Trends in Applied Sciences Research

ISSN 1819-3579

Academic
Journals Inc.

www.academicjournals.com

Trends in Applied Sciences Research 3 (1): 45-52, 2008
ISSN 1819-3579

Optimization of α-amylase Production by Actinomycete Strain AE-19 Isolated from Shrimp Pond

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Abstract: Actinomycetes were isolated from the sediment samples collected from the shrimp pond and examined for their α-amylase activity. Among many strains, the strain AE-19 which was tentatively identified as *Streptomyces aureofasciculus* showed higher α-amylase activity and it was taken for further study. Impact of various physical and chemical factors such as pH, temperatures, sodium chloride concentrations, carbon and nitrogen compounds on the α-amylase activity of *S. aureofasciculus* was studied. It was found that the enzyme activity was maximum at pH 9, temperature 45°C, 0.05% NaCl concentration, carbon compound mannose and nitrogen compound L-histidine. These findings suggest that the strain can effectively be used in large scale production of α-amylase enzyme for commercial purposes, after testing and ascertaining the strain's capability in large scale fermentations.

Key words: Shrimp pond, α-amylase, *Streptomyces aureofasciculus*

INTRODUCTION

Several enzymes are involved in hydrolysis of starch. Among them, α-amylase (endo-1,4, α-D-glucan glucohydrolase) which is an extracellular enzyme, randomly breaks 1,4 α-D-glucosidic linkage in the linear amylose chain (Patel *et al*., 2005). Though α-amylase can be obtained from various sources, this enzyme from microbial sources generally meets the industrial demands. The amylase family enzymes are of great significance due to their wide application potentials. Interestingly, the first enzyme produced industrially was an amylase from a fungal source in 1894, which was used as pharmaceutical aid for the treatment of digestive disorder (Crueger and Crueger, 1989). Amylases find potential applications in a number of industrial processes such as food, fermentation, textiles and paper industries.

Microbial amylases have successfully replaced the chemical hydrolysis of starch in starch processing industries. They would be potentially useful in pharmaceutical and fine chemical industries if enzymes with suitable properties could be prepared (Fogarty and Kelly, 1980). Considering its wide applications, a significant interest exists in α-amylase enzyme research especially for microbial resources. Though major portion of commercially important α-amylase is derived from bacterial sources such as *Bacillus* species, fungal α-amylase is preferred more in human consumption applications. From the available literature, it is found that there are studies on actinomycetes from different marine environs but there is not even a single work on the actinomycetes of shrimp ponds. Hence, the present investigation aims to study the α-amylase activity of actinomycetes isolated from

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the sediments of a shrimp pond under different experimental conditions with pH, temperatures, sodium chloride concentrations, carbon and nitrogen compounds and identify the potential α-amylase producing actinomycetes using chemotaxonomical and conventional methods of identification.

MATERIALS AND METHODS

Isolation of Actinomycetes

The sediment sample was collected from a shrimp pond (Lat. 11° 28' 53.7" N and Long. 79° 45' 31.1" E), located opposite to the Vellar estuary, southeast coast of India, by inserting a sterilized polyvinyl corer (10 cm) into the sediments. The center portion of the 2 cm sediment sample was taken out with the help of a sterile spatula. The collected sample was transferred to a sterile polythene bag and taken immediately to the laboratory. After arrival to the laboratory, the sample was air-dried aseptically for one week. The air-dried sediment sample was incubated at 55°C for 5 min (Sivakumar *et al*., 2005); then, 10-fold serial dilutions of the sediment samples were prepared, using filtered and sterilized 50% seawater. One of the serially diluted samples was plated in the Actinomycetes Isolation Agar medium (Hi-Media, Mumbai) in duplicate petriplates after suitable dilution. To minimize the bacterial and fungal contaminations, all agar plates were supplemented with 20 and 100 mg L^{-1} of nystatin and cycloheximide respectively (Kathiresan *et al*., 2005). The actinomycete colonies that appeared on the petriplates were counted from 5th day onwards, upto 28th day. All the colonies that were growing on the petriplates were separately streaked in petriplates, subcultured, ensured for their axenicity and maintained in slants.

