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SOLID SUBSTRATE FERMENTATION FOR PECTINASES BY *ASPERGILLUS FOETIDUS*

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The biosynthesis of pectinases by *Aspergillus foetidus* was studied in litre conical flasks by the surface culture method using wheat bran as solid substrate. The parameters studied were the depth of the wheat bran, selection of diluents and methods for the extraction of the enzyme. Wheat bran was found to be an ideal substrate providing all nutrients essential for mould growth and enzyme formation. 0.1N HCl as diluent gave optimum results of both pectinesterase and polygalacturonase. The enzyme production reached maximum 48 hr. after incubating at 30°.

INTRODUCTION

In continuation of our work reported earlier on the biosynthesis of microbial enzymes, such as carboxymethyl cellulase [1], amyloglucosidase [2], bacterial proteases [3] and alpha-amylase [4] by *Trichoderma viride, Aspergillus-phoenicis* and *Bacillus subtilis* respectively, the present investigation describes the production of enzyme pectinases by *Aspergillus foetidus.* The enzyme pectinases [5] finds commercial applications in the extraction of juices from fruits like apple, banana, guava, papaya, mango; in the manufacture of the hydrolyzed products of pectin; in the retting of textile fibres; in the manufacture of pectin-free starch; in the refinement of vegetable fibres; in the curing of coffee, cocoa and tobacco; and also as an analytical tool in the estimation of plant products. The percentage yield of fruit juices is greatly improved by enzymic treatment. The use of pectolytic enyzmes in industry is less when compared to other hydrolytic enzymes, such as amylases and proteases. Extensive work, however, has been carried out on the biosynthesis of pectolytic enzymes by both surface (Koji) and submerged culture methods [10]. The present work describes the results of laboratory-scale studies on the biosynthesis of pectin-esterases and polygalacturonases in wheat bran used as solid substrate.

MATERIALS AND METHODS

Organism: The strain of *Aspergillus foetidus* PCSIR-47 derived from NRRL 341 was used in the investigations. The mould culture was maintained on glucose-yeast-extract agar medium of the composition g/l): glucose, 10.0; ammonium sulphate, 2.5; KH_2PO_4, 2.5: yeast extract, 2.5; pectin, 1.0; and agar, 20.0, pH 3.8. All media unless otherwise stated were sterilized at 121° for 15 min. The cultures were incubated at 30° for 5-6 days for maximum sporulation and then kept in a refrigerator.

PROCEDURE

Wheat bran was moistened with 20 ml. N/10 HCl and auto-claved at 121° for 15-20 min. Spore inoculum prepared by suspending spores in 10 ml. sterile 0.05% Monoxal O.T Solution (diacetely-ester of sodium sulphosuccinic acid) was used to inoculate wheat-bran (20 g) medium in 1-litre conical flasks. After cooling the flasks were inoculated with 3 ml of spore suspension and incubated at 30 ± 2° for 42 hr. The flasks were daily shaken during mould growth.

Preparation of enzyme extract. The pectolytic enzymes at the end of the fermentation were extracted adding 200 ml phosphate buffer pH 6.1 (0.2 M Na_2HPO_4 0.2M NaH_2-PO_4) and the flasks were shaken on a rotary shaker for 1 hr. Wheat bran was removed by filtration and the clear filtrate was used for enzyme assay.

Enzyme Assay. (a) pectin methyl-esterase was determined by the method of Macdonnel *et al.* [6] and defined as milliequivalent of ester hydrolyzed per min per g/ml enzyme.

$$\text{P.E. Units/ml} = \frac{\text{ml. of NaOH used} \times 3.1 \times 1 \text{ min}}{\text{ml. of enzyme extract} \times \text{total time in min.}}$$

(b) The polygalacturonase activity was estimated by the method of Kertesz [7] and is defined as millimole of the reducing group liberated per min per ml. of enzyme. A standard curve was prepared with galacturonic acid monohydrate solution.

RESULTS AND DISCUSSION

The optimum time of incubation for the synthesis of pectin esterase (P.E.) and polygalacturonase (P.G.) was studied in wheat bran culture (Fig. 1). The production of both enzymes by mould reached maximum 48 hr. after spore inoculation and further incubation of the wheat bran culture resulted in a decrease of enzymic activity. The actual mechanism for the decrease in enzyme synthesis may be due to the exhaustion of nutrients in the substrate, thus inactivating enzymes with the passage of time. In subsequent experiments, therefore, all wheat bran cultures were incubated for 48 hr.

Selection of the diluent. The moisture level in wheat bran cultures is of great significance for the maintenance of porosity for an adequate aeration of the substrate during microbial growth. Wheat bran mashes were prepared with 1 part of bran to 1.5 parts of diluents such as distilled water, 0.01N or 0.1N HCl (Table 1). The production of both enzymes was maximum when 0.1N HCl was used as diluent, i.e. P.E. = 0.76 and P.G. 23.2 unit/g wheat bran, and it decreased in the order of 0.01N HCl and tap water. It follows that a low pH due to the addition of HCl (0.1N) may be responsible for maximum enzyme formation. This is in accordance with the findings of Oxford [8] who reported that optimum pH for the synthesis of pectinases was about 3.5 – 4.0.

