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ISOLATION AND IDENTIFICATION OF GALACTOSIDASES PRODUCING MOULD CULTURES FROM SOIL

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More than thirty mould cultures capable of hydrolysing α-and β-galaccoside were isolated from local soil samples. *Aspergillii* and *Penicillia* were found to be dominant among the isolates. Members of the same species also exhibited variable enzyme producing capability.

INTRODUCTION

Raffinose in one of the impurities in sugar beet juice which can not be removed by ion exchange demineralisation or by saccharate process. Similarly lactose is undesirable in milk which is fed to lactose intolerants. Presence of raffinose in beet sugar syrups hinders normal crystalization of sucrose. Sugar crystalization becomes uneconomical when content of raffinose are in range of 6% to 10% and the juice is discarded.

Hydrolysis of raffinose into sucrose and galactose by α-galactosidases of microbial origin has been reported by several workers [1-5]. Microbial β-galactosidases have been successfully applied for hydrolysis of lactose present in cheese, whey and milk as a means of improving the nutritional quality of milk and for preparation of low lactose milk for lactose intolerants.

Microorganisms capable of producing α and β-galactosidases which hydrolyse raffinose and lactose were isolated from local soil samples and promising strains were identified.

MATERIALS AND METHODS

Isolation of micro-organisms: Raffinose or lactose hydrolysing strains were isolated by enrichment culture technique [9]. Raffinose or lactose was the sole source of carbon in the medium for the respective strains.

Samples of soil were suspended in 0.8% NaCl solution (1 part of soil to 9 parts of saline) One ml of the soil suspension was added to 9 ml of the modified Czapek broth [10] in which raffinose or lactose replaced sucrose for α or β-galactosidase producing microorganims respectively. The growth of the organisms became visible within 48 hr. of incubation and thereafter six transfers were made in the same medium, at daily intervals, and finally the cultures were plated out on their respective media (modified Czapek Dox broth + 2% agar). The colonies which grew vigorously were isolated 72 hr. after incubation. The incubation temperature was maintained at 32 ± 2°C throughout the present study.

Screening of strain: Screening of cultures was done on the basis of enzyme producing capacity by submerged fermentation method in shake flasks. The fermentation medium was composed of g/L raffinose or lactose, 30.0; $NaNO_3$, 3.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5 and $FeSO_4$, 0.1. Distilled water was used for medium preparation.

1. Spores from 96 hr. old slant cultures were suspended in 0.005% monoxal O. T. (Diacetyl ester of sodium sulfo succinic acid). Spores were counted by serial dilution method using heamacytometer.

2. 4×10^7 spores were inoculated into 50 ml fermentation medium contained in 250 ml conical flask. The shake flask culture were incubated at 150 r.p.m. for 96 hr.

3. The mycelial pellets were recovered by filteration from fermented broth and crushed with sand. The homogeneous mass was diluted with distilled water, centrifuged at 2500 r.p.m. for 5 min. and supernatent was used for assay of enzymic activities.

Assay of Galactosidases: 1. *α-Galactosidase.* α-Galactondase was assayed by the modified method of Dey and Pridham [11]. To 8 ml of 0.05 M citerate buffer pH 4.4 added to 1 ml of appropriately diluted enzyme solution and incubated at 50 ± 1°C with 1 ml of 2.5 mM ρ-nitrophenyl α-D-galactopyranoside (PN PG) solution for 15 min. The reaction was stopped by adding 1 ml of 0.1 M sodium

carbonate solution. The yellow colour of *p*-nitrophenol thus developed was measured by determining the absorbance at 400 nm. A blank using inactivated enzyme, treated at 100°C for 3 min was conducted in parallel.

2. *β-Galactosidase:* β-Galactosidase was assayed by modified Lederberg method [12]. To one ml of enzyme extract was added 8 ml of 0.05 M citrate buffer pH 4.0 and 1 ml of M/200 *o*-nitrophenyl β-D-galactopyranoside (ONPG). After 20 min. of incubation at 37 ± 1°C, 1 ml of 1 M sodium carbonate was added to stop the reaction. Colour intensity was read at 420 nm on spectrophotometer. A blank test with heat inactivated enzyme was conducted in parallel.

3. *Standard curves:* Standard curves of *p*-nitrophenol and *o*-nitrophenol were prepared to estimate α-or β-galactosidic activity using PNPG and ONPG as substrates. Fig. 1 and 2 show the curves.

