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Production of Extracellular Lactase from *Fusarium moniliforme*

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Summary. A strain of *Fusarium moniliforme*, previously used for microbial protein production, excreted lactase (β-D-galactosidase, EC. 3.2.1 23) when cultivated either in a whey liquid medium or on a wheat bran solid medium. The enzyme produced in both media had pH and temperature optima of 4–5 and 50–60 °C respectively and was particularly suitable for processing acid whey.

In the whey culture, maximum lactase yield was observed after 95 h of growth at 30 °C and whey lactose concentration of 9%. The addition of ammonium, potassium and sodium ions to the growth medium considerably enhanced lactase production. A maximum enzyme yield corresponding to hydrolysis of 3 nmoles o-nitrophenyl-β-D-galactopyranoside sec^{-1} ml^{-1} of growth medium, at pH 5 and 60 °C, was obtained.

In the wheat bran culture, the maximum enzyme yield was obtained after 140 h of growth at 28–30 °C. A marked increase in the enzyme production was observed when nitrate or phosphate was added to the growth medium. Also, the addition of certain agricultural by-products (molasses, whey) enhanced lactase production. The observed maximum yield corresponding to the hydrolysis of 182 nmoles of ONPG sec^{-1} g^{-1} of wheat bran, at pH 5 and 60 °C, is comparable to that reported for certain microorganisms used commercially for lactase production.

Introduction

β-D-galactosidase catalyzes the splitting of the β-gallactosidic bond of lactose to glucose and galactose. Lactose or milk sugar is important to milk and milk products for the flavor, texture and nutritional value it imparts to these products. Lactose presents a number of problems; it is poorely tolerated by many people (Rosensweig 1969), a pollutant in the form of untreated whey and easily crystalizes out in frozen and condensed milk products (Holsinger 1978). Several investigators have, therefore, tried to find suitable sources of lactase as large scale production of low-lactase milk and milk products has been delayed by the lack of inexpensive sources of this enzyme (Wierzbicki and Kosikowski 1973; Blankenship and Wells 1974; Sorrensen and Crisan 1974; Rao-Ramana and Dutta 1977; Park et al. 1979; Pastore and Park 1979; Mustranta et al. 1980).

The main sources of commercial lactase are miroorganisms. Among them *Saccharomyces lactis* and *Aspergillus niger* are the most common sources of lactases used in the food industry (Holsinger 1978). These enzymes, however, suffer from the disadvantage of being intracellular. Extracellular lactase from *Aspergillus foetidus* and *Aspergillus oryzae* have already found commercial application in food processing (Park et al. 1979).

The present work deals with the study of certain conditions affecting extracellular lactase production by *F. moniliforme* grown in whey and on wheat bran. This fungus has previously been reported for the good quantity and nutritional quality of its protein (Drouliscos et al. 1976; Macris and Kokke 1977). Also, other *Fusarium* species (*F. graminearum*) have recently received approval for human consumption (Newmark 1980).

Materials and Methods

Organism. A laboratory strain of *F. moniliforme*, previously employed for microbial protein production, was used (Macris and Kokke 1977). The stock culture was maintained on potato-dextrose-agar.

Growth. The liquid growth medium consisted of a solution of commercial spray-dried cow's whey supplemented with 0.5% (w/v) $(NH_4)_2SO_4$. Prior to heat sterilization (121 °C for 30 min), the medium was adjusted to pH 4.5 with 1 N HCl. An inoculum of 10^6 conidia was transferred from the stock culture to 250 ml

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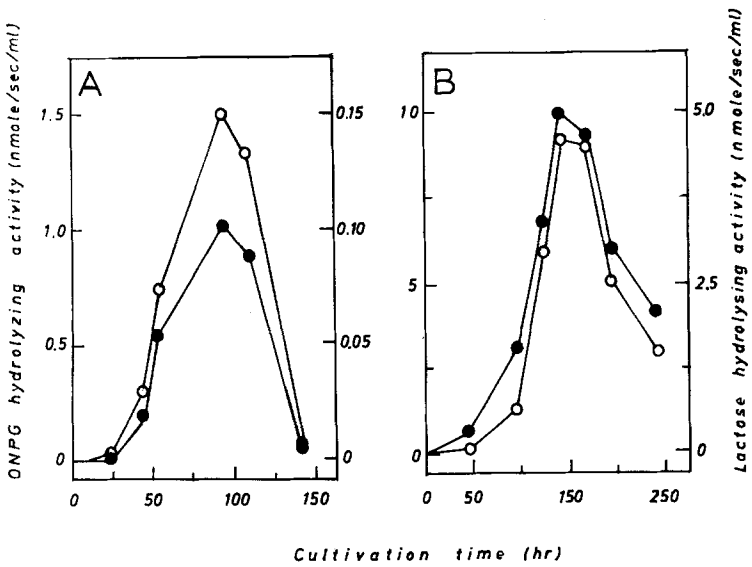

