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Amylase of *Trichoderma viride*

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Optimal production of amylase by *Trichoderma viride* and its study of some crude state characteristics were carried out. The organism was grown in 3 % wheat bran medium containing 1 % glucose as carbon source and later 1 % starch as carbon source. The growth was fairly large and enzyme production was fairly high when glucose was used as source of carbon. In case of starch as carbon source, the growth was very small and the quantity of amylase produced was also small. The comparison showed that glucose is a good carbon source for production of amylase by *Trichoderma viride* as compared to starch.

Key Words: Amylase, *Trichoderma viride*.

INTRODUCTION

Since the first recognition of amylase in second half of 20th century, most of the resources of this enzyme that have been under intensive investigation in applied context have been of vegetable origin. Amylase is now commercially obtained from barley, sweet potatoes, cereals, soybeans and wheat. The studies on microbial production of amylases are comparatively recent and started around the midpoint of 20th century.

Different microbes that have been investigated for different aspects of amylases such as production, methods of assay, characterization, *etc.*, include, mostly, the bacteria and fungi. A few bacteria have been the major focus but the fungi particularly the moulds have been thoroughly thrashed for the study of amylases. A few bacteria that may be quoted in this context are *Clostridium acetobutyllinum*[1], *Bacillus coagulan*[2,3], *Bacillus sterothermophilus*[2], *Bacillus subtilis*[3], *B. diastaticus*[4,5], *etc*. Similarly, a large number of fungi have been under intensive investigation in said context. A few quotable examples from the recent literature may be *Aspergillus* species[6,7].

Following the track traced above, some work was also undertaken in Pakistan on the amylase of some important fungi studied extensively as producers of different enzymes. Malik and Khan[8], presented their results on the production of amylase by *Aspergillus niger*. They grew the organism in 3 % wheat bran medium in presence of 1 % glucose as a source of carbon. They found that the organism did produce more amylase than that reported by previous workers. The growth was subsequently carried out in the above medium in presence of 2 % calcium carbonate to maintain pH constant. There was a 33 % increase in enzyme production.

The purpose of work being reported here was an extension of the work carried by Malik and Khan[8]. To extend, a similar study was carried out after scheduling another organism and that was *Trichoderma viride*.

EXPERIMENTAL

Culture and inoculum: *Trichoderma viride* was obtained from Botany Department, Government College Lahore. The organism was grown on solidified potato-dextrose-agar slants at 28 ºC. For preparation of culture medium, 200 g potato slices were boiled for 1 h in about 500-700 mL distilled water. The resulting thick syrup was drained through a clean muslin cloth. To this was added glucose and agar. The final volume was made up to 1000 mL. The mixture was cooked for 1 h in a water bath. The pH of the medium was maintained at 4.5 *ca.* 10 mL of medium was poured in each test tube. All the test tubes were cotton plugged. The medium was sterilized in a pressure cooker at 15 psi for 15 min. The test tubes were allowed to set for 24 h to prepare the slants. The slants were inoculated with a sterilized needle loop and incubated at 30 ºC The growth was allowed to occur for 4-6 days. The slants were preserved in a refrigerator. The culture medium had the following composition:

Potatoes	Dextrose	Agar	pH
200 g/L	20 g/L	15 g/L	4.5

To prepare the inoculum, the slants were washed carefully with sterilized distilled water and a sporal suspension was obtained. The spores were centrifuged at 2500 rpm for 20 min in a sterilized centrifuge tube. The supernatant was discarded and the pallet was suspended in an adequate volume of sterilized distilled water. The optical density of the suspension was determined in a spectrophotometer. The suspension of the same optical density was transferred each time to keep the total population of spores constant and 10-20 mL of sporal suspension was transferred to each of the flasks containing 250 mL wheat bran medium and 30 mL glucose solution.

Fermentation medium: Wheat bran was chosen for the growth of *Trichoderma viride* as it was considered to be a suitable medium for the production of extra-cellular cellulase. The growth medium for *Trichoderma viride* was prepared by mixing the following quantities of ingredients per liter.

Wheat bran	K_2HPO_4	KCl	$MgSO_4 \cdot 7H_2O$
30 g	2.0 g	0.54 g	0.5 g

The above quantities of ingredients were mixed in distilled water to make 1 L suspension. The pH of the suspension was adjusted to 4.5. The suspension was then sterilized for half an hour at 15 psi. 20 g glucose was dissolved in 250 mL of distilled water and was sterilized separately for 0.5 h.

250 mL of wheat bran medium was taken in different 500 mL conical flasks. To each flask then was added 30 mL of sterilized glucose solution to make final concentration of 1 % glucose. The flasks were cotton plugged and were ready for inoculation.

Fermentation: Two types of fermentations were carried out. *Trichoderma viride* was grown by surface culture technique. In the first type, the growths were carried in 250 mL flasks each containing 100 mL wheat bran medium followed by transfer of 12 mL glucose solution. The flasks were inoculated using 4 mL of inoculum and subsequently incubated in an incubator. The growth temperature was 30 ºC. After 3 or 4 days when the growth of the organism had started, first flask was removed. Its contents were filtered and the extra-cellular amylase present in the filtrate was assayed taking 1 mL filtrate.

In the second type of fermentation, the organism was grown in one 500 mL Erlenmeyer flask containing 250 mL medium to which 30 mL glucose solution had been added. The flasks were inoculated using 10 mL of inoculum and subsequently incubated in an incubator. The growth temperature was 30 ºC. After 3-4 days when the growth of the organism had started, *ca.* 5 mL suspension was taken out with a sterilized pipette. The suspension was filtered and the enzyme activity of the extra-cellular amylase present in the filtrate was assayed taking 1 mL filtrate.