4. *Unit of α-β-Galactosidase:* One unit of enzyme is that which releases one μ mole of *p*-/*o*-nitrophenol under assay conditions.

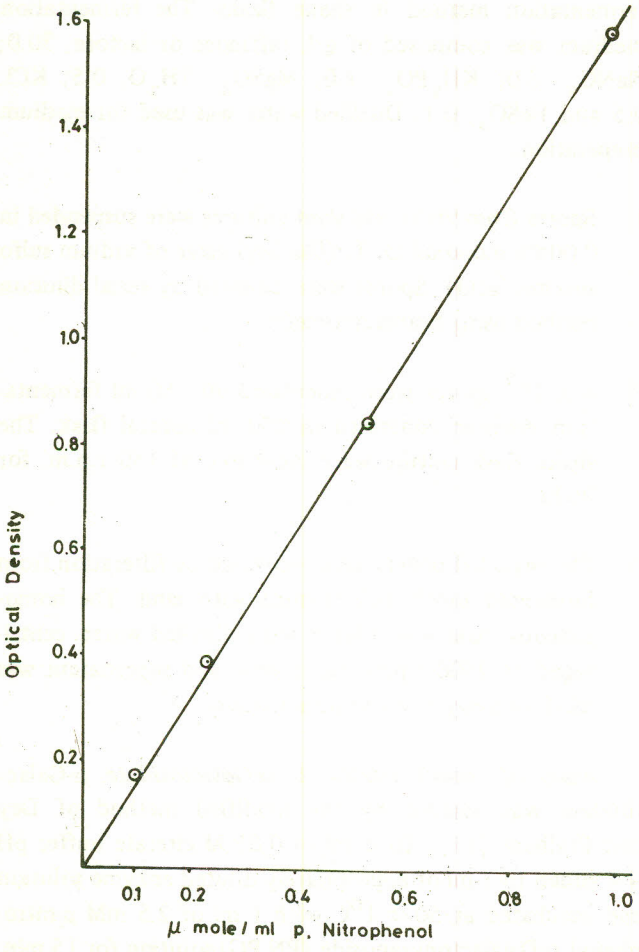

Fig. 1. Standard curve for estimation of *p*. Nitrophenol at 400 nm.

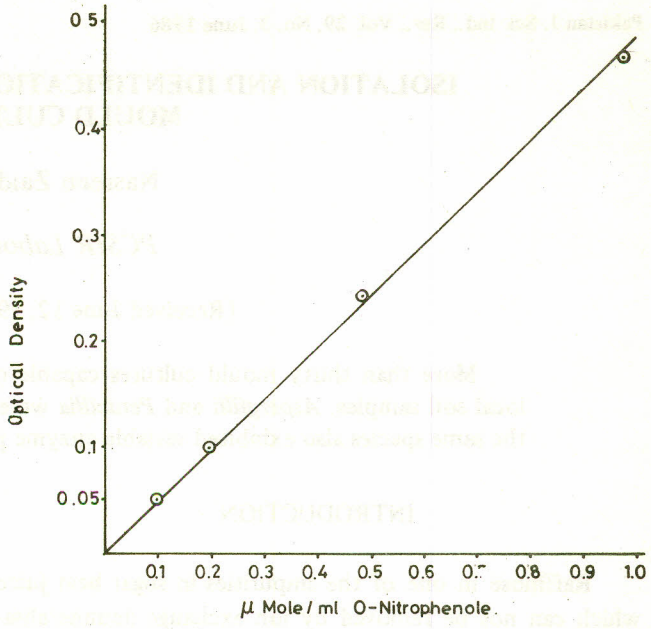

Fig. 2. Standard curve for *o*-Nitrophenol estimation at 420 nm.

Identification of selected culture.

1. Strains were subcultured on Czapek solution agar medium in petri plates for identification based on cultural and microscopic characteristics.

2. Slide culture was done after Raper and Fennell [13]. Slide cultures were prepared by placing a drop of melted agar on flame – sterilized microscope slide. The agar was then inoculated, covered with a sterile coverslip, and incubated in a moist atmosphere at 25°C for 96 hr. Culture were examined under microscope.