Assay of α-amylase Activity

α-amylase activity was assayed, following Summer's method. One ml of buffered substrate prepared by dissolving 1 g starch in 100 mL of phosphate buffer (pH-6.9) by boiling for 5 min at 80 to 85°C was added to 1 mL of phosphate buffer in a series of tubes and equilibrated for 5 min at 28°C. To all these tubes, 50 µL of tissue enzyme was added and allowed for reaction for 10 min. The reaction was stopped by adding 2 mL of DNSA (3, 5-di-nitro-salicylic acid). The contents were boiled exactly for 3 min. in water bath and cooled for 20-25 min after which, the colour developed was read at 540 nm in a spectrophotometer. α-amylase activity represented as total activity has been expressed as the amount of micromoles of maltose liberated per minute of incubation per gram of sample at 28°C.

Effects of pH, Temperature, Sodium Chloride, Carbon and Nitrogen Compounds on α-Amylase Activity of the Actinomycete

Amylase activities of the actinomycete strain was measured at different pH, temperature, sodium chloride concentration, carbon compounds and nitrogen compounds. The experiments were conducted in 250 mL Erlenmeyer flasks containing the sterilized Amylase Production Medium (APM) (Na_2 HPO_4 6 g, KH_2 PO_4 3 g, NH_4Cl 1 g, NaCl 0.5 g, $CaCl_2$ 0.15 g, $MgSO_4$, $7H_2O$ 0.25 g, Casein hydrolysate 0.20 g, Yeast extract 0.10 g, Starch 20 g, Water 1000 mL) broth at 15 lbs pressure for 15 min. After sterilization of the broth by autoclaving, the flask was cooled and the strain was inoculated and incubated differently for different parameters as described in the following paragraphs by taking one parameter at one time. Appropriate controls were maintained in all the experiments.

Effect of pH

Effect of pH was studied by varying the pH from 4 to 9. After inoculation of the strain, it was incubated for seven days at 45°C.

Effect of Temperature

After the inoculation of the strain in APM broth, it was incubated at various temperatures viz. 35, 40, 45, 50, 55 and 60°C for seven days.

Effect of Sodium Chloride Concentrations

To study the tolerance towards sodium chloride, the APM broth prepared with distilled water was added with sodium chloride of varying concentrations viz., 0.05, 0.5, 1, 2, 3 and 4%. After the inoculation of the strain, it was incubated at 45°C for seven days. Simultaneously, a control was maintained without sodium chloride.

Effect of Various Carbon Compounds

The APM broth was used for studying the effect of various carbon compounds such as arabinose, xylose, inositol, fructose, rhamnose, sucrose, raffinose and mannose. The broth was distributed into different flasks and 1% of each carbon source was then added before inoculation of the strain and incubated for seven days at 45°C.

Effect of Various Nitrogen Compounds

The APM broth was used for studying the influence of different nitrogen sources viz. L-asparagine, L-phenylalanine, L-histidine and L-hydroxyproline. The broth was distributed into various flasks and 0.8 mL of each nitrogen compound was then added and incubated for seven days at 45°C.

At the end of the incubation period, the α-amylase activity was determined by the procedure as described earlier.

Taxonomic Investigation

The genus level identification was made for the strain AE-19 using the cell wall composition analysis and micromorphological studies (Lechevalier and Lechevalier, 1970). Characterization of the strain AE-19 was made by following the methods described by Shirling and Gottlieb (1966) using standard yeast extract-malt extract agar (ISP-2). The species level identification of the strain was based on the keys of Nonomura (1974) and Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

RESULTS AND DISCUSSION

Enzyme Assay

Among 20 strains of actinomycetes, only 8 strains showed α-amylase activity. Out of these 8 strains, one strain AE-19 exhibited maximum enzymatic activity (2.09 μg maltose mL^{-1} h^{-1}) and it was selected for further studies. Umamaheswary *et al*. (2005) isolated 40 strains of *Streptomyces* from the fish, *Mugil cephalus* collected from the Vellar estuary and out of them, only the strain LG-33 showed good L-glutaminase activity. Sivakuamr *et al*. (2006) reported that strain (LG-10) of *Streptomyces* sp. isolated from the fish, *Chanos chanos* showed good L-glutaminse activity. Sahu *et al*. (2007) isolated 40 strains of *Streptomyces* from different parts of three estuarine fishes viz., *M. cephalus*, *C. chanos* and *Etroplus suratensis* for L-asparaginase and out of the 40 strains, only six strains showed significant L-asparaginase activity. From the present findings and also from other studies, it could be inferred that though the marine environs are harbouring a good number of actinomycete strains, only a few of them are capable of to producing novel enzymes which have very good industrial and commercial applications.