Fig. 1 A and B. Effect of cultivation time on lactase production. The quantities of concentrated enzyme powder used for hydrolysis of lactose were equivalent to the volume on non-concentrated whey lactose filtrate or wheat bran extract used for ONPG hydrolysis: **A** Liquid culture containing 6% whey lactose; **B** Wheat bran culture supplemented with 0.25 M $(NH_4)_2SO_4$. •, Non-concentrated enzyme and ONPG as substrate; ○, concentrated enzyme and lactose as substrate

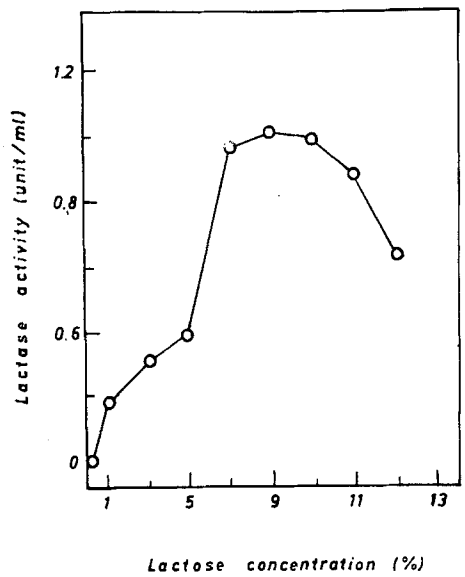

Fig. 2. Effect of whey lactose concentration on lactase production

Erlenmeyer flasks containing 100 ml of sterile growth medium. The flasks were incubated at 30 °C in a rotary shaker operating at 180 rpm. At the end of the incubation period the fungal biomass was harvested by centrifugation at 5,000 g for 30 min. The supernatant was further clarified by passing it through a membrane filter (pore size 1.2 μm) and used as a source of the enzyme.

The solid growth medium consisted of 5 g of wheat bran and 10 ml tap water in a 100 ml Erlenmeyer flask. The flasks were sterilized, inoculated and incubated under the same conditions used for the whey liquid culture. Following incubation, 50 ml of distilled water was added to each flask and the enzyme was extracted by agitation (150 rpm) at room temperature for 1 h. The extract was centrifuged at 5,000 g for 10 min and the supernatant served as an enzyme source.

Preparation of Enzyme Concentrate. The enzyme was precipitated from the whey culture filtrate or the wheat bran extract at 4 °C by adding ethanol to a final concentration of 70% by volume. The precipitate was freeze dried.

Assay of Enzyme. A quick assay method using o-nitrophenyl-β-D-galactopyranoside (ONPG) as substrate was employed. One ml of an appropriate enzyme dilution was incubated for 10 min at 60 °C with 4 ml of 0.1 M acetate buffer, pH 5, containing 3 mM ONPG. Denatured enzyme (heated at 100 °C for 10 min) was used in the blank. The reaction was stopped by adding 1 ml 30% $NaCO_3$ solution (w/v) and the liberated o-nitrophenol was measured spectrophotometrically at 420 nm. One unit of lactase activity was defined as the amount of enzyme required to liberate 1 n mole of o-nitrophenol per sec under the conditions described above.

Lactase activity was also determined from the amount of glucose liberated during lactose hydrolysis. One ml of an appropriate enzyme dilution was incubated for 10 min at 60 °C with 4 ml of 0.1 M acetate buffer, pH 5, containing 30 mM lactose. The liberated glucose was estimated with Glucinet reagent (I.S.V.T. Sclavo, Divisione Diagnostici, Siena, Italy). Lactose was determined colorimetrically (Nickerson et al. 1976).

Results and Discussion

The effect of cultivation time on enzyme activity is shown in Fig. 1. Optimum activities were obtained by growing the fungus for 85–100 and 140–160 h in whey and wheat bran media respectively. The optimum cultivation times reported for certain fungi, producing extracellular lactase in a commercial scale, were 65 and 120 h for *A. oryzae* grown on wheat bran and in a starch liquid medium respectively (Park et al. 1971). Also, *Scopulariopsis sp.* had an optimum cultivation time of 110 h when grown on wheat bran (Pastore and Park 1979).

