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Screening and production of bacterial amylase from different *Strepomyces* species

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Abstract
The enzymes from the microbial sources are more stable and can be obtained from cheap sources. Amylase is most important industrial enzyme which is widely used in food and biofuel industries. *Streptomyces* is a group of bacteria belongs to largest genus of Actinobacteria and commonly isolated from the soil. *Streptomyces* species have been widely used as microbial cell in industries for production of antibiotics. For the production of antibiotics, Streptomyces species are frequently used as microbial cells. This study reported that screening and production of amylase from different *Streptomyces* species *i.e. Streptomyces microflavus*, *Streptomyces cyaneus* and *Streptomyces diastaticus*. The cultures were screened for the production of amylase by starch agar plate assay. Result shown the *Streptomyces diastaticus* was better amylase enzyme producer compare to *Streptomyces microflavus* and *Streptomyces cyaneus*. *Streptomyces diastaticus* gives maximum starch hydrolysis by showing clear zone (4.4 mm), enzyme production (1.60 U/ml) and protein concentration (2.25 mg/ml).
***Key words**: Amylase, Streptomyces, Antibacterial, Antibiotic, Screening*

Introduction
Amylase is a high-valuable biocatalysts that used in hydrolysis process of starch. Amylase is present in all life domains, but microbial amylase easily culturing and economically produced which is broadly used in industries. Amylases have found their applications in analytical chemistry, fine chemical industries, starch saccharification, pharmaceuticals, syrup production, food fermentation, beverages, brewing, distillation and textile industries (Tonkova, 2006; Li *et al*., 2007; Vidyalakshmi *et al*., 2009; Sanghvi *et al*., 2011). It is derived from the plants, animals and many microorganisms. The well-known thermostable *Bacillus* produces amylase *i.e. Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis* and *Bacillus stearothermophilus* have used in various commercial purpose (Pandey *et al*., 2000; Prakash and Jaiswal, 2010).
Streptomyces are an economically important group of bacteria among Actinobacteria family. They produces antibiotics, antimicrobial agent, antioxidant agent, antimicrobial agent, immunosuppressive agent, enzyme and enzyme inhibitor (Bull *et al*., 2005; Sreejetha *et al*., 2012).Soil is the main source for isolation of *Streptomyces*. Although these group of bacteria can be isolated from aquatic habitat (Takahashi and Omura, 2003). *Streptomyces* are an active source for amylase enzyme. Notably, *Streptomyces rochei* BTSS 1001, *Streptomyces avermitilis*, *Streptomyces sp*. S2BA-08 and *Streptomyces* strain A3 used for production of amylase (Vigal *et al*., 1991; Chakraborty *et al*., 2012; Acharyabhatta *et al*., 2013).

Materials and Methods
Collection of culture
The *Streptomyces* culture namely *Streptomyces microflavus*, *Streptomyces cyaneus* and *Streptomyces diastaticus* were provided by Modern Biotech Research Lab Raipur.
Screening for amylase production
Streptomyces were screened for amylolytic properties by starch hydrolysis test on starch agar plate. The organism were streaked as a line on the starch agar plate and plates were incubated at 45ºC for 48 hours (Hamilton *et al*., 1999).

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Amylase production

Freshly prepared inoculum was used to inoculate the production medium. For the preparation of inoculum a loop full of bacterial isolates were transferred in 50ml of inoculum medium contained (g/L) starch 10, peptone 10, yeast extract 20, KH_2PO_4 0.05, $MnCl_2.4H_2O$ 0.015, $MgSO_4$ 0.25, $CaCl_2.2H_2O$ 0.05 and $FeSO_4.7H_2O$ 0.01. Amylase production was carried out by submerged fermentation. 500ml of the production medium (same as inoculation medium) was inoculated with 10ml of bacterial inoculum. The flask was loaded on a rotary shaker incubator at a speed of 2000 rpm at 45ºC for 24 hours. After incubation, fermented broth was centrifuged at 7000 rpm for 15 min in a cooling centrifuge. Supernatant was collected and used for the estimation of amylase.

Enzyme assay

Amylase activity were determined by spectrophotometric 0.5ml crude enzyme was taken in test tube in triplicate and 0.5ml of substrate (starch) was added in the test tube. The test tube were covered and incubate at 37ºC for 30 min in the water bath. Then 0.5ml of 0.1N HCl was added in each tubes to stop the reaction and 0.025ml of iodine solution was added. After cooling at room temperature, the absorbance was read at 620nm by spectrophotometer.

Protein estimation

Protein were estimated by Folin Lowry method. Take sterilized test tube and make a standard series of protein in it. Add 0.5ml of alkaline solution at each tubes. Then added standard protein solution in each of the tubes except one tube which is known as blank. Then after add 0.5ml of Folin Ciocalter reagent and makeup the volume with distilled water. Kept it in room temperature for 30 min and read at 750nm.

