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Isolation of fungus from damp wall with amylolytic activity

Sanjay Sahay

Department of Botany and Biotechnology,
Government Science & Commerce College, Benazir, Bhopal-462 008 (India)

ABSTRACT

Constitutive amylase producing fungal isolates were screened from among those isolated from the damp wall. Two species of *Aspergillus* producing green (AG1) and yellow (AY1) conidia were found to produce amylase constitutively in potato dextrose broth. While AY1 showed increase in amylase activity only upto three days of growth, the increase in amylolytic activity was found till sixth day in case of AG1.

Key words: Fungus; constitutive amylase production; *Aspergillus* spp.

Amylases (EC3.2.1.11, 4-D-glucan-glucanohydrolase) representing approximately 30% of the worldwide industrial enzyme production[4], applied in baking, brewing, textile and detergent and other fields, such as clinical, medicinal and analytical chemistry[4]. The amylases with a specific set of properties are required for various applications.

The amylases have been obtained from bacteria, fungi and yeasts. But the amylases of fungal origin are more stable than those of bacterial origin[2]. The objective of this work was to isolate fungi from damp wall in the rainy season as source of amylases.

Fungus was isolated from damp wall showing fungal growth during rainy season. With the help of sterile ear bud, fungal spore was picked up from the wall of botany department. The bud carrying spore was dipped in 10 ml saline water. The water was diluted to 10-6 and 0.1 ml of sixth dilution was spread on to PDA (potato dextrose agar) medium. The plate was incubated at 37°C in an incubator for two days. The fungal colonies growing on this medium was then purified and maintained on the PDA slant.

Screening of amylase producing fungus:

Minimal synthetic medium[5] containing starch as sole carbon source was then inoculated with all the fungal isolates. The fungus showing growth and transparent halo around the colony was picked as the fungus producing amylase.

Liquid culture:

The fungus was grown in potato dextrose broth on shaking incubator at 37°C. After three,

four and five days of growth, the broth was centrifuged at 10000 rpm and the supernatant was used as source of enzyme.

Amylase assay:

The Amylase enzyme was assayed according to the method described by Miller[3]. One ml. of culture extract was pipetted in a test-tube. Then 1 ml of 1%soluble starch was added into citrate phosphate buffer (pH6.5). It was incubated in water bath at 25°C for 30 min. Blank was set consisting of 2 ml of extract that has been boiled for 20 min (boiling inactivates enzymes) added to the starch solution and treated with the same reagent as the experimental tubes. Reaction was stopped by adding 2 ml of Dinitrosalycilic acid (DNSA). Boil it for 5 minutes, cool and add 20 ml of distilled water. Determine colour intensity at 540 nm. One unit of amylase activity was defined as the amount of enzyme that releases 1 mg of reducing sugar as glucose per ml per min under the assay condition.

Effect of pH on enzyme activity:

The pH optimum of the enzyme was determined by varying the pH of the reaction mixtures using the following buffers (100 mM): sodium acetate (pH 3.0-5.5), sodium phosphate (pH 6.0-7.0) and Tris-HCl (pH 7.5-8.0).

Of the four different morphotypes, two fungal isolates were found to use starch as sole carbon source. These isolates also produced halo. On the basis of macro and micromorphological studies, they were found to be species of *Aspergillus*. One was green conidia producing fungus (AG1) while other was yellow conidia producing isolate (AY1).

Amylase activity was assayed in the broth itself. The amylase activity shown by the isolates is given in Table 1.

As Table 1 indicates AG1 shows increase in amylase activity till the sixth day while in case of AY1 the saturation in activity is reached very soon *i.e.* on the fourth day. Industrially, AG1 seems to be better than AY1. Both the isolates showed constituent production of amylase which is very important industrially.

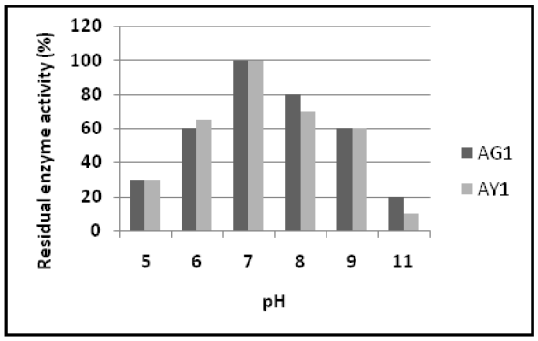

Fig. 1. Effect of different pHs on the activity of enzyme

Table-1. Amylase activity shown by the fungal isolates in different periods of growth

Fungal isolates	Amylase activity (unit/ml) in the broth of different periods of growth			
	Third day	Fourth Day	Fifth day	Sixth day
AY1	3	4.4	7	7
AG1	2	5	9	11

The effect of pH on the enzyme activity has been shown in Fig. 1. The enzyme obtained the isolates showed highest activity at neutral pH suggesting that the enzyme is a neutral amylase.

Amyalses have earlier been isolated from bacteria[6], fungus[2], and other species[7] earlier. There is also report of constitutive amylase producing fungal species *Aspergillus oryzae*[1], however this is first time the fungal isolate from damp wall is being reported producing amylases constitutively.

The fungus may be important industrially. A neutral amylase is useful in sachharification industry. A further characterization of amylases from these isolates is required.

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