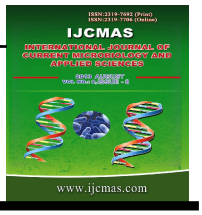
Int.J.Curr.Microbiol.App.Sci (2013) 2(8): 206-214

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 8 (2013) pp.206-214**
http://www.ijcmas.com



Original Research Article

Production, purification and characterization of α - amylase using *Streptomyces* spp. PDS1 and *Rhodococcus* spp. Isolated from Western Ghats

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Keywords

Western ghats; amylase; *Stroptomyces* spp. Pds1; *Rhodococcus*; Siruvani forest of Tamilnadu optimum activity.

A B S T R A C T

S*treptomyces* spp. Pds1 and *Rhodococcus* spp. were isolated from soil samples collected from Western ghats of Siruvani forest of Tamilnadu. They were used for the production of α amylase in the chemically defined medium . Various physico and chemical parameters such as pH, temperature , incubation time were determined. Amylase production was maximum on 3rd day of incubation. (56U/mL) by *Streptomyces* spp.pds1 whereas *Rhodococcus* produced only 50U/Ml on 3rd day of incubation. The optimum pH and temperature were found to 7.0 for both the strains. The optimum temperature was 40^0C for both the strains . The amylase from the *Streptomyces* spp. pds1 was partially purified and its molecular weight was determined using SDS- PAGE. The molecular weight was found to be 44kDa. Among the strains tried *Streptomyces* spp. pds1 was found to be good candidature for amylase production.

Introduction

Actinomycetes are known to produce several enzymes, degrading complex organic materials in soil OR sediments. Gluve and Desmukh, 2011, reported various enzymes such as protease, gelatinase , amylase , lectinases , cellulases and ureases from the actinomycetes strains isolated from the coastal segments of kongan coast of Maharashtra. Actinomycetes are gram + filamentous bacteria which are freely OR saprophytically in different habitats such as soil, warm water, marine water etc…. Some important species are Micromonospora, Nocardia & *Streptomyces* , whose large genomes enable them to produce some types of secondary metabolites, antibiotics and industrially enzymes (Salami . 2004 ; Padmadhas and Ragunathan, 2010 & Santos *et al* ., 2012).

Industrial enzyme sector in India and the world is developing fast for meeting the

needs of food processing, pharma and textile industries. Research on enzyme producing actinomycetes re very limited in our country. Enzyme production using actinomycetes is interesting in nowadays by the researchers. Today morethan 4000 enzymes are known of which many are commonly produced. Majority of the enzymes are from bacterial and fungal origin. Among microorganism actinomycetes produces different enzymes and emergence as rich source for the production of industrially important enzymes. (Suneethat *et al* .,2004 ; Dietera *et al* ., 2004 ; Oyeleke *et al* ., 2010 & Kafilzadeh *et al* ., 2012).

Amylases is one of the most commonly used enzyme in dofferent starch industries . It has many applications in the syrup production, hydrolysis of starch et… (Tonkova 2006). α amylases (indo-1, 4 α D- glucan glucano hydrolase ,F.C.3.2.1.1) are extracellular enzymes , that cleaves α 1-4 linkages between glucose units and leave the glucose, maltose and maltotriose units . Enzymatic hydrolysis of starch has now replaced acid hydrolysis in over 75% of starch hydrolyzing process (Gupta and Grautam , 1995, Ragunathan and Swaminathan,2005 and Vidyalakshmi *et al* .,2009). Applications of amylases includes , processing of fruits like mango, banana, papaya and citrus fruits (Sharanappa *et al* .,2012); used in laundry and dish washing detergents (Vander Maarel *et al* .,2002) ; food fermentation , textile and paper industries have been reported (Sanghvi *et al* .,2011). Amylases have been derived from plants, animals , several fungi , yeast, bacteria and actinomycets. Microbial source of amylase is preferred to other sources because of its plasticity and vast availability. (Mishra & Behera 2008 ; Li *et al* .,,2011). There are various reports on α amylase production by actinomycetes. A promising *Streptomyces clavifer* and *Streptomyces* spp. (Yassien and Astuur, 2011; Hoque *et al* .,2006 ; Kar and Ray, 2008) have been reported. Recently, Kafolzadeh *et al* .,2012 isolated *Streptomyces* genus from aquatic sediments for the production of amylase & screening of marine actinomycetes for production of enzymes (Selvam. *et al* .,2012) have been reported.