RESULTS

Galactosidase producing microorganisms were isolated from soil samples collected from gardens and nurseries which are considered to be rich source of microflora. The isolates included mould, yeast and bacterial cultures. Among those mould were selected for further studies. Collection was confined to thirty-three cultures dominated by *Aspergillii* and *Penicilia.* Critarion of primary selection was luxarient growth of microorganisms capable of producing α- and β-galactosidase.

Seventeen raffinose utilizing and sixteen lactose utilizing cultures were propagated on raffinose and lactose containing media respectively. Enzyme producing capacity of each culture was compared on the basis of enzyme units/4 x 10^7 spores, as shown in Table 1 and 2.

Table 1. α-Galactosidic activity of mould cultures capable of hydrolysing raffinose.

Culture No.	Enzymic Activity U/4 x 10^7 spores
MRU – 18	2.9
MRU – 19	20.0
MRU – 20	1.4
MRU – 21	8.1
MRU – 22	Nil
MRU – 23	Nil
MRU – 24	1.6
MRU – 25	20.8
MRU – 26	58.0
MRU – 27	Nil
MRU – 28	Nil
MRU – 29	48.0
MRU – 30	Nil
MRU – 31	48.8
MRU – 32	48.0
MRU – 33	48.0

MRU = Mould raffinose utilizer

Table 2. β-Galactosidic activity of lactose hydrolysing mould cultures.

Culture No.	Enzymic activity U/4 x 10^7 spores
MLU – 1	Negligible
MLU – 2	4.2
MLU – 3	6.1
MLU – 4	2.6
MLU – 5	2.6
MLU – 6	4.0
MLU – 7	2.6
MLU – 8	5.0
MLU – 9	Negligible
MLU – 10	"
MLU – 11	"
MLU – 12	"
MLU – 13	2.0
MLU – 14	0.8
MLU – 15	4.0
MLU – 16	4.0
MLU – 17	3.5

MLU = Mould lactose utilizer

Table 3. Identification of selected cultures.

Culture No.	Nomenclature
MLU – 2	*Aspergillus terreus*
MLU – 3	*Aspergillus terreus*
MLU – 8	*Aspergillus niger*
MLU – 15	*Aspergillus oryzae*
MRU – 25	*Aspergillus flavus*
MRU – 26	*Aspergillus terreus*
MRU – 31	*Pencillium frequentans*
MRU – 33	*Aspergillus terreus*

Four strains from each group which produced maximum amount of enzyme were studied in detail. Identification of the selected cultures was based on morphological and microscopic examination. Culture No. MRU-25, 26, 31 and 33 yielded 20.8, 58.0, 48.8 and 48.0 u of α-galactosidase/4 x 10^7 spores (inoculam) respectively. While culture ML U. 2, 3, 8 and 15 yielded 4.2, 6.1, 5.0 and 4.0 U of β-galactosidase/4 x 10^7 spores (inoculum) respectively.

Culture No. MLU – 2, – 3, MRU – 25, – 26 were identified as *Aspergillus terreus;* MLU-8 was *Aspergillus niger;* MLU – 15 was *Aspergillus oryzae;* MRU 25 was *Asperigillus flavus* and MRU – 31 was *Penicillium frequentans,* as shown in Table 3.

DISCUSSION

Cultural and morphological characteristics of selected cultures *A. terreus, A. niger, A. oryzae, A. flavus* and *P. frequantans* were compared with those reported in "The genus Aspergillus" [13] and "A manual of Penicillia [14] Taxanomic identity of the selected culture and the reported one almost matched each other. Varieties of one species *Aspergillus terreus* yielded maximum α-/β-galactosidase. Enrichment culture technique favoured adaptive enzyme formation hence either of the two strain of the same species adapted α-/β-galactosidase synthesis. Although each strain was capable of producing both galactosidases at a time but spontaneous induction due to raffinose/lactose (inducers) resulted in high yielding α-/β-galactosidase synthesizing varieties.

Maximum α-galactosidic activity 58 U were assayed when 4 x 10^7 spores of *Aspergillus terreus* (culture No. MRU – 26) were propagated. The other strain which yielded maximum β-galactosidase (6.1 U/4 x 10 spores) was culture No. MLU-3. The strains on examination exhibited

taxanomical identity with that of *Aspargillus terreus.*

It was evident from the above results that either of the two cultures were varieties of the same species i.e.

1. *Aspergillus terreus Var. raffinose utilizer* MRU-26
2. *Aspergillus terreus Var. lactose utilizer* MLU-3.

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