a 4-day growing, the biomass of *Penicillium canescens* was filtered through gauze, washed with distilled water and after repeated centrifugation it was mounted in acetic buffer of pH 4.5, resulting in the volume of 40 cm^3, frozen and disintegrated.

After disintegration, the obtained suspension was centrifuged at 1800 g/15 min in order to remove the cell walls.

The activity of β-galactosidase with o-nitrophenyl β-D-galactopyranoside (ONPG) was determined in the preparations obtained from the biomass of yeast or hypha fungus as well as in the liquid left after the biomass was separated from the culture medium [Greenberg & Mahoney, 1981]. A unit of the β-galactosidase activity was the amount of enzyme which releases 1 μmole of o-nitrophenyl in 1 min under the determination conditions (optimum temperature and pH). The activity with lactose as a substrate was determined with the enzymatic method [Jasewicz & Wasserman, 1961]. An activity unit (AU) was the amount of micromoles of glucose released from lactose within 15 min under optimum conditions of the enzyme activity. The protein content was determined with Lowry method [Mejbaum-Katzenellenbogen & Mochnacka 1969].

The influence of the selected cations and EDTA on the activity of β-galactosidase was examined with the addition of cation salt solution to the reaction mixture in such concentration that the addition of 0.1 cm^3 ensured its 0.02% concentration in the sample.

Hydrolysis of lactose was performed on the solutions of milk permeate. The lactose content in the solution of permeate was determined with the AOAC method [AOAC, 1990a].

The content of glucose in hydrolysates was determined with the AOAC method [AOAC, 1990b] to calculate the degree of lactose hydrolysis. The saccharides contained in the hydrolysates were identified with thin layer chromatography [Kowalewska-Piontas & Bednarski, 2001].

RESULTS AND DISCUSSION

Evaluation of biosynthesis of β-galactosidase by the examined strains of yeast *Candida sphaerica* WKIIW3b and *Kluyveromyces fragilis* 28 and a strain of hypha fungi *Penicillium canescens* grown in culture media with lactose as a source of carbon (culture media 1, 3 and 4) and without lactose (culture media 2, 5 and 6) confirms that not only lactose is an inductor of β-galactosidase biosynthesis. It was found that also oligosaccharides present in soybean flour or wheat bran perform this function (Table 1). Yeast *Kluyveromyces fragilis* 28 synthesised β-galactosidase in both culture media with lactose (No. 1) and culture media without lactose (No. 3, 5 and 6). The activity of β-galactosidase as well as the yield of its synthesis expressed as total activity were the highest (16.18 AU/cm^3 of the preparation and 517.8 AU/dm^3 of the culture medium, respectively) when only lactose was the source of carbon in the culture medium. The replacement of lactose or its part with oligosaccharides present in soybean flour or wheat bran contributed to a decrease in both the activity of the synthesised β-galactosidase and total activity of the β-galactosidase obtained from 1 dm^3 of the culture medium. On the other hand, yeast *Candida sphaerica* WKII3b synthesised the largest amount and the most active β-galactosidase in the culture media where a source of carbon was lactose combined with oligosaccharides from soybean flour (culture medium No. 3) or from wheat bran (culture medium No. 4). The presence of oligosaccharides from wheat bran in the

TABLE 1. Influence of carbon source in culture medium on the activity of β-galactosidase synthesised by yeast intracellularly.

Specification		Carbon source in culture medium											
		Yeast type											
		Kluyveromyces fragilis 28						*Candida sphaerica* WK II W3b					
No. of culture medium **		1	2	3	4	5	6	1	2	3	4	5	6
pH of culture medium after growth		3.46	4.46	3.98	3.00	3.51	5.82	3.89	5.99	4.28	4.46	6.72	6.25
Yield of wet biomass [g/dm^3 of culture medium]		28.40	15.58	28.03	27.64	16.51	13.50	28.04	20.72	28.92	44.96	18.20	38.26
β-Galactosidase preparation after disintegration and centrifugation of cell walls	Protein content [mg/cm^3]	13.60	3.5	3.08	3.10	5.60	3.08	4.22	3.99	4.78	4.95	2.62	3.70
	Activity*** of β-galactosidase [AU/cm^3]	16.18	1.85	1.20	4.06	1.12	1.08	1.73	1.32	3.35	3.46	0.76	0.89
	Specific activity [AU/mg of protein]	1.19	0.53	0.39	1.31	0.20	0.35	0.41	0.33	0.70	0.70	0.29	0.24
	Total activity of β-galactosidase [AU/dm^3 of culture medium]	517.8	59.4	46.1	129.9	35.8	34.5	69.2	52.6	133.8	138.6	30.4	35.5