Until, now no extensive studies have been carried out to isolate the novel actinomycetes from Western ghats for the production of α amylase. Based on this, the present investigation focused on the production of α amylase from two strains of actinomycetes isolated from soils of Western ghats , viz. *Streptomyces* spp. Pds1 and *Rhodococcus* spp. The amylase was optimized, purified and its molecular weight was also determined.

Materials and Methods

Source of inoculums

Two strains of actinomycetes *Streptomyces* spp. pds1 and *Rhodococcus* spp. (Padhmadas and Ragunathan ,2010) were obtained from the Dept. of Biotechnology, SNMV College of Arts & Science, Coimbatore – 50 and used for the production of amylase and preserved on actinomycetes slants.

α amylase production

The production medium (25mL) was prepared in 100mL Erlenmeyer flasks, containing basal medium (g/L). 10.0g starch; 5.0g yeast extract; 10.0g Na_2HPO_4; 0.5g KCl and 0.15g $MgSO_4$ and pH was adjusted to 7.0 before sterilization. After sterilization the two strains were incoculated separately and incubated at

37^{o}C for 5 days in rotary shaker (120rpm). After 5th day the culture was centrifuged (7000rpm for 10 minutes) and the clear supernatant was used as crude enzyme extract.

Biomass Estimation

The biomass of actinomycetes culture was estimated after filtering the culture medium through pre weighed Whatman Number 1 filter paper and dried to a constant weight at 80^{0}C and reweighed . The difference in weight denoted the biomass .

Enzyme assay

A amylase activity was determined as described by Okolo *et al* .,(1995). The reaction mixture consists of 1.25ml of 1%starch in 0.1M acetate buffer ; 0.25ml of 0.1M acetate buffer (ph7.0) ; 0.25ml of distilled water and 1.0ml of crude enzyme. After 10 minutes of incubation at 50^{0} C , the liberated reducing was estimated by the addition of 3,5 dinitrosalicylic acid (DNS) followed by boiling for10 minutes (Bernfield *et al* .,.,1955) and the OD was measured at 540nm and one unit of the enzyme required to release 1µ mol of reducing sugar in one minute under the assay condition.

Optimization of culture conditions

The various parameters like pH (4.0 to 8.0), temperature (30°C to 50°C) and incubation time (1 to 5 days) were determined.

Amylase purification

The crude enzyme was subjected to ethanol precipitation (70%) to remove the unwanted protein compounds. After that ,the filtrate was dialyzed in dialysis membrane with 0.1M acetate buffer (ppH7.0) and then it was eluted using the same buffer in DEAE- Sephadex A – 100 column equilibrated with the same buffer .

Determination of molecular weight (Lamelli, 1970)

SDS- PAGE was carried out and molecular weight was determined. The protein content was estimated by using Lowry *et al* ., (1951).

Results and Discussion

The two novel actinomycetes strains *Streptomyces* spp. Pds1 and *Rhodococcus* spp. were used for the α amylase production (Figure 1). The optimization and partial purification were also carried out.