Effects of pH, Temperature, Sodium Chloride, Carbon Compounds and Nitrogen Compounds on Amylase Activity

The selected isolate AE-19 was allowed to grow in different pH, temperatures, salinities, carbon sources and nitrogen sources. pH of the medium played an important role in amylase production. The enzymatic activity was optimum at pH 9 (Fig. 1). Hamiton *et al*. (1999) observed the maximum amylase activity at pH 6 while Patel *et al*. (2005) reported maximum amylase activity at pH 7. Whereas, Sodhi *et al*. (2005) found that the amylase activity was higher at pH 6.5 and temperature 60°C. But in the present study, the maximum activity was recorded at pH 9 which indicates that the strain prefers alkaline conditions for better enzyme production. The strain AE-19 showed maximum amylase activity at the temperature of 45°C (Fig. 2) which indicates that the enzyme is not thermostable. Stamford *et al*. (2001) reported 70°C as optimum temperature for the production of amylase enzyme by *Nocardiopsis* sp. Kundu (2006) reported the maximum amylase activity at 50°C while Nipkow *et al*. (1989) observed the maximum enzyme activity at 60°C. This reveals the fact that the optimum conditions for enzyme production differ species to species.

Since the strain was isolated from the shrimp pond, it was felt that is necessary to understand the influence of different sodium chloride concentrations on the enzymatic activity of AE-19. Good activity was recorded at 0.05% sodium chloride concentration (Fig. 3). Saramma *et al*. (1994) reported that the amylase from *Vibrio* sp. required 1-2% sodium chloride concentration for the maximum enzyme activity. Likewise, Kundu (2006) also observed the maximum amylase activity at 1-2% sodium chloride concentration by the actinomycete strain. However, in the present study, the test strain was isolated from the shrimp pond. Thus the isolated strain could not be a true marine form thereby it might not be able to produce higher amount of the enzyme at higher sodium chloride concentrations. This could be ascribed to the fact that the enzyme production starts to decrease with the increasing NaCl concentration.

Among the different carbon sources used, the enzyme activity was maximum in rhamnose and mannose and minimum was recorded in sucrose (Fig. 4). Hamilton *et al*. (1999) tested the amylase activity in various carbon sources and found that the highest amylase activity was shown by the *Bacillus* sp. with lactose as carbon source and the enzyme was not produced when glucose and fructose were used as carbon sources.

Among the different nitrogen sources used, the enzyme activity was maximum in L-histidine and minimum in L-hydroxyproline (Fig. 5). But Patel *et al*. (2005) and Kundu (2006) observed maximum amylase activity in L-Phenylalanine as nitrogen source in *Aspergillus oryzae*, *A. awamori* and *Streptomyces galilaeus*.

Present investigation on the determination of optimal process parameters for the strain AE-19 for its better enzyme activity has yielded important results, indicating that the strain can be used for large scale industrial production of the enzyme. However, these parameters have to be tested in large scale cultures in automated incubators so as to confirm the optimum conditions for enzyme production before industrial application.

Taxonomic Investigation

The strain AE-19 possesses LL-Diaminopmelic (LL-DAP) and the test strain contains glycine in its cell wall. Presence of LL-DAP along with glycine indicates the cell wall chemotype-I. The strain with chemotype-I does not have any characteristic pattern of sugars (Lechevalier and Lechevalier, 1970). The species belonging to the wall type I are *Streptomyces*, *Streptoverticillium*, *Chainia*, *Actinopycnidium*, *Actinosporangium*, *Elyptrosporangium*, *Microellbosporia*, *Sporichthya* and *Intrasporangium* (Lechevalier and Lechevalier, 1970). The micromorphological observations of the strain AE-19 reveal that it belongs to the genus *Streptomyces*. The morphological, physiological and

