A C T A A L I M E N T A R I A P O L O N I C A
Vol. XII (XXXVI), No. 1 1986

JADWIGA KOWALEWSKA
WŁODZIMIERZ BEDNARSKI
MARIAN MIECZKOWSKI

PRODUCTION AND USE OF β-GALACTOSIDASE FROM *ASPERGILLUS NIGER*

Institute of Food Engineering and Biotechnology,
University of Agriculture and Technology, Olsztyn

Key words: β-galactosidase, lactose fermentation, whey utilization, biomass production.

Fungi strains best suited for the biosynthesis of β-galactosidase were selected. β-galactosidase formation, biomass production and lactose fermentation by *Aspergillus niger* 3 grown on whey were investigated. A partly purified and fixed enzyme preparation was used to hydrolyse lactose in acid whey.

The production of cottage cheeses and edible casein is currently being increased in Poland with the view of a fuller utilization of milk proteins in human nutrition [3]. Each year about 1.5 bilion dm^3 of the so called acid whey is produced during the manufacture of the mentioned protein products and most of it is not utilized rationally. One of the factors hampering the use of whey concentrates in food industry is the poor solubility of lactose which is responsible for the grainy texture unwellcome in concentrates [4, 5, 12].

The reduced usability of lactose is due, among others, to the fact that it is less sweet than other sugars and that it is not fermented by the majority of microorganisms [4].

The research was performed within the problem MR.II.17.

All the mentioned drawbacks of milk sugar and the lack of tolerance to it on the part of some groups of people may be eliminated by enzymatic hydrolysis of lactose with β-galactosidase. After hydrolysis of lactose to glucose and galactose, the mixture of saccharides is better soluble, sweeter and easier assimilated by humans and microorganisms.

The lactose in acid whey produced during the manufacture of cottage cheeses or casein is best hydrolyzed by β-galactosidase from molds [4, 9, 13].

In this research we studied the following:

(i) The selection of a mold strain predisposed to the biosynthesis of β-galactosidase;

(ii) The optimization of culture conditions for the selected mold strain;

(iii) The obtaining and preliminary purification and fixing of the β-galactosidase preparation;

(iv) The evaluation of the suitability of this preparation for hydrolysis of lactose in acid whey.

MATERIAL AND METHODS

SUBSTRATE AND CONDITIONS OF MOLD CULTURE

The study was performed with mold strains obtained from strain collections of the Institute of Food Engineering and Biotechnology of the University of Agriculture and Technology in Olsztyn, the Institute of Microbiology of the Łódź Technical University, and of the Pilot Plant of Acetic Acid Production in Zgierz.

The molds were cultured on whey-agar slants. Mold spores were washed from the slants with 5 cm^3 of sterile physiological fluid and the obtained suspension was used to inoculate 50 cm^3 of whey medium prepared according to the method described in our previous work [1].

The mold culture was maintained in 200 cm^3 Erlenmeyer flasks placed on a shaker for 48 h at 30°C.

The obtained inoculum was introduced into the following two kinds of culture (in amounts corresponding to 10% of culture volume):

(i) Stationary mold culture in "Roux" bottles at 30°C for 120 h, with periodic shaking;

(ii) Submerged mold culture in 30 dm^3 „Simax" fermenters containing 12-15 dm^3 of the medium. The mold culture was maintained in the following conditions: temperature — 30°C, time — 96-120 h, mixing — 170 r.p.m., aeration — 0.14-1.12 dm^3 air per min per dm^3.

OBTAINING OF β-GALACTOSIDASE PREPARATION

Specific amounts of culture fluid were taken during the mold culture and after its conclusion and centrifuged at 45 000 g for 15 min. After separating the biomass, the activity of β-galactosidase was determined in the supernatant, while the biomass was washed with acetate buffer of pH 4.5 and again centrifuged as above. The mold biomass was frozen and disintegrated in a Biotex X 25 apparatus, or cooled to 2-4°C and disintegrated in a Dyno Mill KDL apparatus.

After disintegration, the mold biomass was centrifuged in 15 000 g a Beckman J 21 C centrifuge at 45 000 g for 15 min, and cell wall sediment together with a solution containing enzymatic proteins were obtained.

The sediment was again dissolved in acetate buffer and macerated for 24 h. Next it was centrifuged once again as described above. The sediment was discarded and the supernatant combined with the solution from the first centrifugation. The supernatant was thickened osmotically in the presence of a 60% saccharose solution until the dry substance value reached 30%.