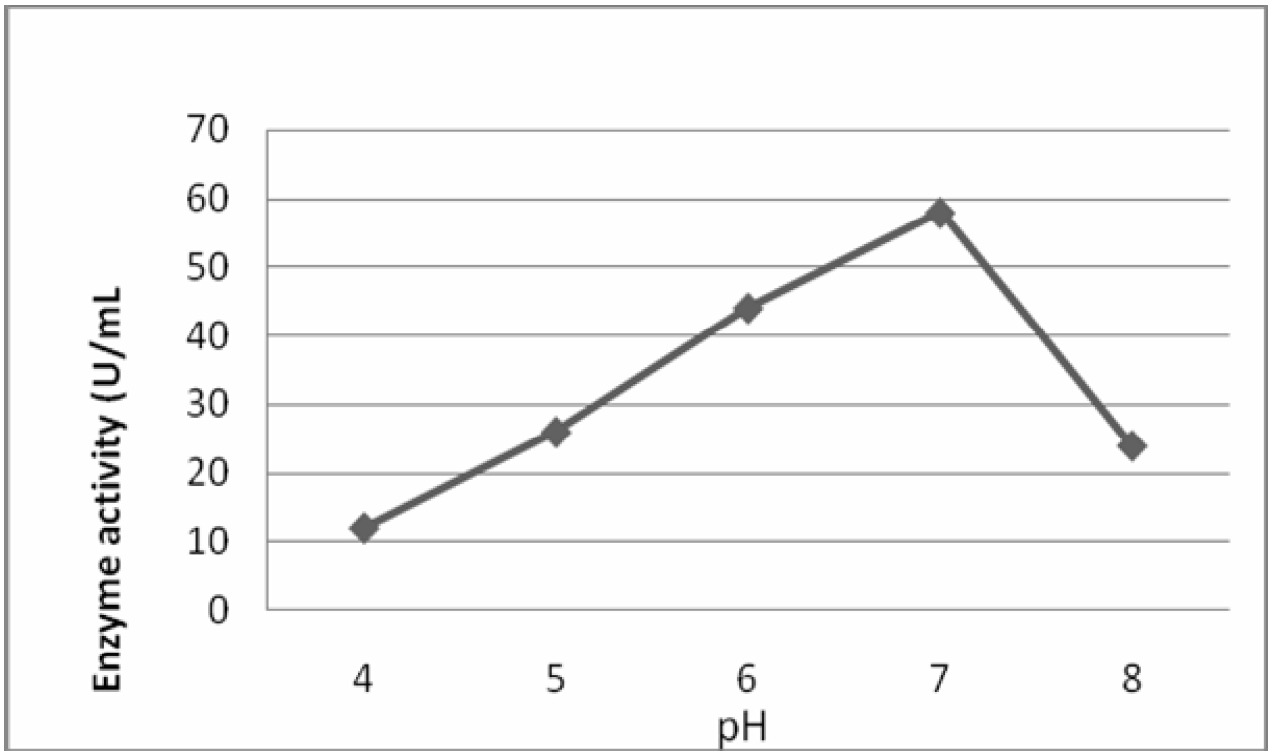
Effect of incubation time on α amylase production

The two strains were inoculated on production media and incubated for 5days (30^{0}C at 120rpm). Increase in enzyme activity was occurred on 2day of incubation. Among the two strains tried *Streptomyces* spp. Pds1 have produced maximum enzyme activity on 4th day of incubation (56U/mL). Whereas *Rhodococcus* spp. produced on 4th day with less enzyme activity(Table1). After the 4th day of incubation the enzyme activity was declined. Ashar Mohammed *et al* ., (2000) reported that, maximum production of amylase was occurred on 48 hr of incubation by *Arachriotus* spp ; 72 hrs of incubation by *B. amyloliquefaciens* (Dhanya and Gangadharen) ; 96th hrs of incubation by soil thermophilic strains of actinomycetes (Salahuddin *et al*., 2011); 120 hrs of incubation for amylase production by *Aspergillus oryazae*

Table.1 Effect of incubation time of α-amylase

Day	*Streptomyces* pds1 spp.		*Rhodococcus* spp.	
	Biomass (mg/L)	**Enzyme activity (U/mL)**	**Biomass (mg/L)**	**Enzyme activity (U/mL)**
1	24	12	20	16
2	38	26	32	26
3	56	56	42	54
4	28	23	12	32
5	20	12	10	25

Figure.2 Effect of pH on amylase production **by** *Streptomyces* spp. Pds1



(Vasuleo Zambare, 2010); 7th day of incubation for marine water actinomycetes (Selvam *et al* .,2012) have been reported.

Influence of pH on amylase production

pH (biocatalyst) is the most important factor which markedly influence the enzyme activity . In the present study both the strains required an initial pH of 7.0 (Figure 2). The Biocatalyst was able to present a good performance within the complete range, retaining the activity still in pH 8.0 (24U/mL). For *Streptomyces* spp. Pds1 (ph -7.0 – 58U/mL) and *Rhodococcus* spp the pH was 7.0 with enzyme activity of 42 U/mL. Earlier reports also witnessed our findings. pH 7.0 by Krishna and Chan , 1996 ; Negi and Banerjee (2009) (ZB) observed the activity of pH betewwen 5.0 and 9.0 in *Aspergillus awamori.* Yassien and Asfoar (2012) obtained the optimum pH of 6.0 for *Streptomyces clavifer* ; the pH 7.0 for amylase from *Streptomyces* sp. SLBA-08 was observed recent studies (Santos *et al* ., 2012).