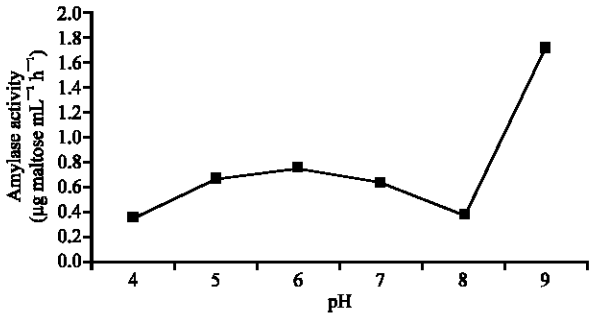

Fig. 1: Effect of pH on α-amylase activity of the strain AE-19

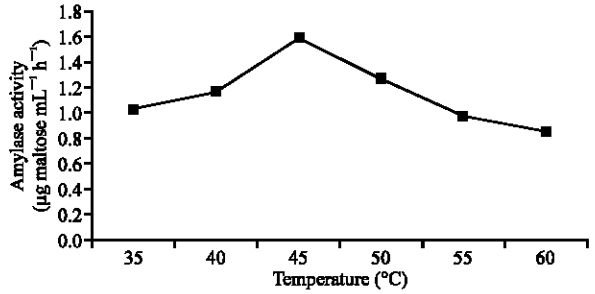

Fig. 2: Effect of temperature on α-amylase activity of the strain AE-19

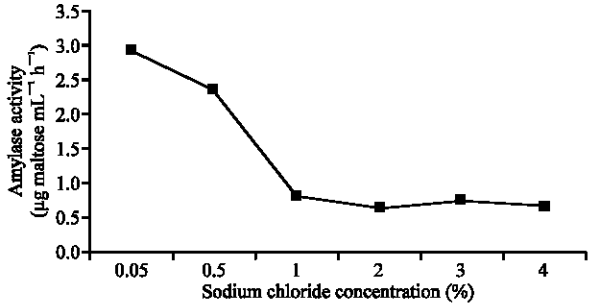

Fig. 3: Effect of sodium chloride concentration on α-amylase activity of the strain AE-19

biochemical characteristics of the amylase producing strain AE-19, tested in the present study, are shown in Table 1. These characteristics were compared with those of the *Streptomyces* species, given I the Key of Nonomura (1974) and the Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). The comparison showed that the present strain differed with the reverse side pigmentation and utilization of carbon source i.e., sucrose. The test strain coagulates the milk whereas the reference strain *S. aureofasciculus* is not coagulating the same. Except these, all the other characters are exactly similar to those of *S. aureofasciculus.* Hence, the strain AE-19 has been tentatively identified as *S. aureofasciculus.*

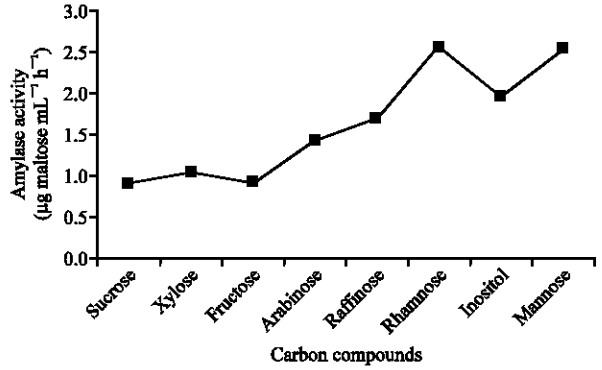

Fig. 4: Effect of carbon compounds on α-amylase activity of the strain AE-19

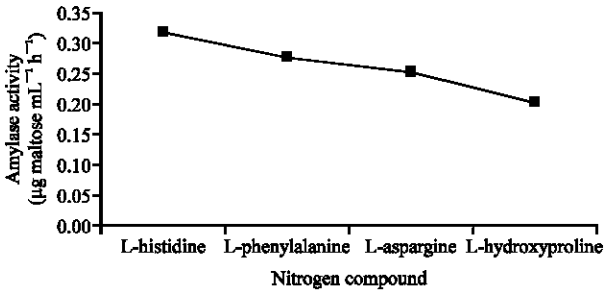

Fig. 5: Effect of nitrogen compounds on α-amylase activity of the strain AE-19

Table 1: Comparison between the strain AE-19 and *Streptomyces aureofasciculus*

Characters studied	Strain AE-19	*Streptomyces aureofasciculus*
Aerial mycelium	White	White
Melanoid pigment	-	+
Reverse side pigment	+	+
Soluble pigment	-	-
Spore chain	Rectiflexibiles	Rectiflexibiles
Utilization of sole carbon sources		
Arabinose	+	+
Xylose	+	+
Inositol	+	+
Manitol	+	+
Fructose	+	+
Rhamnose	+	+
Sucrose	+	-
Raffinose	+	+
Utilization of sole nitrogen sources		
L-asparagine	+	+
L-hydroxyproline	+	+
L-histidine	+	+
L-phenylalanine	+	+
Biochemical properties		
Cellulose degradation	-	-
Hydrogen sulphide production	+	+
Melanin production	-	-
Nitrate reduction	-	-
Starch hydrolysis	+	+
Milk coagulation	+	-

+: Positive; -: Negative

CONCLUSION

From the present study, it is concluded that the sediments of the shrimp pond are good source materials for the isolation of potential actinomycetes. The present study has revealed that the tentatively identified species, *S. aureofasciculus* isolated from the sediments possesses good α-amylase activity. The study has also standardized the growth parameters of the actinomycetes for the maximum enzyme production, which can be effectively used in the large scale production of enzyme for commercial purposes.