Assay of amylase activity: The amylase activity was assayed by Bernfeld method[9]. It is based on the principle that when the enzyme is incubated with the starch substrate, maltose is produced, which is a reducing sugar that develops color with 3,5-dinitrosalicylic acid whose optical density is read in a colorimeter.

To assay, 1 mL enzyme sample was incubated with 1 mL of starch substrate (1 % starch in citrate-phosphate buffer) at 37 ºC for 3 min.

The enzyme action was stopped by adding 2 mL of dinitrosalicylic acid reagent[10]. 1 mL 3,5-dinitro-salicylic acid reagent was then added to 1 mL aliquot of the mixture. The mixture was heated for 5 min in a boiling water bath and cooled under cold running tap water and filtered. The optical; density of the color developed was read in a spectrophotometer at 625 nm. A blank was prepared in another test tube as above but 1 mL heat denatured enzyme was used in place of the enzyme sample. The denaturation was accomplished by prolonged heating of the enzyme at 100 ºC.

The unit of amylase activity was defined as the amount of the enzyme which produced one micromole of maltose in 3 min at 37 ºC and pH 4.5 when incubated with 1 % starch solution under the assay conditions defined.

A standard curve was constructed by plotting optical density against known concentrations of maltose. The optical density units were converted to the micromoles of maltose by comparison with the standard curve.

Amylase production with glucose as major carbon source: The organism was grown in ten 250 mL flasks each containing 100 mL 3 % wheat bran medium containing 1 % glucose at 30 ºC. The growth was carried out as above and extracellular enzyme activity was assayed after every 48 h.

Amylase production with starch as major carbon source: The growth was carried taking 250 mL medium in 500 mL flasks. In place of 1 % glucose, 1 % starch was used as major carbon source. The progress of fermentation was followed by withdrawing 10 mL samples and assaying amylase activity as described above.

RESULTS AND DISCUSSION

The amylase production by *Trichoderma viride* during its growth at 30 ºC in 3 % wheat bran medium containing 1 % glucose as carbon source is shown in Fig. 1.

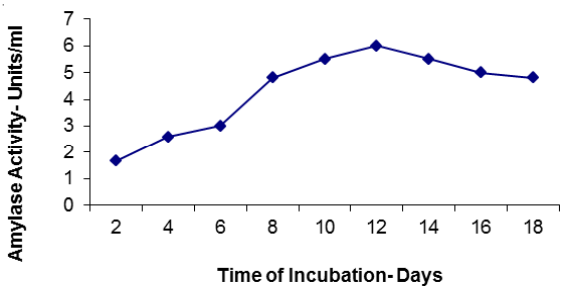

Fig. 1. Variation of amylase activity during the growth of *Trichoderma viride* in 3 % wheat bran medium in presence of 1 % glucose

A follow up of the progress shows that the amylase production was fairly large. The activity profile (Fig. 1) indicates that *Trichoderma viride* produces significant quantity of amylase. The optimum production of the enzyme takes place on 12th day (6 units/mL).

The amylase production by *Trichoderma viride* during its growth at 30 ºC in 3 % wheat bran medium containing 1 % starch as carbon source is shown in Fig. 2.

The activity profile shows that *Trichoderma viride* produces a very small quantity of amylase. The optimum production of the enzyme takes place in the earlier period of growth that is on 4th day (1.3 unit/mL).

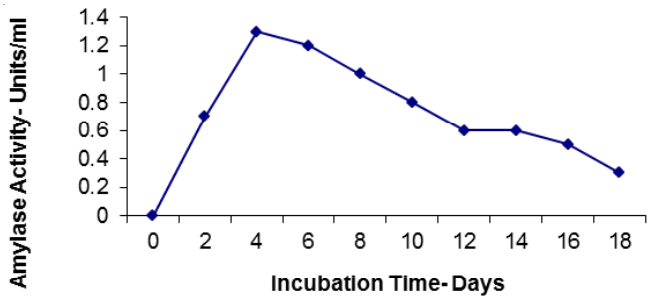

Fig. 2. Variation of amylase activity during the growth of *Trichoderma viride* in 3 % wheat bran medium in presence of 1 % starch

Fig. 3 the comparison of activity profile in Fig. 1 with the activity profile in Fig. 2 is made in the comparison shows that *Trichoderma viride* produces much greater quantity of amylase when grown in 3 % wheat bran medium in presence of glucose as carbon source as compared to that it produces in this medium in presence of starch as carbon source and as an enzyme inducer.

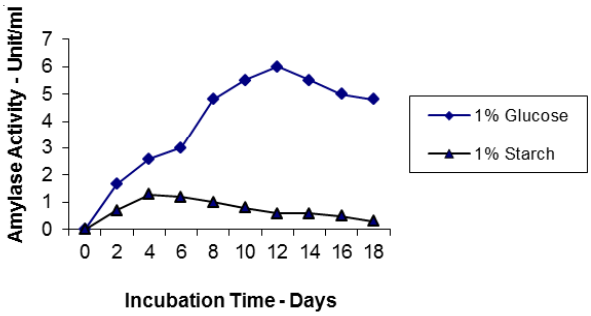

Fig. 3. Comparison of activity profile in Fig. 1 with the activity profile in Fig. 2

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