The production of lactase by *F. moniliforme* was studied over a wide range of whey lactose concentration. The best results were obtained with lactose concentrations of 7–11% (Fig. 2). Lower or higher concentrations resulted in lower enzyme activities. Similar results were reported for a cell-

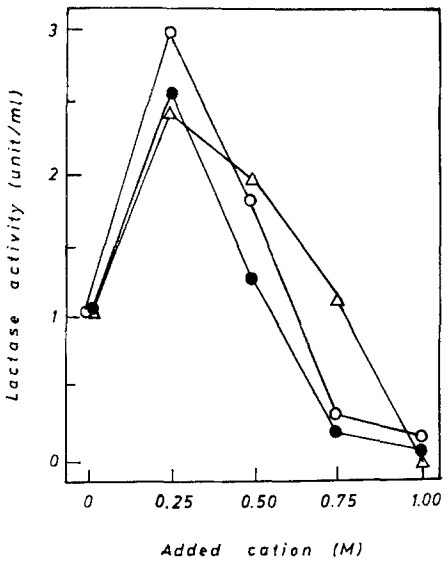

Fig. 3. Effect of cation supplementation of whey liquid medium on lactase production. ○, KCl; ●, NaCl; △, NH_4Cl

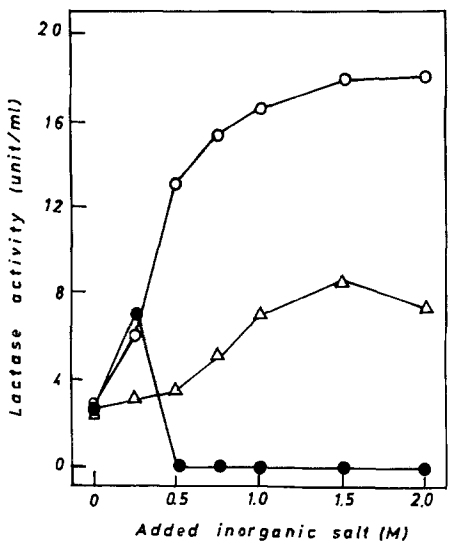

Fig. 4. Effect of certain inorganic salts, added to wheat bran medium, on lactase production. ●, $(NH_4)_2SO_4$; △, $NaNO_3$; ○, NaH_2PO_4

Table 1. Comparison of extracellular enzyme produced by *F. moniliforme* and certain other fungi used commercially for lactase production

Microorganism	Growth medium	Optimum		Enzyme yield[a]	References
		pH	Temp (°C)		
A. oryzae	Wheat bran[b]	4–5	55–60	150.0	Park et al. (1979)
A. oryzae	Starch[c]	4–5	55–60	8.5	Park et al. (1979)
Scopurariopsis sp.	Wheat bran[b]	4–5	50–65	233.3	Pastore and Park (1979)
F. moniliforme	Wheat bran[b]	4–5	50–60	182.0	
F. moniliforme	Whey[c]	4–5	50–60	3.0	

[a] nmoles of hydrolyzed ONPG s^{-1} ml^{-1} (liquid medium) or g of wheat bran (solid medium) at pH 5 and 60 °C
[b] Solid growth medium
[c] Liquid growth medium

bound lactase of *Saccharomyces fragilis* grown in whey (Wendorff er al. 1970).

The concentration of monovalent cations in the whey growth medium affected lactase production. The results appear in Fig. 3 and show that the enzyme activity increased 2–3 fold when the added NH_4^+, K^+ and Na^+ reached a concentration of 0.25 M. Higher concentrations of the tested cations had a marked inhibitory effect on enzyme activity. Stimulatory effects of the same cations on cell-bound lactase were reported previously (Davies 1964; Rao-Ramana and Dutta 1977).

Inorganic nitrogen and phosphorous added to the wheat bran medium markedly affected lactase production (Fig. 4). Nitrogen concentrations up to 0.25 M, in the form of an ammonium salt, increased the enzyme yield about 2 fold, which declined thereafter. Elevated concentrations of nitrogen in the form of a nitrate salt were less inhibitory and increased the enzyme yield about 3 times. The best results were obtained when sodium dihydrogen phosphate was used and the observed enzyme yields were comparable to those reported for certain molds producing extracellular lactase on a commercial scale (Table 1). Enhanced enzyme yields were also obtained when molasses and whey were added to the wheat bran medium. The results are shown in Fig. 5 and show that whey and molasses increased enzyme production about 2.5 and 4 times respectively.