Effect of temperature on amylase activity

Temperature was also considered a important parameter to study the activity of α-amylase. In the present study the enzyme activity was found upto 45^0C . The optimum temperature was 40^oC (52U/mL) for *Streptomyces* spp. PDS1 and *Rhodococcus* spp.. Increase in enzyme activity from 35^oC to 45^OC was observed. This value is similar or even higher than the optimal temperature reported by Chakraborty *et al* .,(2007) for *Bacillus* spp. Asgher *et al* .,(2007) observed an optimum temperature at 70^0C. from moderately thermophilic *Bacillus subtilis*. (ZB).

An optimum temperature of 45^0C for *Streptomyces gulbargensis* was reported (Syed *et al* ., 2009). Amylase from soil thermophilic actinomycetes showed the highest activity at 55^oC in the presence of starch as substrate (Salahuddin *et al*., 2011). Santos *et al* ., (2012) observed an optimum temperature of 50^oC for amylase activity by *Streptomyces* sp. SLBA -08.

Enzyme purification

The crude enzyme was first concentrated with ethanol (2X volume)_ followed by dialysus against the buffer overnight and then gel filterated using column DEAE SephadexA-100 chromatography (2.5 X15cm) that was equilibrated by 0.1M acetate buffer (pH 7.0). The fractions were collected and subjected to SDS- PAGE. The molecular weight was found to be 44kDa for the *Streptomyces* spp. PDS1. The molecular weight of amylase produced by *S. gulbargensis* was 55kDa (Syed *et al* .,2009) and 50kDa for *Streptomyces clavifer* (Yassien *et al* ., 2012).

Based on the above investigation it can be concluded that the two novel isolates viz. Streptomyces spp. PDS1 and Rhodococcus isolated from Western ghats can be good candidate for the amylase production . The optimum day for the amylase production was found to 3rd day for both strains. The pH and temperature were 7.0 and 35^OC for both strains. Among the two strains tried *Streptomyces* spp. PDS1 was found to be best candidate for the amylase production. Hence, the partial purification was carried out only for the amylase of Streptomyces spp., PDS1 . The enzyme was partially purified and the molecular weight was found to be 44kDA using SDS- PAGE.

Figure.3 Effect of pH on amylase production by *Rhodococcus* spp.