The concentrate was then joined with glicerine in 1:1 proportion and the obtained solution served as "crude" β-galactosidase preparation. The activity of β-galactosidase was determined according to the method of Salomon and Johnson [7]. We assumed the activity unit to be the amount of enzyme liberating 1 μM of glucose from lactose in 15 min at 37°C.

EVALUATION OF THE SUITABILITY OF THE β-GALACTOSIDASE PREPARATION FOR HYDROLYSIS OF LACTOSE IN ACID WHEY

100 cm^3 whey portions were poured into Erlenmeyer flasks and pasteurized for 30 min at 65°C. The lactose content in whey was determined according to the method of Bertrand [2]. Next, the effect of whey acidity (pH 3.5, 4.0, 4.5, 5.0, 5.5) and temperature (30, 35, 40, 45 and 50°C) was studied. 1 cm^3 of the preparation was added. Lactose hydrolysis was carried out for 5 h, mixing the medium with a magnetic mixer. After selecting the most favourable conditions of lactose hydrolysis, we studied the effect of Mg^{++}, Mn^{++}, Ca^{++}, K^+ and Na^+ ions on the degree of lactose hydrolysis in whey containing 0.02% additions of $MgSO_4$ or $MnSO_4$, or $CaCl_2$ or KCl or NaCl. Next, we determined the volume of the β-galactosidase preparation addition (0.5, 1.0, 1.5 and 2.0 cm^3) and studied the degree of lactose hydrolysis after 3, 4, 5 or 6 h of reaction with the enzyme.

For the sake of comparison, the hydrolysis of lactose in whey was also performed at 4°C, with the remaining conditions left unchanged.

RESULTS AND DISCUSSION

The mold strains selected in the preliminary stage of the study were evaluated as to their ability to biosynthesize of β-galactosidase. The activity of the enzyme was determined in mold mass after disintegration and in the fluid left after the separation of biomass from the medium. This procedure was inspired by reports of other authors that some mold strains synthesises extracellular β-galactosidase [9, 10]. Out of the 31 available mold strains, only seven proved capable of producing intracellular β-galactosidase, and none synthesized extracellular β-galactosidase.

Next, bearing in mind the results of Zalokar [14] who found that the activity of β-galactosidase synthesized by Neurospora crassa increases with the increase of culture age, we studied the effect of time of culture of the selected seven mold strains on β-galactosidase activity. The results presented in Table 1 show that the most active β-galactosidase was produced by the *A. niger* 3 strain after six days of culture and by *A. niger* E-1 after three days. These strains were used in the subsequent studies aimed at optimizing the conditions of their culture.

Table 1. Effect of time on the activity of β-galactosidase produced by selected fungi strains

Origin of strain	Strain	β-galactosidase activity (in μM glucose (cm^3) following disintegration of mold biomass, after different duration of culture (days)				Strains selected for further experiments
		3	4	5	6	
Collection of strains of the Institute of Food Engineering and Biotechnology, University of Agriculture and Technology in Olsztyn	*Aspergillus niger 3*	0.161	0.182	0.201	0.521	×
Collection of strains of the Institute of Microbiology of the Łódź Technical University	*Aspergillus niger* E-1	0.217	0.170	0.168	0.160	×
Collection of strains of the Pilot Plant of Acetic Acid Production in Zgierz	*Asp. niger* 54	0.050	0.075	0.100	0.050	
	Asp. niger 81	0.149	0.172	0.197	0.160	
	Asp. niger 85	0	0	0.170	0.160	
	Asp. niger 86	0	0	0	0	
	Asp. niger 91	0	0	0.153	0.150	

The productivity of β-galactosidase biosynthesis by molds was studied in dependence on the lactose content in the substrate, the time of culture, the total content of nitrogen substances in the substrate, and the active acidity of the substrate. The following set of parameters was selected by the variance method: lactose content in the substrate — 3%, duration of mold culture — 108 h, addition of $(NH_4)_2SO_4$ to the substrate — 1%, active acidity of the substrate — pH 5.0.