The effect of pH and temperature on the enzyme activity were investigated since both properties are important for the industrial application of this enzyme. The results

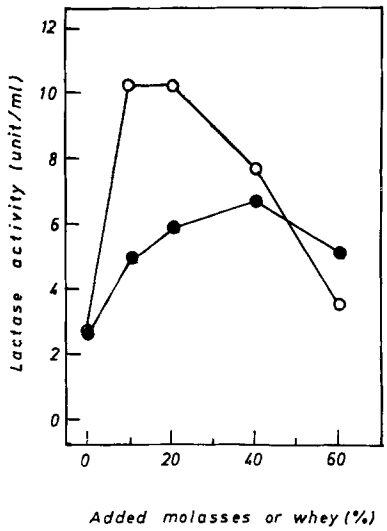

Fig. 5. Effect of supplementation of wheat bran medium with certain agricultural by-products (% of wheat bran) on lactase production. ○, molasses; ●, whey powder containing 80% lactose

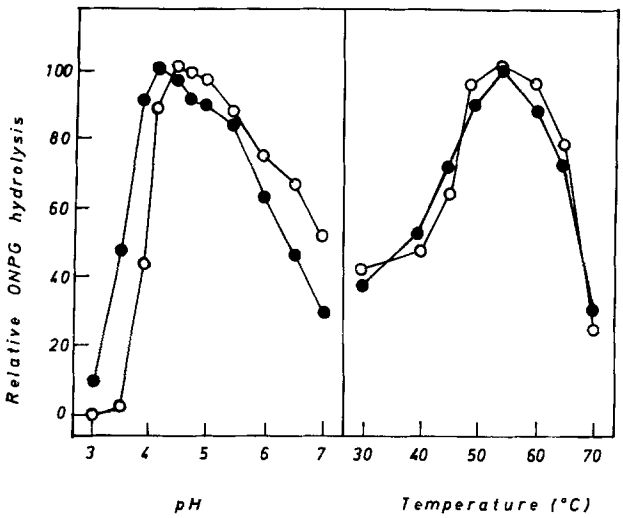

Fig. 6. Effect of pH and temperature on enzyme activity. ●, Concentrated lactase produced from the whey liquid medium; ○, concentrated lactase produced from the wheat bran solid medium

are shown in Fig. 6 and show that the enzyme functions optimally at pH 4–5 and 50–60 °C. The acidic pH optimum makes the enzyme particularly suitable for processing acid whey whereas the high temperature optimum is very advantageous because it depresses microbial growth (Sorrensen and Crisan 1974).

In conclusion, *F. moniliforme* is worth considering for the production of microbial protein, and is potentially useful for producing lactase from agricultural by-products.

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References

Blankenship LC, Wells PA (1974) Microbial beta-galactosidase: a survey for neutral pH optimum enzymes. J Milk Food Technol 37:199–202

Davies R (1964) Lactose utilization and hydrolysis in *Saccharomyces fragilis.* J Gener Microbiol 37:81–89

Drouliscos NJ, Macris BJ, Kokke R (1976) Growth of *Fusarium moniliforme* on carob aqueous extract and nutritional evaluation of its biomass. Appl Environ Microbiol 31(5):691–694

Holsinger VH (1978) Application of lactose-modified milk and whey. Food Technol 32(3):35–40

Macris BJ, Kokke R (1977) Kinetics of growth and chemical composition of *Fusarium moniliforme* cultivated on carob aqueous extract for microbial protein production. Eur J Appl Microbiol Biotechnol 4:93–99

Mustranta A, Karvonen E, Linko M (1980). Production of mold lactases. VI Intern Ferm Symp Abstracts, London Ontario Canada

Newmark P (1980) Fungal food Nature 287:6

Nickerson TA, Vujicic JF, Lin AY (1976) Colorimetric estimation of lactose and its hydrolytic products. J Dairy Sci 59:386–390

Park YK, De Santi MSS, Pastore GM (1979) Production and characterization of β-galactosidase from *Aspergillus oryzae.* J Food Sci 44:100–103

Pastore GM, Park YK (1979) Screening of high β-galactosidase-producing fungi and characterizing the hydrolysis properties of a selected strain. J Food Sci 44:1577–1580

Rao-Ramana MV, Dutta SM (1977) Production of beta-galactosidase from *Streptococcus thermophilus* grown in whey. Appl Environ Microbiol 34(2):185–188

Rosensweig N (1969) Adult human milk intolerance and intestinal lactase deficiency. A review J Dairy Sci 52:585–587

Sorrensen SG, Crisan EV (1974) Thermostable lactase from thermophilic fungi. J Food Sci 39:1184–1187

Wendorff WL, Amudson CH, Olson NF (1970) Nutrient requirements and growth conditions for production of lactase enzyme by *Saccharomyces fragilis.* J Milk Food Technol 33:451–455

Wierzbicki LE, Kosikowski FV (1973) Lactase potential of various microorganisms grown in whey. J Dairy Sci 56:26–32

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