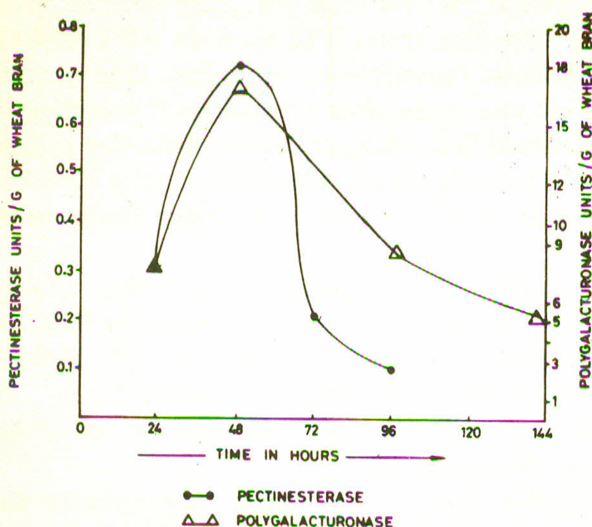

Fig. 1. Biosynthesis of enzymes pectinesterase and polygalacturonase by *Aspergillus phoetidus*.

Extraction of enzymes. The extraction of the enzyme pectinases from the fermented wheat bran was studied using a phosphate buffer, saline solution or water (Table 2). The ratio of wheat bran to the solution for the extraction of enzyme was kept at 1:10 in these experiments. The extraction of enzyme was maximum in the presence of the phosphate buffer; that is, the amounts of pectinestrase and polygalacturonase were 0.23 and 31.4 units/g wheat bran respectively. It decreased in the order of saline solution and distilled water. In subsequent experiments, therefore, a phosphate buffer was employed for enzyme extraction

Table 1. Selection of Diluents

Substrate	Diluent	P.E. units/g wheat bran	P.G. units/g of wheat bran
Wheat bran	0.1 N HCl	0.763	23.2
Wheat bran	0.01 N HCl	0.501	21.6
Wheat bran	Distilled water	0.123	15.7

* Samples were analyzed after 48 hr. incubation at 30 ± 2°.
** Every reading is a mean of triplicate readings.
*** Fermented wheat bran was extracted with a phosphate buffer.

Table 2. Extraction of Enzyme from the Fermented Wheat Bran

Substrate	Extracting Material	P.E. units/g wheat bran	P.G. units/g of wheat bran
Wheat bran	Phosphate pH 6.1 buffer	0.231	31.4
Wheat bran	Saline solution (0.9%)	0.089	29.6
Wheat bran	Distilled water	0.054	24.3

* The samples were analyzed after 48 hr. incubation.
** 20 g. wheat bran was used in each flask.

from fermented wheat bran. Sreakantiah [9] used a citrate buffer for the extraction of enzyme.

Effect of depth of wheat bran on enzyme synthesis. The depth of wheat bran is very critical in solid substrate fermentation. Thus the effect of varying concentrations of wheat bran (10-60 g/flask) which resulted in different depths of the substrate (0.4-2.5 cm) in litre conical flasks was studied on enzyme formation (table 3). The ratio of the diluent to wheat bran was kept at 1:1.5. The production of both enzymes was maximum in flasks containing 10 g wheat bran (0.4 cm depth) but this is not economically feasible for large scale production due to large areas. Qadeer and Anjum [2] have already reported that the depth of 2.5 cm in trays was adequate for the synthesis of enzyme amyloglucosidase by *Aspergillus phoenicis.*

Effect of different nitrogen sources. The production of microbial enzymes is greatly influenced by both the concentration and source of nitrogen. Enzyme synthesis by *A. foetidus* was investigated by adding nitrogen from different sources (Table 4) The level of nitrogen in all cultures was kept at 0.1 g per 20 g medium. Urea was found to be an ideal source for maximum enzyme formation. The amount of pectin esterase (P.E.) and polygalacturonase

Table 3. Effect of thickness of wheat bran Layer on enzyme synthesis

Wheat bran in gram/ flask	Depth in cm	Pectinesterase (P.E.) units/g of wheat bran	Polygalacturonase (P.G.) units/g of wheat bran
10	0.4	0.934	34.3
20	0.8	0.726	33.6
30	1.6	0.553	31.2
40	1.8	0.173	28.7
60	2.5	0.083	23.5

(P.G.) was 0.36 and 46.4 units/g wheat bran and it decreased in the order of $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$ or $NaNO_3$.

Table 4. Effect of different nitrogen sources on the biosynthesis of Pectinases by Aspergillus foetidus

Nitrogen sources	P.E. Units/g of wheat bran	P.G. Units/g of wheat bran
Sodium nitrate	0.164	32.0
Ammonium sulphate	0.213	34.3
Di-ammonium hydrogen phosphate	0.224	42.1
Urea	0.360	46.4
0.1 N HCl (control)	0.205	31.3

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