The above parameters were observed during the biosynthesis of β-galactosidase by the studied molds in submerged culture (Table 2). The culture of *A. niger* E-1 yielded an average of 260 g dm^3 of "wet" biomass (with an 82% water content) with an activity of 488,59 μM glucose from 1 dm^3 of substrate. The culture of *A. niger* 3 maintained in identical conditions gave 257 g of biomass from 1 dm^3 of substrate but with a much

Table 2. Yield of β-galactosidase biosynthesis by *A niger* E-1 and *A. niger* 3 in submerged culture (mean values from 3 cultures)

Strain	Lactose content in medium (%)	Time of culture (h)	$(NH_4)_2SO_4$ addition (%)	Acidity of medium before culture (pH)	Yield of wet biomass from 1 dm^3 of medium (g/dm^3)	Total solids obtained from the medium (g dry substance/dm^3)	Lactose content in medium after culture (%)	Acidity of medium after culture (pH)	Enzyme activity in biomass after disintegration (μM glucose /dm^3 medium)
A. niger E-1	3	108	1	5	260.0	16.08	0.27	2.02	488.59
A. niger 3	3	108	1	5	257.0	12.57	0.59	1.68	791.22

[59]

Table 3. Effect of selected cations on whey lactose hydrolysis by β-galactosidase from *Aspergillus niger* 3

Ion addition	Temperature	Hydrolysis time (h)	Reduction capability (cm^3/0.1 N $KMnO_4$)	Glucose (mg/100 cm^3 medium)	Degree of lactose hydrolysis (%)
Control sample	40	4	11.5	750	30.0
Mn^{++}	40	4	12.2	1170	56.4
Mg^{++}	40	4	11.8	930	37.2
Ca^{++}	40	4	11.4	700	28.0
K^+	40	4	13.8	2190	87.6
	4	24	12.9	1680	68.8
Na^+	40	4	11.2	580	23.2

higher activity of 791,22 μM glucose from 1 dm^3 of substrate. On the basis of these results we selected the strain *A. niger* 3 as the source of enzyme for the hydrolysis of lactose in acid whey.

In the subsequent stage of research we found that the optimum acidity for the activity of the *A. niger* 3 enzyme is pH 4.5 and the optimum temperature is 40°C (Fig. 1). We determined that by adding 1.5 cm^3 of pre-

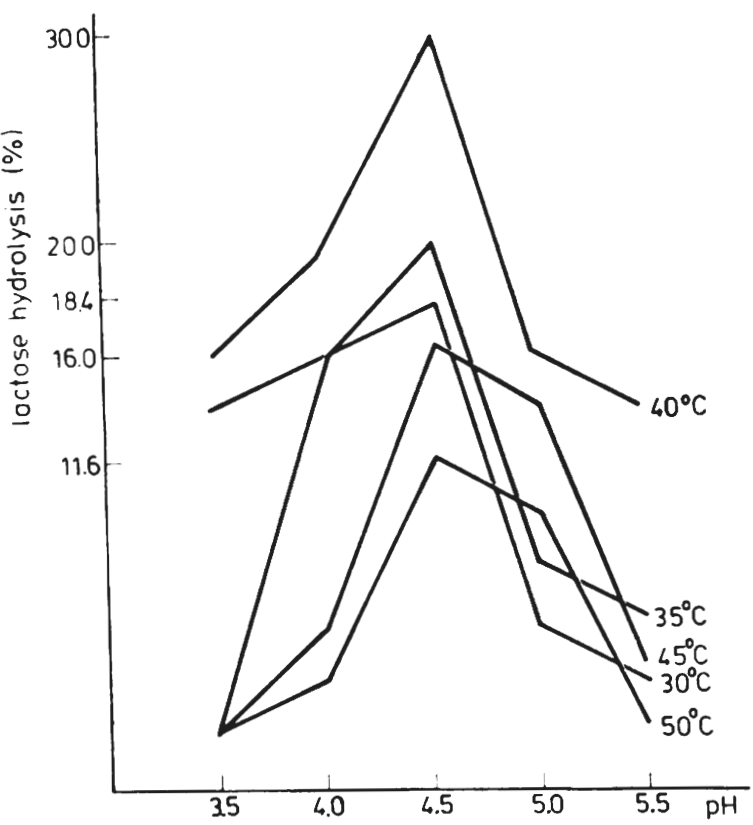

Fig. 1. Effect of pH and temperature on whey lactose hydrolysis by β-galactosidase from *Aspergillus niger* 3 (enzyme dose — 1 cm^3/100 cm^3 of whey, hydrolysis time — 5 h)

paration with an activity of 0.35 activity units (a.u) to 100 cm^3 of whey, it is possible to obtain a decomposition of up to 98% of lactose after 4 h (Fig. 2). Moreover, we observed the positive effect of K^+ and Mn^{++} ions on the activity of the β-galactosidase from *A. niger* 3 and, consequently, on the degree of lactose hydrolysis in whey. According to other authors [5, 9, 12], ions of metals have no effect on the activity of enzymes produced by molds.

