

Soil *Streptomyces* – A Potential Source of the Industrial Enzyme, Glucoamylase

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ABSTRACT. The potentials of soil *Streptomyces* for the production of enzymes has not been realised adequately compared to bacteria and fungi. *Streptomyces* exhibit a high degree of starch hydrolysing activity. Isolates of thermotolerant *Streptomyces* from several soil samples and also those obtained from starch enrichment technique displayed glucoamylase (E.C. 3.2.1.3 α -1, 4 glucan glucohydrolase) activity. The two *Streptomyces* isolates VPG-10 and VPG-11 registered very high glucoamylase activity (specific activities 440 and 340). Cassava thippi, a waste product from cassava-sago industry could serve as a cheap substrate for glucoamylase production. By stepwise purification of the crude enzyme through dialysis, acetone precipitation and ammonium sulphate precipitation, the activity of the enzyme increased six folds. The enzyme was stable at 70°C and has a temperature optimum of 40°C.

INTRODUCTION

Enzymes play a vital role in several industrial processes like food fermentation, textile and pharmaceutical preparations. In recent years, microbial enzymes have assumed greater importance in industries than the enzymes derived from plants or animals. Starch hydrolysing enzymes, generally recognised as 'amylases' have been in use for long years, in food preparations, brewing and distillery industries (Toldra *et al.*, 1992). The enzyme glucoamylase often called 'saccharifying amylase' is widely used for the production of high fructose and glucose syrups usable in pharmaceutical industries (Fullbrook, 1984). On an industrial scale, this enzyme is mainly produced by strains of *Aspergillus*, *Rhizopus* and by the bacteria *Clostridia*. While soil actinomycetes have been reported to produce α and β amylases, information on the production of glucoamylase is scarce. It has been the aim of the present investigation to explore the potentials of *Streptomyces* for glucoamylase production.

MATERIALS AND METHODS

Source of *Streptomyces* isolates

The *Streptomyces* used were isolated from different soils in the Tamil Nadu Agricultural University, Coimbatore, India by enrichment technique. Ken Knight's medium was used for the isolation of actinomycetes.

Isolation of thermophilic starch hydrolysing actinomycetes by soil enrichment technique

Red loamy soil (25.0 g) was mixed with 5.0 g of potato starch. Sufficient water was added to keep the moisture content around 40%. The soil cultures were incubated at 50°C for 15 days and moistened daily. Five grams of samples were withdrawn, serially diluted in sterile water blanks and plated using Ken Knight's agar medium. The plates were incubated at 50°C for 7 days and the colonies of actinomycetes were picked up, purified and maintained on Ken Knight's agar slopes (Agate and Bhat, 1963).

Rapid screening of isolates for amylolytic activity

Purified isolates were streaked on starch agar medium. The plates were flooded with Lugol's iodine after sufficient growth of the isolate occurs. Isolates exhibiting halo around them were selected for further study (Smale and Keil, 1966).

Production of glucoamylase by actinomycetes

Starch broth (100 ml) of the following composition was prepared and dispensed in 250 ml Erlenmeyer flasks (Soluble starch : 10.0 g; yeast extract : 2.0 g; ammonium chloride: 1.0 g; magnesium sulphate : 0.5 g; potassium phosphate: 0.5 g; sodium chloride: 1.5 g; distilled water : 1000 ml; pH-6.5). The medium was autoclaved for 15 min at 120°C (Bajpai and Bajpai, 1989).

After cooling, a quantity of 5 ml spore suspension of the actinomycete was added and placed over an incubator cum shaker (New Brunswick, USA) set at 37°C at 100 strokes per min. At desired intervals of 3rd, 5th, 10th and 15th days of growth, the flasks were withdrawn. The broth was centrifuged at 5,000 G for 15 min and the clear supernatant served as the source of enzyme.

Assay of glucoamylase

The method described by Elegado and Fugio (1993) was used. The assay system consisted of 1.0 ml of 1% soluble starch in sterile distilled water, 2.0 ml of 0.1 M phosphate buffer (pH-6.5) and 2.0 ml of enzyme source. The reaction proceeded for 60 min at ambient temperature. After terminating the activity, 1 ml of the enzyme reaction mixture was withdrawn and the quantity of reducing sugars liberated was determined (Nelson, 1944). One unit of enzyme activity is defined as the amount of enzyme that released one μ mole of glucose at 28°C in 1 h (Campus *et al.*, 1992). Protein contents of the culture filtrate were determined (Lowry *et al.*, 1951).

Thermostability of glucoamylase

As the enzyme was derived from thermophilic actinomycetes, the thermostable nature of the enzyme was examined. The enzyme assay as detailed above was performed at temperatures of 10°C to 70°C in thermostatically controlled water bath. Glucoamylase activity was determined (Nelson, 1944).

