Amylase Production in Solid State Fermentation by the Thermophilic Fungus Thermomyces lanuginosus

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The production of extracellular amylase by the thermophilic fungus Thermomyces lanuginosus was studied in solid state fermentation (SSF). Solid substrates such as wheat bran, molasses bran, rice bran, maize meal, millet cereal, wheat flakes, barley bran, crushed maize, corn cobs and crushed wheat were studied for enzyme production. Growth on wheat bran gave the highest amylase activity. The maximum enzyme activity obtained was 534 U/g of wheat bran under optimum conditions of an incubation period of 120 h, an incubation temperature of 50°C, an initial moisture content of 90%, a pH of 6.0, an inoculum level of 10% (v/w), a salt solution concentration of 1.5:10 (v/w) and a ratio of substrate weight to flask volume of 1:100 with soluble starch (1% w/w) and peptone (1% w/w) as supplements.

[Key words: Thermomyces lanuginosus, amylase, solid state fermentation, wheat bran, optimization]

Amylases (a term that refers here to α-amyrase, β-amyrase and glucoamylase [GA]) are among the most important enzymes in present-day biotechnology. Although amylases can be derived from several sources, including plants, animals and microorganisms, microbial enzymes generally meet industrial demands. Currently, a large number of microbial amylases are available commercially and they have almost completely replaced the chemical hydrolysis of starch in the starch processing industry (1). Amylases find potential application in a number of industrial processes such as in the food, fermentation, textile and paper industries.

Traditionally, amylase has been produced by submerged fermentation (SmF) and used in a one-way process in solution. In recent years, however, solid state fermentation (SSF) processes have been increasingly utilized for the production of this enzyme.

SSF holds tremendous potential for the production of enzymes (2). It can be of particular relevance in those processes where a crude fermented product may be used as an enzyme source (3). The selection of a particular strain, however, remains a tedious task, particularly when commercially significant enzyme yields are required. Agro-industrial residues are generally considered the best substrates for the SSF processes and enzyme production in SSF. The use of SSF for the production of enzymes and other products has many advantages over submerged fermentation (4) and these have been widely discussed in the literature (1, 4, 5).

SSF has gained renewed interest from researchers for the production of these enzymes in view of its economic and engineering advantages and is often employed to produce amylases (2, 5). Selvakumar et al. (6) reviewed the microbial synthesis of starch-saccharifying enzymes in solid cultures. Since thermostability is a feature of most of the enzymes sold in bulk for industrial application, thermophilic microorganisms are of particular interest for the production of thermostable amylases. Recent research on thermostable amylases has concentrated on the enzymes of thermophiles and extreme thermophiles.

The production of amylolytic enzymes from the thermophilic fungus Thermomyces lanuginosus has only been reported from submerged cultivations (7–9). T. lanuginosus grows on different carbon sources and produces a wide range of industrially important amylase enzymes (8). Reports on amylase production by SSF using wheat bran and thermophilic fungi are scanty. The purpose of the present study was to investigate the production of amylase under SSF conditions. In this paper, we report a number of factors that influence amylase production by Thermomyces lanuginosus through SSF.

MATERIALS AND METHODS

Microorganism and growth Thermomyces lanuginosus ATCC 58160 was grown at 50°C in submerged cultivation on a medium containing the following (g/l distilled water): soluble starch, 15.0; yeast extract, 5.0; K2HPO4, 5.0; MgSO4, 5.0, with a pH of 6.0. All chemicals used were of analytical grade.

The experiments were conducted in 250-ml Erlenmeyer flasks containing 10 g of substrate and 1 ml of salt solution containing the following (g/l distilled water): K2HPO4, 5; MgSO4, 7H2O, 5. Distilled water was added to a achieve final substrate moisture content of 80%. After sterilization, the flasks were cooled and inoculated with a 10% (v/v) inoculum and incubated at 50°C for 96 h. After fermentation, the contents of the flasks were harvested and assayed.

Commercial quality wheat bran, molasses bran, rice bran, maize...
meal, millet cereal, wheat flakes, barley bran, crushed maize, corn- 
cobs and crushed wheat were procured from the local market and 
used as solid substrates and their effect on the production of amy-
lase was determined. The best solid substrate was selected and 
used in subsequent experiments.

Various process parameters were optimized by conventional 
methods for maximal enzyme production as follows: incubation 
period (24, 48, 72, 96, 120, 144 and 168 h), incubation temperature 
(40°C, 50°C, 60°C, 70°C and 80°C), initial total moisture content 
(50%, 60%, 70%, 80%, 90%, 100% and 110%), initial pH (4.0– 
8.0) and inoculum level (5%, 10%, 15%, 20%, 25% and 30% 
[w/v]). Wheat bran, the best solid substrate, was supplemented 
with different carbon sources (soluble starch, sucrose, lactose, mal- 
tose, dextrose, fructose and glucose) and nitrogen sources (pep-
tone, tryptone, meat extract, ammonium sulphate, yeast extract, 
soybean meal, urea, ammonium sulphate and sodium nitrate) at 1% 
(w/w) and the ratios of salt solution concentration to substrate 
weight (v/w) (1.0:10, 1.5:10, 2.0:10 and 2.5:10) and substrate 
weight to flask volume (w/v) (1:50, 1:100, 1:150, 1:200, 1:250 
and 1:300) were varied.