An addition of Mn^{++} ions caused a twofold increase of the degree of lactose hydrolysis, and the K^+ ions activate the β-galactosidase from *A. niger* vere clearly: the obtained degree of lactose hydrolysis in the studied whey samples was three times that in control (Table 3).

We know from the literature [6] that an addition of K^+ ions to a β-galactosidase preparation obtained from yeast stabilizes the activity of the enzyme. The positive effect of K^+ ions was also apparent during the hydrolysis of lactose in whey at 4°C; this indicates that the enzyme from *A. niger* 3 is also active in low temperatures.

The results concerning the productivity of β-galactosidase biosynthesis as well as the assessment of its applicability in the hydrolysis of lactose in acid whey are in agreement with the results of other authors [8, 9, 13].

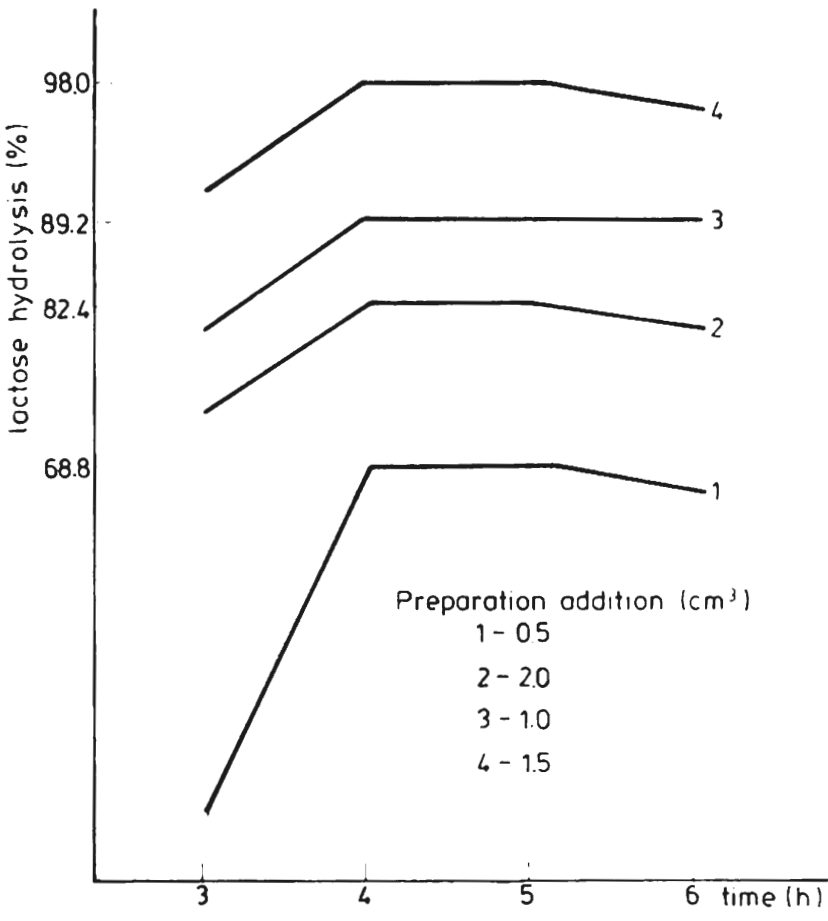

Fig. 2. Effect of time and enzyme dose on whey lactose hydrolysis by β-galactosidase from *Aspergillus niger* 3 (hydrolysis temperature — 40°C, pH 4.5. addition of K^+ ions — 0.02%)

The encouraging results in the production of β-galactosidase preparation from molds and the sufficient degree of lactose hydrolysis in acid whey suggest that the findings of this research might be used in industrial practice, for instance to obtain sugar concentrates that can be used in the technology of beer production [11].

The studies currently under way are aimed at obtaining purified β-galactosidase preparations which may be used in lyophilized or immobilized form in the production of glucose-galactose syrups from whey.

CONCLUSIONS

1. Among the 31 investigated mold strains, *Aspergillus niger* 3 gave the highest yield of β-galactosidase.

2. It is possible to obtain 12.57 g dry substance of mold from 1 dm^3 of substrate when *Aspergillus niger* 3 is cultured under the following conditions: lactose content in substrate — 3%, addition of $(NH_4)_2SO_4$ to the whey — 1%, acidity of substrate — pH 5, time of culture — 108 h. The activity of the preparation obtained in such conditions is 791.22 a.u. from 1 dm^3.