* No activity of β-galactosidase was found in after-growth liquid ; ** marking as in methodology; *** activity of β-galactosidase from *K. fragilis* was determined at 37°C and pH 6.8 and that from *C. sphaerica* WKIIW3b at 43°C and pH 6.8.

culture medium seems to have more influence on the growth of cells of yeast *Candida sphaerica* WKII3b. The highest yield of wet biomass of such yeast was obtained from the culture media No. 4 and 6 and it accounted for 44.96 g and 38.26 g, respectively (Table 1). Lactose present in the culture media No. 1, 3 and 4 contributed to obtaining a higher β-galactosidase activity which amounted to 1.73; 3.35; 3.46 AU/cm^3, respectively, compared to a lower β-galactosidase activity of 1.32; 0.76 and 0.29 AU/cm^3 obtained from the culture media without lactose No. 2, 5, 6, respectively.

It was found that lactose is not an inductor of β-galactosidase synthesis for each strain. Hypha fungi strain *Penicillium canescens* grown in a culture medium only with lactose made more biomass than in the culture media without lactose or culture media mixed with lactose. However, β-galactosidase activity obtained after the growth of fungi in a culture medium with lactose was the lowest in both biomass and liquid after separating it and reached to 0.55 and 0.15 UA/cm^3, respectively (Table 2, culture medium 1). However, after the growth in the culture media without lactose (culture media 2, 5 and 6), β-galactosidase activity in both biomass of *Penicillium canescens* and after-growth liquid was high and varied from 60.38 to 67.43 AU/cm^3 in biomass and from 6.20 to 9.88 AU/cm^3 in after-growth liquid.

A relationship between the enzyme activity determined with ONPG as the substrate and the activity determined with lactose is observed in the biosynthesis of β-galactosidase by yeast *K. fragilis* 28. The highest activity of β-galactosidase was obtained after the growth in the culture medium No. 1. It was determined with both ONPG and lactose and accounted for 12.9 AU/cm^3 and 27.8 μmol of glucose/cm^3, respectively (Table 3). β-Galactosidase obtained from yeast *Candida sphaerica* WKIIW3b which was multiplied in the culture media 3 and 4 showed a similar activity determined with ONPG which amounted to 3.36 and 3.48 AU/cm^3, respectively. On the other hand, the activity of β-galactosidase from the biomass multiplied in the culture medium No. 4 determined with lactose as the substrate was higher than that obtained in the culture medium No. 3 and reached 9.79 and 6.66 μmol glucose/cm^3, respectively (Table 3).

TABLE 3. Influence of carbon source in culture medium on the affinity of synthesised β-galactosidase to ONPG and lactose.

Yeast strain	No. of culture medium*	β-Galactosidase activity determined:			
		with ONPG [AU/cm^3]		with lactose [μmol of glucose/cm^3]	
		biomass	after-growth liquid	biomass	after-growth liquid
Candida	1	1.73	0	4.32	0
sphaerica	2	1.30	0	2.03	0
WK II W3b	3	3.36	0	6.66	0
	4	3.48	0	9.79	0
	5	0.77	0	3.49	0
	6	0.88	0	2.67	0
Kluyveromyces	1	12.9	0	27.80	0
fragilis	2	2.6	0	2.19	0
28	3	1.43	0	2.59	0
	4	8.55	0	4.76	0
	5	0.99	0	2.09	0
	6	1.19	0	0.94	0
Penicillium	1	0.55	0.20	15.30	0.40
canescens	2	60.40	9.88	26.30	8.81
	3	1.61	2.09	29.50	5.73
	4	3.07	5.26	38.0	6.60
	5	67.43	6.20	56.26	1.72
	6	60.69	8.70	28.93	2.64

* marking as in methodology

Penicillium canescens grown in the culture media with lactose synthesised extracellular β-galactosidase whose activity determined with ONPG was clearly lower than that obtained in the nutrient media without lactose (Table 3). It seems interesting that although the activity of β-galactosidase obtained from the biomass of *P. canescens* multiplied in the culture medium with lactose was the lowest and reached 15.3 μmol of glucose/cm^3 it was still only 4-fold lower than the highest activity obtained from the culture medium No. 5 which amounted to 56.26 μmol of glucose/cm^3. On the other hand, the activity of β-galactosidase in the liquid after the

TABLE 2. Influence of carbon source in culture medium on the activity of β-galactosidase synthesised by *Penicillium canescens* extra- and intracellularly.