Partial purification of the enzyme

Glucoamylase was produced in 2 l round bottom flask in modified starch broth. After 3 days of incubation at 37°C in a wrist action shaker (100 strokes per min), the clear culture filtrate was obtained through filtration and centrifugation.

Enzyme purification

Dialysis

Clear culture filtrate (100 ml) was dialysed against changes of distilled water over 24 h at 10°C. The dialysate was assayed for enzyme activity.

Ammonium sulphate precipitation

The culture filtrate was treated with ammonium sulphate at different per cent of concentrations (Jayaraman, 1981). The culture filtrate was left at 10°C for 24 h and the precipitate formed was collected by centrifugation and assayed for glucoamylase activity.

Acetone precipitation

A known quantity of culture filtrate was added with equal volume of acetone, mixed well and left at 10°C for 24 h. The enzyme protein precipitation was collected by centrifugation. At every stage of purification, the activity of the enzyme was assayed.

Production of glucoamylase by solid state fermentation

Agro-wastes like cassava thippi, cassava rind, infested sorghum grain and wheat bran were taken for the study. The starch content of these wastes was determined by using Anthrone reagent. The thippi upon analysis revealed that it contained 7.3 ± 0.6 per cent of starch w/w (Lonsane *et al.*, 1992).

A quantity of 100 g of starchy wastes (cassava thippi, cassava rind, infested sorghum grain and wheat bran) were taken in 500 ml Erlenmeyer flasks separately. Tap water was used as the moistening agent and the ratio of the solids to the moistening agent was 2:1 (w/v). The flasks were autoclaved at 120°C, for 15 min and allowed to cool. Then

the wastes were inoculated with 5 ml of spore suspension of actinomycete isolate VPG-11. The flasks were incubated in slating position at 37°C in an incubator.

The fermented substrate after drying at 40°C was flooded with 5 volumes of 0.1 M phosphate buffer (pH-6.5) at 28°C for 60 min. and stirred well. The slurry was squeezed through layers of moist cheesecloth. The extract was centrifuged at 5000 G for 15 min. and clear supernatant obtained served as the glucoamylase enzyme source.

Characterization of selected actinomycete isolates

To study the morphological characterization of actinomycetes, the culture were grown on starch agar slides in Petridish moist chamber (Waksman and Henrici, 1943). After sporulation, the slides were examined microscopically. The nature of aerial and submerged mycelium, sporulation, branching of the mycelium and other morphological characters were observed.

RESULTS AND DISCUSSION

Of 237 isolates of thermophilic actinomycetes obtained by enrichment technique, 8 isolates were selected as good starch hydrolysers (Fig. 1) as they formed larger halos around them when they were grown in starch agar plates and flooded with Lugol's iodine.

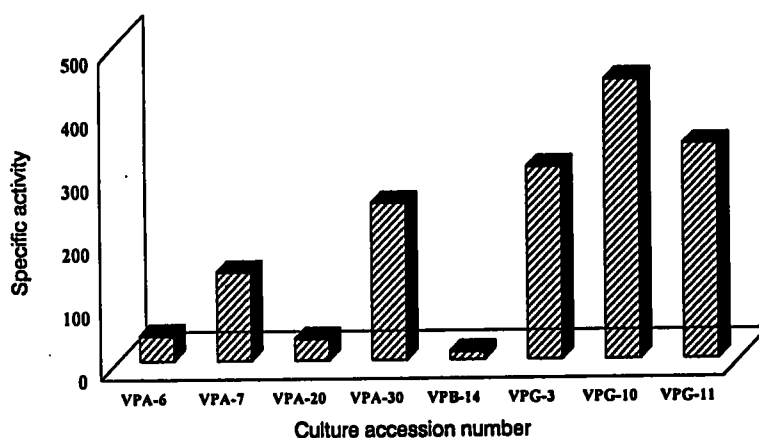


Fig. 1. Specific enzyme activity of selected actinomycetes on the 3rd day of inoculation in starch broth.

Among the eight, isolates VPG-10 and VPG-11 were selected for detailed study as they were most promising, based on their specific activity. VPG-10 and VPG-11

isolates exhibited specific activity of 440 and 340 respectively and the enzyme activity was observed to be 2.3 in both isolates at 72 h of fermentation.

The purer the enzyme, greater would be the quantity of end product. In the present experiment, acetone precipitation has shown 4.7 and 6.1 folds increased activity for VPG-10 and VPG-11 respectively, and ammonium sulphate 20-50% precipitation has shown 1.8 and 2.4 folds increased activity for VPG-10 and 11 respectively (Table 1).

Table 1. Activity and purification of glucoamylase from culture filtrate.

S. No.	Culture Accession No.	Process	Glucoamylase activity (E.U.)	Specific activity	Purification factor
1	VPG-10	Culture filtrate	2.3	444.4	1.0
		Ammonium sulphate (20-50%) precipitation	4.1	810.2	2.0
		Acetone precipitation	1.6	2083.3	5.0
2	VPG-11	Culture filtrate	2.3	341.9	1.0
		Ammonium sulphate (20-50%) precipitation	2.4	833.3	2.0
		Acetone precipitation	1.0	2083.3	6.0

Of the two isolates, the performance of purified VPG-11 was better and hence, VPG-11 was used for glucoamylase production under solid state fermentation.