3. Partly purified and osmoactively fixed β-galactosidase preparations may be used to hydrolyse lactose in acid whey.

4. Additions of K^+ and Mg^{++} greatly increase the activity of β-galactosidase from *Aspergillus niger* 3.

LITERATURE

1. Bednarski W., Jakubowski J., Poznański S., Surażyński A.: Zeszyty Naukowe AR-T Olsztyn 1973, 2, 3.
2. Budsławski J., Drabent Z.: Metody analizy żywności. WNT, Warszawa 1972.
3. Czerwiński S., Imbs B.: Wykorzystanie serwatki w przemyśle mleczarskim. CRS, Warszawa 1974.
4. Dłużewski M.: Roczn. Techn. i Chemii Żywności 1961, 16, 21.
5. Greenberg N. A., Mahoney R. R.: J. Food Sci., 1981, 46, 684.
6. Jacober-Pivarnik L. F., Rand A. G. jr.: J. Food Sci., 1984, 49, 435.
7. Jasewicz L., Wasserman A. E.: J. Dairy Sci., 1961, 44, 393.
8. Okos M., Grulke A. R., Syrerson A.: J. Food Sci., 1978, 43, 566.
9. Park Y. K., de Santi M. S., Pastore G. M.: J. Food Sci., 1979, 44, 100.
10. Pastore G. M., Park Y. K.: J. Ferment. Technol., 1980, 58, 59.
11. Poznański S., Leman J., Bednarski W., Szmelich W., Kowalewska J., Chodkowski M., Wieliczko W.: Przemysł Fermentacyjny i Rolny 1977, 31, 3.
12. Shukla T. P.: CRC Crit. Rev. Food Technol., 1975, 5, 325.
13. Whitaker J. R.: Conference on Enzyme Economics. Chicago Marcel Dekker Inc. 1978.
14. Zalokar M.: Am. J. Botany 1959, 46, 555.

Manuscript received: November, 1984
Authors address: 10-719 Olsztyn 5, blok 31

J. Kowalewska, W. Bednarski, M. Mieczkowski

OTRZYMYWANIE I ZASTOSOWANIE β-GALAKTOZYDAZY Z *ASPERGILLUS NIGER*

Instytut Inżynierii i Biotechnologii Żywności,
AR-T, Olsztyn

Streszczenie

Spośród 31 dostępnych szczepów pleśni wybrano *Aspergillus niger* 3, który podczas wgłębnej hodowli na podłożu z serwatki w ustalonych warunkach syntetyzował zadowalające ilości biomasy — 12,57 g s.s./dm^3 o aktywności całkowitej 791,2 j.a. Optymalna kwasowość działania enzymu otrzymanego z *A. niger* 3 wynosi pH 4,5 w temp. 40°C. Dodatek 1,5 cm^3 preparatu do 100 cm^3 serwatki pozwala uzyskać 98% rozkładu laktozy w ciągu 4 h. Obecność jonów Mn^{++} podnosi dwukrotnie stopień rozkładu laktozy, natomiast jony K^+ aktywują β-galaktozydazę z pleśni *A. niger* 3 w najwyższym stopniu, powodując trzykrotny wzrost stopnia rozkładu laktozy w porównaniu z próbą kontrolną.

FIRST ANNOUNCEMENT

The V-th European Nutrition Conference is organized by:
The Polish Society of Nutritional Sciences and the Human Nutrition Committee of the Polish Academy of Sciences
Warsaw, 20-23 May, 1987

Sponsored by:
The Federation of European Nutrition Societies
Main topics of the conference
— Food quality
— Animals in nutrition experiments
— Food consumption and nutritional status assessment
— Food and drug interaction
— Nutrition education and extension
— European food and nutrition policies
— Nutrition and mental and physical function
— Nutrition and cancer
— Nutrition and hypertension
— Parenteral and enteral nutrition
Language of the Conference: English
Official Travel Company: Polish Airlines "LOT"
Telex: 814327
Tourist information: "Orbis"
Telex: 814728
Correspondence of the Conference Bureau: Fifth FENS European Nutrition Conference
c/o Institute of Human Nutrition
Warsaw Agricultural University, SGGW-AR
ul. Nowoursynowska 166
02 766 Warsaw, Poland
Telex: 814790 SGGW PL
Telephone: 431878