Specification	Carbon source in nutrient medium											
	milk permeate 1		soybean flour, wheat bran 2		milk permeate, soybean flour 3		milk permeate, wheat bran 4		soybean flour 5		wheat bran 6	
	Type of β-galactosidase preparation											
	from biomass*	after-growth liquid	from biomass*	after-growth liquid	from biomass*	after-growth liquid	from biomass*	after-growth liquid	from biomass*	after-growth liquid	from biomass*	after-growth liquid
pH of culture medium after growth	-	4.35	-	6.27	-	5.47	-	5.85	-	6.53	-	6.65
Yield [g or cm^3/dm^3 of culture medium]	75.2	930	35.3	970	28.9	950	32.8	940	35.3	950	48.7	890
Protein content [mg/cm^3]	1.90	0.7	2.40	0.9	2.02	0.7	2.34	1.1	2.48	0.9	1.92	1.1
β-Galactosidase activity** [AU/cm^3]	0.55	0.15	60.48	9.88	0.87	2.09	1.85	5.26	67.43	6.20	60.38	8.70
Specific activity [AU/mg of protein]	0.29	0.21	25.2	10.98	0.43	2.98	0.79	4.78	27.19	6.89	31.45	7.91
Total activity of β-galactosidase	22.0	136.7	2419.2	9583.6	34.9	1985.5	73.9	4944.4	2697.2	5890.9	2415.3	7743.9
[AU/dm^3 of culture medium]	158.7		12002.8		2020.4		5018.3		8588.1		10159.2	

* – β-galactosidase preparation after disintegration of fungi biomass and centrifugation; ** – β-galactosidase activity determined at 45°C and pH 4.5.

growth of fungi in the culture medium with lactose amounted to only 0.40 μmol of glucose/cm^3 and the activity of β-galactosidase in the liquid after the growth in the culture medium without lactose amounted to 8.81 μmol of glucose/cm^3. Thus it was as much as 22 times higher. It confirms the fact that *P. canescens* synthesises extracellular β-galactosidase.

It is worth emphasising that as early as in 1984 Jacober-Pivarnik & Grand [1984] suggested that β-galactosidase isolated from various sources can show different hydrolytic activity depending on the substrate used to determine it.

Kim *et al.* [1997] noticed that even small changes in the concentration of metal ions or buffer could influence the activity determined with ONPG and with lactose to various extent. The addition of various cations and EDTA to the solutions of the substrates, *i.e*. to ONPG or lactose, confirms the findings of Kim *et al.* [1997] (Table 4). Namely, the addition of Co^{++} resulted in a significant increase in the activity of β-galactosidase determined with ONPG obtained from the culture of *C. sphaerica* in the culture medium 1, 2 and 4. The activity of β-galactosidase synthesised in the culture medium 1 and 4 and determined with lactose was lower than the control one and amounted to 81.4% and 60.5%, respectively, while that of the enzyme obtained from the culture of *C. sphaerica* in the culture medium No. 2 amounted to as much as 110.9%. β-Galactosidase from yeast *K. fragilis* 28 in the presence of Co^{++}, Zn^{++}, Cu^{++} ions and EDTA determined with ONPG did not show any activity while it grew in the reaction with lactose compared to the control sample and amounted to 369.8; 493.1; 109.6 and 123.3%, respectively.

It also results from Table 4 that the addition of cations and EDTA in the procedure for the determination of the activity of β-galactosidase from *P. canescens* has a much smaller influence than on β-galactosidase from yeast. Only