Results and Discussions

In the present work all test *Streptomyces sp*. showing the amylase production. *Streptomyces diastaticus* had maximum clear zone (4.4 mm) shown in Figure no. 1, maximum amylase assay activity (1.60U/ml) shown in Figure no. 2 and maximum protein concentration (2.25 mg/ml) shown in figure no. 3. Similar results found by the other authors, Islam *et al*. (2014) isolates the *Streptomyces* from Bangladeshi soil and determine the amylase activity and conclude the *Streptomyces* are a good amylase producer, Rengasamy and Thangaprakasam (2018) isolates the *Streptomyces* from marine and screened for production of amylase enzyme and Al-Dhabi *et al*. (2020)isolated the Streptomyces species from Jazan region of Saudi Arabia and purified the starch hydrolysis amylase enzyme.

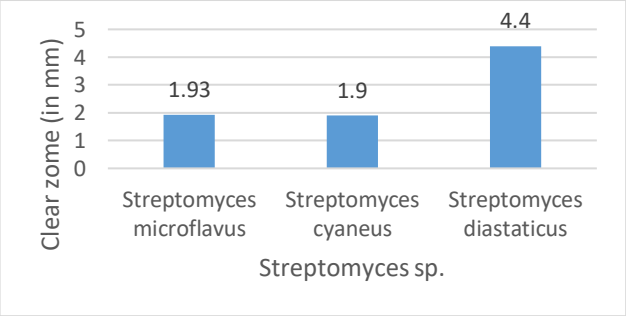

Figure no. 1:- Primary screening for amylase production

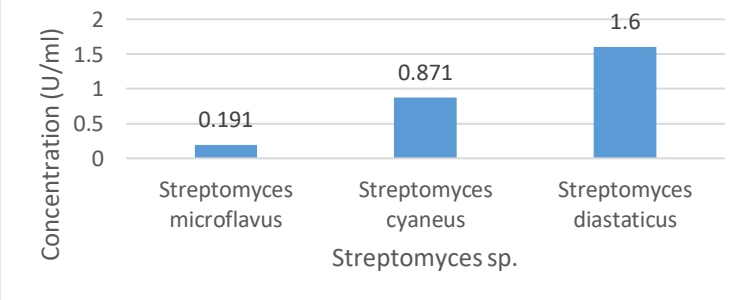

Figure no. 2:- Enzyme assay for amylase production

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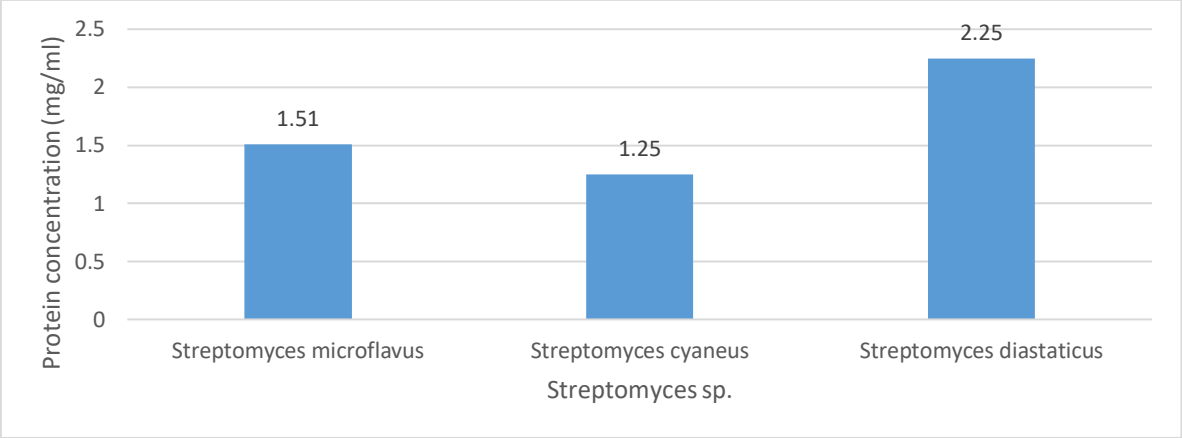

Figure no. 3:- Protein estimation by Folin Lowry Method

Conclusion

Amylases are the most important enzymes in present day in Microbiology and Biotechnology. Starch degrading enzyme like amylase have received great deal of attention because of their perceived technological significance and economic benefits. The amylase can be derived from several sources such as plant, animals and microorganisms. The microbial amylases meet industrial demands, a large number of them are available commercially, and they have almost completely replaced chemical hydrolysis of starch in starch processing industry. The main purpose of this work was wide production of amylases using different strains of *Streptomyces*. These studied was showed that one of the *Streptomyces* species was capable to produce amylase enzyme. The best amylase production was shown by *Streptomyces diastaticus*.

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