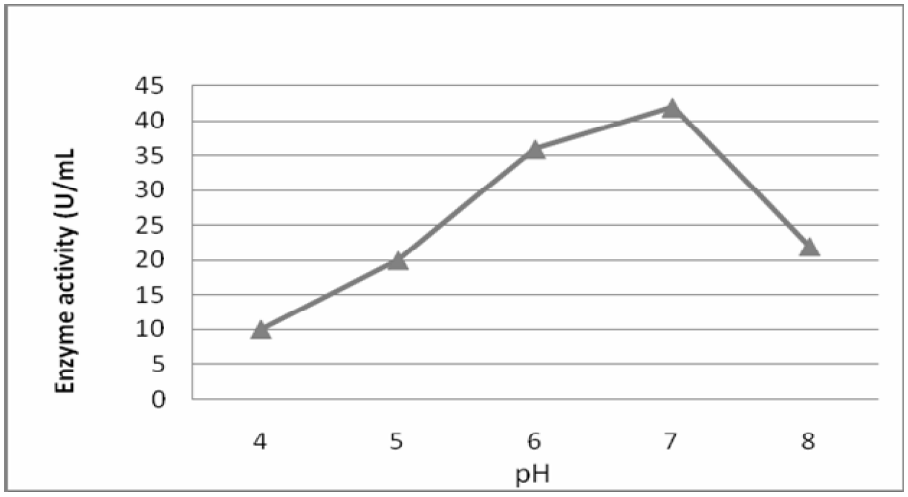


Figure.4 Effect of temperature on amylase production by *Streptomyces* spp. Pds1

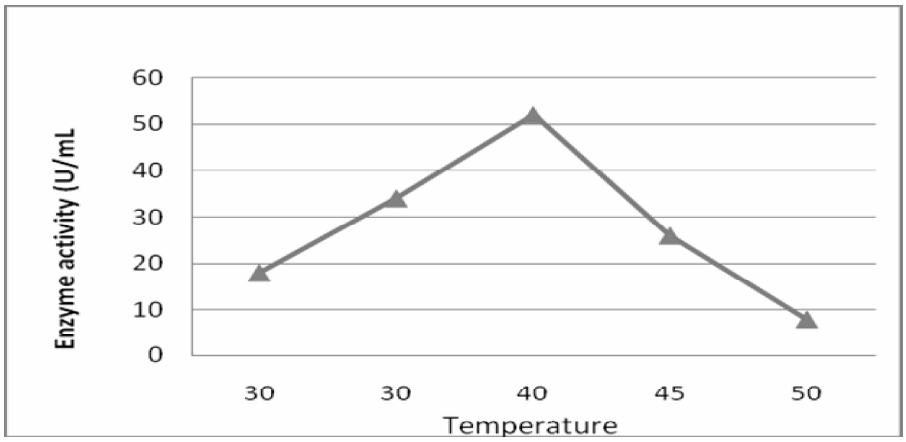


Figure.5 Effect of temperature on amylase production by *Rhodococcus* spp.

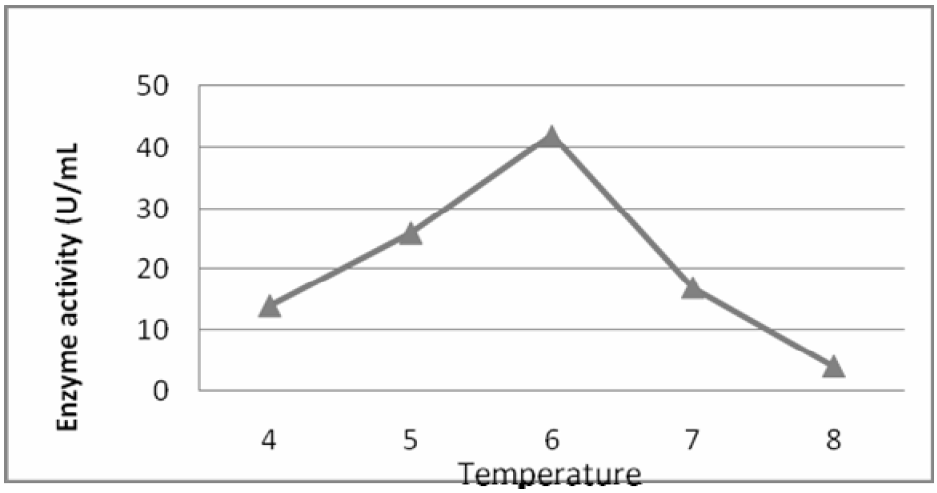
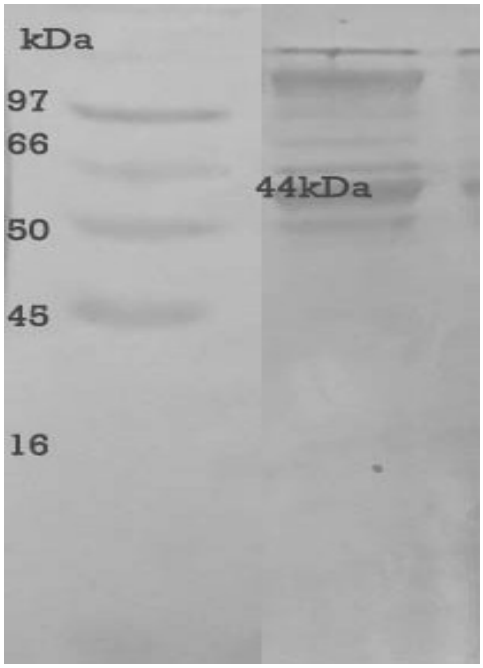


Table.2 Purification of amylase by *Streptomyces* spp. pds1

Purification steps	Total activity	Total protein (mg)	Specific activity	Purification fold	Recovery (%)
Crude extract	1400	0.42	66.7	100	1
Ethanol ppt	340	0.21	161.90	242.73	2.42
Dialysis	210	0.18	233.33	61.76	3.49
DEAE Sephadex	108	0.06	900.0	51.43	3.86

Figure.6 SDS - PAGE result of amylase produced by *Streptomyces* spp. Pds1



Acknowledgement

Corresponding author is thankful to Centre for Bioscience and Nanosciecne Research for their timely help to do part of the work in the institute.

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