Cassava thippi, cassava rind, infected sorghum grain and wheat bran were used as the substrate under solid state fermentation. Similar reports were published by many authors (Hari and Purushothaman, 1997 and Pandey *et al.*, 1995). Of these, cassava thippi was found to be the best substrate for glucoamylase production as the specific activity (200) of the enzyme produced by VPG-11 on thippi was greater than other substrates (Table 2).

Table 2. Activity of glucoamylase produced by the *Streptomyces* isolate, VPG-11 on different substrates under Solid State Fermentation.

S. No.	Substrate	Glucoamylase activity (E.U.)	Specific activity
1	Cassava thippi	14.4 ± 0.5	200.0
2	Cassava rind	7.2 ± 0.4	44.4
3	Infested sorghum grain	14.4 ± 0.6	88.9
4	Wheat bran	9.6 ± 0.4	29.6

It was confirmed that the actinomycete isolates VPG-10 and 11 were *Streptomyces* spp. as they showed the characteristic aerial mycelium profusely branched, bearing spores. Solitary sporangia are also borne by aerial mycelium.

The glucoamylase enzyme produced by actinomycete isolates showed activity even at 70°C though activity was less. The optimum temperature for glucoamylase activity was 40°C and the optimum pH for enzyme activity was 6.0 (Fig. 2 and 3). Similar report was published by some authors (Tsuchida and Irie, 1978).

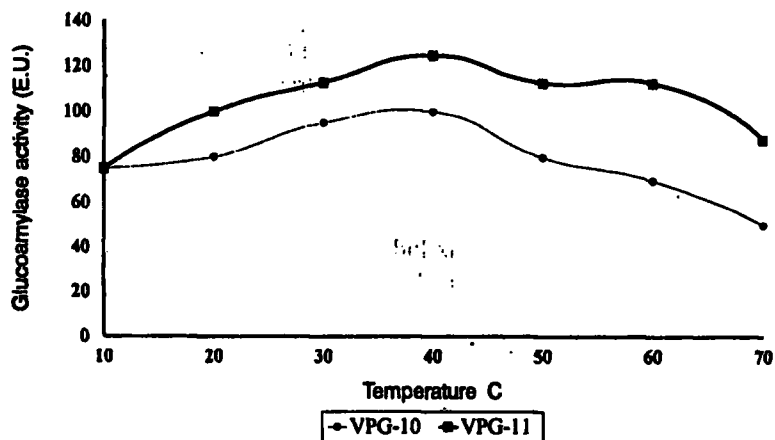


Fig. 2. Effect of temperature on glucoamylase activity.

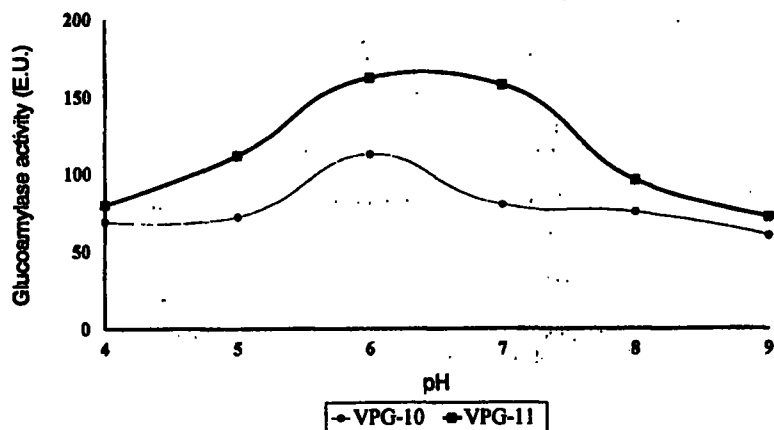


Fig. 3. Effect of pH on glucoamylase activity.

About 1% soluble starch was used throughout the experiment, but agro-waste cassava thippi having 7.3% starch was used in the solid state fermentation. This yielded the highest specific activity. The isolate VPG-11 exhibited enzyme activity of 135 E.U. and 14.4 E.U. for 1% soluble starch and 7.3% starch concentration of cassava thippi respectively. The *Streptomyces* isolate, VPG-11 could grow and produce enzyme even at higher substrate concentration.

CONCLUSIONS

The present study indicated that *Streptomyces* from soil are potential producers of starch hydrolysing enzymes. Thermotolerant isolates of *Streptomyces* exhibited high glucoamylase activity. The 2 isolated VPG-10 and VPG-11 were best. It is possible to use cassava thippi, a waste from sago industry as a substrate to produce glucoamylase.

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