Production of amylase under optimum conditions On the 
basis of the results obtained with all the optimum parameters, 
namely viz, wheat bran containing soluble starch and peptone as 
supplements (1% w/w), pH of 6.0, moisture content of 90%, salt 
solution concentration of 1.5:10 (v/w), inoculum level of 10%, incu-
bation temperature of 50°C and incubation period of 120 h. The 
extent of improvement in the optimized medium was evaluated us-
ing basal medium (10) as a control. The fermentation was carried 
out as described above.

Analytical methods At the end of fermentation, the solid 
biomass was treated with 50 ml of distilled water and agitated 
thoroughly on a magnetic stirrer for 30 min. The entire contents 
were filtered through muslin cloth and the residue was again 
treated with another 50 ml of distilled water in the same manner 
and filtered. The filtrates were pooled together and centrifuged 
(10,000 rpm, 15 min), and the clear supernatant was used as the en-
zeyme source.

Amylase activity was determined at 50°C by mixing appropri-
ately the diluted enzyme with 1% (w/v) soluble starch dissolved in 
0.1 M sodium acetate buffer (pH, 6.0) and determining the reduc-
sing sugars, in the form of glucose, after 5 min (11). One unit of 
activity was defined as the amount of enzyme releasing 1 mg of 
glucose equivalents from the substrate per min at 50°C. All the 
experiments were conducted in triplicate and the mean of the three 
was recorded. To fix the initial moisture content of the solid 
medium containing wheat bran alone as the substrate. The 
order of substrate suitability was wheat bran > millet cereal > 
crushed maize > crushed wheat > corn cobs > wheat flakes > 
maize meal > barley bran > molasses bran > rice bran. Differently, 
solid substrates were found to effect the production of enzymes (12). It was previously reported that wheat 
bran was found to be the best substrate for glucoamylase 
production by an Aspergillus species (13) and suitable for 
necessary manipulation (13, 14). In subsequent experiments, 
therefore, wheat bran was used as the substrate for the pro-
duction of amylase.

The incubation time for achieving the maximum enzyme 
level was governed by the characteristics of the culture and 
is based on growth rate and enzyme production. The T. 
tamuginosus strain produced high titers of enzyme (262 U/g) 
at 120 h of incubation (Fig. 1). A similar result was reported 
by Ellaiah et al. (13). The optimal temperature for maxi-
mum amylase production (263 U/g) was found to be 50°C 
(Fig. 2). Previously, 30°C and 45°C were reported as opti-
mum temperatures for amylase production by Aspergillus 
flavus and Myceliophora thermophila, respectively (15, 16).

A high enzyme titer (298 U/g) was attained when the initial 
moisture level was 90% (Fig. 3). The critical importance
of moisture level in SSF media and its influence on the biosynthesis and secretion of enzymes can be attributed to the interference of moisture in the physical properties of the solid particles. An increase in moisture level is believed to reduce the porosity of the wheat bran, thus limiting oxygen transfer (17). A low moisture content causes reduction in the solubility of nutrients of the substrate and a low degree of swelling (18).

The maximum amylase production (281 U/g) was obtained at a pH of 6.0. The inoculum level was also an important factor for the production of amylase. High inoculum levels are inhibitory in nature. The highest enzyme production (267 U/g) was obtained at an inoculum level of 10% (v/w).

The influence of supplementary carbon and nitrogen sources was studied. Of the carbon sources tested, soluble starch increased the amylase production (388 U/g) followed by sucrose (Table 1). Earlier workers reported soluble starch increased the amylase production (388 U/g) followed by sucrose (388 U/g) followed by fructose (266 U/g) was obtained at an inoculum level of 10% (v/w). The inoculum level was also an important factor for the production of amylase. High inoculum levels are inhibitory in nature. The highest enzyme production (267 U/g) was obtained at an inoculum level of 10% (v/w).

Among the nitrogen sources, peptone increased amylase production (414 U/g) followed by tryptone and meat extract (Table 2). Previous findings have shown that peptone, sodium nitrate and casein hydrolysate are good nitrogen supplements, as compared with the basal medium (control). Although the results of these investigations are based on experiments conducted in flasks, they provide valuable information for the production of amylase by SSF.

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![FIG. 3. Effect of initial moisture content on amylase production](image-url)