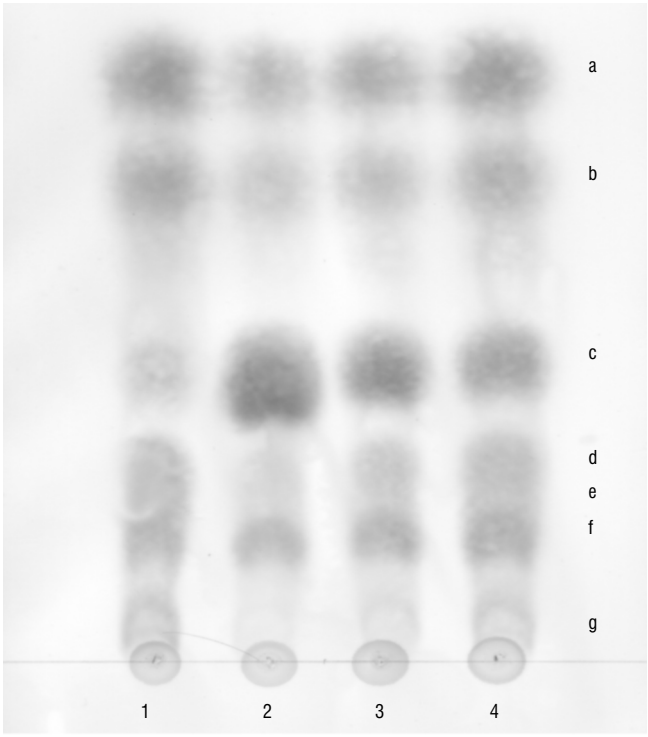

FIGURE 1. The chromatogram of the separation of saccharides present in hydrolysates of milk permeate with lactose content of 28.8%. β-Galactosidase preparations obtained from the biomass of yeast *Candida sphaerica* WKIIW3b multiplied in culture media with various carbon sources. Hydrolysis was performed at the temperature of 43°C for 4h at pH 6.8. 1–4 – hydrolysates (number of hydrolysates corresponds with number of culture medium in methodology); a – glucose, b – galactose, c – lactose and lactulose, d – unidentified gala tooligosaccharide, e – 4-0-(3-0-α-D-galactopyranosyl-β-D-galactopyranosyl)-D--galactopyranose, f – unidentified galactooligosaccharide, g – unidentified galactooligosaccharide.

Table 4. Influence of the addition of various ions or EDTA to the solution of substrate on the activity of β-galactosidase synthesised by fungi. β-Galactosidase activity was determined with ONPG (A) or with lactose (B) under optimum conditions.

Substrate solution with the addition of	β-Galactosidase activity (A) [AU/cm^3] (B) [μmol of glucose/cm^3]									
	β-galactosidase synthesised by									
	Candida sphaerica WK II W3b						*Kluyveromyces fragilis 28*		*Penicillium canescens*	
	Type of culture medium *									
	1		2		4		1		2	
	A	B	A	B	A	B	A	B	A	B
Control ** [%]	8.51	39.3	0.62	5.5	2.51	16.2	13.07	29.1	354.9	5.9
	100	100	100	100	100	100	100	100	100	100
Zn^{++}	5.67	5.1	0.60	2.5	2.51	traces	0	107.6	341.3	6.2
	66.6	13.0	96.8	45.4	100			369.8	96.2	105.1
Co^{++}	26.71	32.0	10.80	6.1	5.67	9.8	0	141.5	354.9	10.4
	313.9	81.4	1741.9	110.9	225.9	60.5		493.1	100	176.3
Mn^{++}	28.54	39.9	1.02	5.7	5.76	10.2	9.8	23.9	1041.3	5.5
	335.4	101.5	164.5	103.6	229.5	62.9	75.0	82.2	293.4	93.2
Na^{+}	5.76	41.2	0.62	3.4	2.01	17.8	–	–	–	–
	67.7	104.8	100.0	61.8	83.7	109.9				
K^{+}	6.68	10.9	0.73	6.9	2.47	7.3	14.9	183.3	354.9	10.4
	78.5	27.7	117.7	125.4	98.4	45.1	114.0	630.1	100	176.1
Cu^{++}	0.12	0	0.13	3.1	0.15	0	0	31.9	424.6	5.1
	1.4	0	20.9	56.3	5.98			109.6	119.6	84.4
Mg^{++}	23.79	135.6	1.12	8.1	5.76	30.9	9.8	27.9	354.9	7.0
	279.9	345.0	180.6	147.3	229.5	190.7	75.0	95.9	100	118.6
EDTA	1.13	0	0.20	2.5	0.21	0	0	35.9	355.0	7.1
	1.6		32.2	45.4	8.4			123.3	100	120.3

* marking as in methodology; ** β-galactosidase activity ; % – activity expressed in percentage of control sample activity