ACKNOWLEDGMENTS

Authors thank Prof. T. Balasubramanian, Director, Center of Advanced Study in Marine Biology and the authorities of Annamalai University for providing with necessary facilities. One of the authors (M.K.S.) is thankful to the Ministry of Environment and Forests, Government of India for granting the fellowship.

REFERENCES

Buchanan, R.E. and N.E. Gibbons, 1974. Bergey's Manual of Determinative Bacteriology. 8th Edn., The Williams and Wilkins Co., Baltimore.

Crueger, W. and A. Crueger, 1989. Industrial Microbiology. Sinauer Associates, Sunderland, M.A.

Fogarty, W.M. and C.T. Kelly, 1980. Amylases, amyloglucosidases and related glucanses. J. Econ. Microb. Biocon., 5: 115-170.

Hamilton, L.M., C.T. Kelly and W.M. Fogarty, 1999. Production and properties of the raw starch-digesting α-amylase of *Bacillus* SP.IMD 435. Proc. Biochem., 35: 27-31.

Kathiresan, K., R. Balagurunathan and M. Masilamani Selvam, 2005. Fungicidal activity of marine actinomycetes against phytopathogenic fungi. Indian J. Biotechnol., 4: 271-276.

Kundu, S., 2006. Isolation and identification of actinomycetes from estuarine fishes and optimization of α-amylase enzyme. M.Phil. Thesis, Annamalai University, India, pp: 46.

Lechevalier, M.P. and H. Lechevalier, 1970. Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol., 20: 435-443.

Nipkow, A., G.J. Shen and J.G. Zeikus, 1989. Continuous production of thermostable α-amylase with *Clostridium thermosulfurogenes*. Applied Environ. Microbiol., 55: 689-694.

Nonomura, H., 1974. Key for classification and identification of 458 species of the *Streptomycetes* included in ISP. J. Ferment. Technol., 52: 78-92.

Patel, A.K., K.M. Nampoothiri, S. Ramachandran, G. Szakacs and A. Pandey, 2005. Partial reification and characterization of α-Amylase produced by *Aspergillus oryzae* using spent-brewing grains. Indian J. Biotechnol., 4: 336-341.

Sahu, M.K., K. Sivakumar, E. Poorani, T. Thangaradjou and L. Kannan, 2007. Studies on L-asparaginase enzyme of actinomycetes isolated from estuarine fishes. J. Environ. Biol., 28: 465-474.

Saramma, A.V., B. Philip and P.G.V. Nair, 1994. Amylase Production by *Vibrio* Species. In: Nutrients and Bioactive Substances in Aquatic Organisms. Devadasan, K., M.K. Mukundan, P.D. Antony, P.G.V. Nair, P.A. Perigreen and J. Joseph (Eds.), Society of Fisheries Technologists, India, pp: 31-43.

Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomycetes* species. Int. J. Syst. Bacteriol., 16: 313-340.

Sivakumar, K., M.K. Sahu and K. Kathiresan, 2005. Isolation and characterisations of *Streptomycetes*, producing antibiotic, from a mangrove environment. Asian J. Microbiol. Biotechnol. Environ. Sci., 7: 457-464.

Sivakumar, K., M.K. Sahu, P.R. Manivel and L. Kannan, 2006. Studies on L-glutaminase producing actinomycetes strain LG-10 from the estuarine fish, *Chanos chanos* (Forskal, 1775). Indian J. Exp. Biol., 44: 256-258.

Sodhi, H.K., K. Sharma, J.K. Gupta and S.K. Soni, 2005. Production of a thermostable α-amylase from *Bacillus* sp. PS-7 by solid state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. Proc. Biochem., 40: 525-534.

Stamford, T.L.M., N.P. Stamford, L.C.B.B. Coelho and J.M. Arauja, 2001. Production and characterization of a thermostable α-amylase from *Nocardiapsis sp.* endophyte of yam bean. J. Biores. Technol., 76: 137-141.

Umamaheswary, K., M.K. Sahu, K. Sivakumar, T. Thangaradjou, D. Sumitha and L. Kannan, 2005. Investigations on L-glutaminase producing actinomycetes strain LG-33 from the estuarine fish, *Mugil cephalus* (Linnaeus, 1758). Environ. Econ., 23: 942-947.