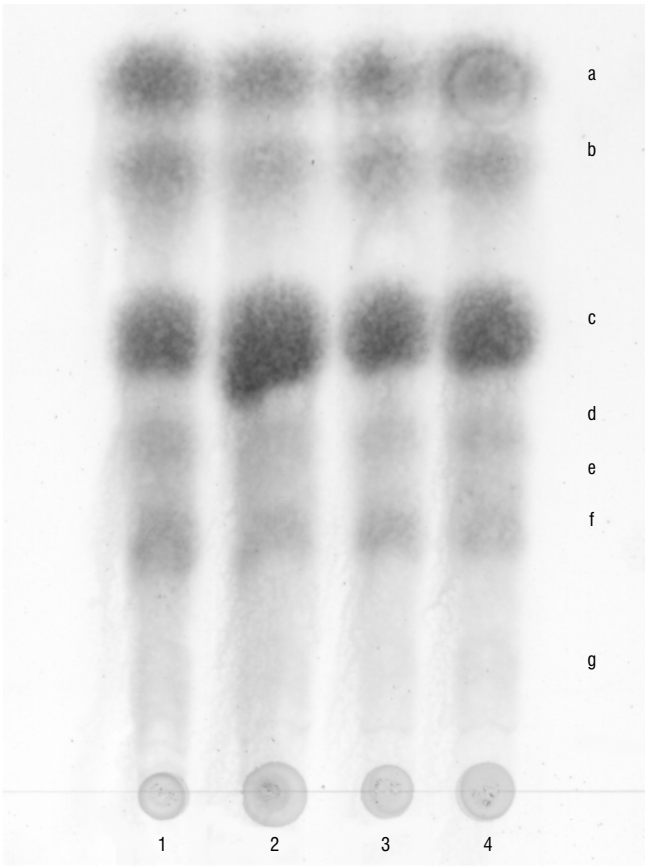

FIGURE 2. The chromatogram of the separation of saccharides in hydrolysates of milk permeate with lactose content of 20.9% by β-galactosidase preparation from *Kluyveromyces fragilis* 28 obtained in culture media with lactose (1 and 2); lactose + wheat bran (3 and 4). Hydrolysis was performed at 37°C for 4 h (hydrolysates 3 and 4) and for 6 h (hydrolysates 2 and 4) at pH 6.8 (marking a–g as in Figure 1).

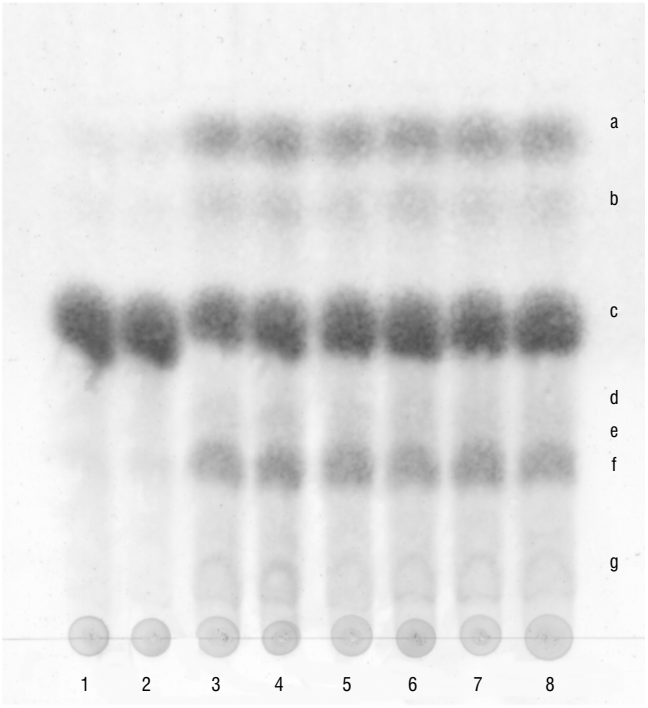

FIGURE 3. The chromatogram of the separation of saccharides in hydrolysates of milk permeate with lactose content of 17.4% by β-galactosidase from the biomass of *P. canescens* grown in culture media with various carbon sources: hydrolysates 1 and 2 – milk permeate; hydrolysates 3 and 4 – flower+wheat bran; hydrolysates 5 and 6 – soybean flour; hydrolysates 7 and 8 – wheat bran. Hydrolysis was performed at the temperature of 45°C for 4 h (hydrolysates 1, 3, 5, 7) and for 6 h (hydrolysates 2, 4, 6, 8) (marking a–g as in Figure 1).

the addition of Mn^{++} ions caused almost a three-fold increase in the activity of β-galactosidase determined with ONPG (1041.3 AU/cm^3) compared to the control sample (354.9 AU/cm^3). On the other hand, the same addition of Mg++ ions reduced the activity of β-galactosidase determined with lactose to a small extent which amounted to 93.2% of the control sample activity.

A significant influence of cations on the activity of β-galactosidase from *C. sphaerica* can result from varied content of metal ions in the culture media obtained from various components.

Determination of differences in the evaluation of hydrolytic activity of β-galactosidase using various substrates is of a great practical significance. Due to a less inconvenient procedure the activity of β-galactosidase is usually determined with ONPG. If such an activity does not correspond with the activity of β-galactosidase determined with lactose, the evaluation of the activity of the applied enzyme can be actually wrong.

Transgalactosilation property of the examined preparations of β-galactosidase seems to be closely related to the degree of lactose hydrolysis. Too low degree of lactose hydrolysis (Figure 1, hydrolysate 2 – 13.7% or Figure 3, hydrolysates 1 and 2 – 3.1 and 3.3%, respectively) does not favour the creation of oligosaccharides. Similar degree of lactose hydrolysis in the samples obtained with β-galactosidase preparation from *Kluyveromyces fragilis* 28 (Figure 2; in hydrolysates 1 to 4: 27.2, 28.8, 23.2, 24.0% respectively) and with β-galactosidase preparation from *P. canescens* (Figure 3, hydrolysates 3–8: 35.6, 42.4, 35.6, 47.3, 31.5 and 44.1%, respectively) caused that the division of saccharides on chromatograms looks very similar. We consider the presented identification of saccharides present in the hydrolysates of lactose preliminary. In our further studies we are going to analyse them quantitatively with HPLC.

According to Huber *et al.* [1976], a favourable relationship of the activity of transgalactosilation to the activity of lactose hydrolysis can be adjusted with a change in pH, usually to more acid. The activity of transgalactosilation can also be improved with a decrease in the temperature of hydrolysis [Cruz *et al.* 1999] or with the addition of an inhibitor of lactose hydrolysis [Kowalewska-Piontas & Bednarski, 2001].

CONCLUSIONS

1. The inductors of β-galactosidase biosynthesis by fungi are, beside lactose, oligosaccharides present in soybean flour and wheat bran.

2. The addition of soybean flour or wheat bran to a culture medium with lactose favours an improvement in the activity of β-galactosidase synthesised by *Candida sphaerica*.

3. Comparison of determinations of the β-galactosidase activity indicates that when selecting the substrates to be used for this purpose (ONPG or lactose) the properties of the enzyme, which are dependent on the source of carbon

used in the growth of fungi used in its biosynthesis, should be taken into account.

4. The activity of β-galactosidase determined with ONPG or with lactose is determined by the presence of ions but the extent of such activity depends on the composition of a culture medium and type of fungi used in the biosynthesis of β-galactosidase.

5. The obtained preparations of β-galactosidase show transgalactosilation properties. The yield of enzymatic synthesis of galactooligosacccharides depends on the degree of lactose hydrolysis.

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WPŁYW WYBRANYCH INDUKTORÓW NA BIOSYNTEZĘ I WŁAŚCIWOŚCI BETA-GALAKTOZYDAZY

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Przeprowadzono hodowlę dwóch szczepów drożdży *Candida sphaerica* WKIIW3b i *Kluyveromyces fragilis* 28 oraz grzybów strzępkowych *Penicilum canescens* na pożywkach, w których źródłem węgla był permeat po UF mleka, mąka sojowa lub otręby pszenne, stosowane oddzielnie lub w różnej kombinacji. W otrzymanych preparatach beta-galaktozydazy oznaczono aktywność hydrolizy z ONPG i z laktozą oraz zbadano wpływ niektórych kationów i EDTA na aktywność beta-galaktozydazy zarówno z ONPG, jak i z laktozą jako substratem.

Właściwości transgalaktolzyacji preparatów beta-galaktozydazy syntezowanych przez grzyby na pożywkach z różnym źródłem węgla badano stosując te preparaty do biokonwersji laktozy w permeacie mleka o jej stężeniu od 17,4 do 28,8%. Sacharydy w otrzymanych produktach oznaczono metodą TLC.