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Original Article

COMPARATIVE ANALYSIS OF AMYLASE PRODUCTION BY *PROTEUS SP* FROM DIFFERENT SOIL HABITAT

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ABSTRACT

Objective: The main goal of our study was to isolate the amylase producing bacteria from three different soil samples collected from sewage dumped lake(L), Agricultural field (A), highway (R) and comparative analysis of amylase production between different bacterial source at various incubation period.

Methods: Isolates from three different sources were characterized through various standard biochemical tests and cultured on a production media, from which the crude enzyme was obtained and quantified in terms of maltose liberated per ml of crude enzyme on 15 min of incubation with the soluble starch substrate using DNS method.

Results: On the biochemical characterization the isolates (L, A and R) were identified as *Proteus sp* but their production rates were distinct. The *Proteus sp* from highway (R) had maximum amylase production rate on 12 hrs of incubation, which was quantified to 650µg/ml of maltose/15 min and production rate was decreased on further incubation period. On contrast, the *Proteus sp* obtained from sewage dumped lake soil (L) had minimum amylase production rate on 12 hrs of incubation which was quantified to 200µg/ml of maltose/15 min and production rate was increased within the incubation period. The *Proteus sp* obtained from agricultural soil (A) had a stable amylase production rate at all incubation period includes 12 hrs, 24 hrs and 36 hrs which were quantified to 250µg/ml of maltose/15 min.

Conclusion: Sewage dumped lake soil was an effective source for isolation of amylase producing bacteria than other sources.

Keywords: Amylase, *Proteus sp*, Incubationn period, Maltose.

INTRODUCTION

Enzymes are responsible for thousands of metabolic processes that sustain life [1] There are about 3000 enzymes known today but only few are industrially exploited[2]. These are mainly extracellular hydrolytic enzymes [3], which degrade naturally occurring polymers such as starch, proteins, pectin and cellulose [4]. Amylase is an enzyme that catalyses the hydrolysis of starch into simple sugars.

Starch is a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. It consists of two types of molecules, the linear amylose and the branched amylopectin. Amylases hydrolysis the glycosidic bonds present between the residues, based on the region of cleavage amylases can be classified as:

1. α-Amylases (1, 4- α-glucan- glucanohydrolases) which are extracellular enzymes [5] which hydrolyze α-1, 4-glycosidic bonds ultimately yielding maltotriose and maltose fromamylose, or maltose, glucose and "limit dextrin's" from amylopectin [6].

2. β- Amylases, (α-1, 4-glucan- glucohydrolases) which acts on starch by splitting glucose units from the non reducing end and

3. Another type of amylase found includes, γ-Amylase which cleaves α (1, 6) glycosidic linkages, as well as the last α (1, 4) glycosidic linkages at the non reducing end of amylase and amylopectin, yielding glucose.

Microbial amylases have successfully replaced chemical hydrolysis in starch processing industries. The major advantages of using microorganisms for the production of amylases are the economical bulk production capacity and microbes are easy to manipulate to obtain enzymes of desired characteristics [7-8].

Microorganisms used in α-Amylases and β-amylases production includes*Bacillus subtilis, B. cereus, B. polmyxa, B. amyloliquefaciens, B. coagulans, B. subtilis, Lactobacillus, Escherichia, Proteus sp, B. lincheniformis,Bacillus steriothermophilu, Bacillus megaterium, Strepotmyces sp., Pseudomonas sp* [9].

Amylases are the most important enzymes and are of great significance for biotechnology, thus constituting a major class of industrial enzymes having approximately 25-30% of the world enzyme market [10]. Although the details of the specific fermentation processes adopted by different manufacturers vary, there are two main methods for amylase production, submerged fermentation and solid–state fermentation [1]. At industrial scale, most of the enzymes are manufactured by submerged fermentation (SmF) techniques. The production of microbial α-amylase by bacteria dependents on the type of strain, composition of medium, methods of cultivation, cell growth, nutrient requirement, metal ions, pH, temperature and time of incubation, thermo stability and the control of contamination during fermentation [8]. *Proteus is* a genus of gram negative bacilli, widely distributed in nature as saprophytes, being found in decomposing animal matter, in sewage, in manure soil, and human and animal feces. In this present study, we have isolated the *Proteus sp* from different soil habitat and comparatively quantified the rate of enzyme production at different incubation period.

MATERIALS AND METHODS

Collection of samples

Soil samples were collected from agricultural field (Guduvancherry). Sewage dumped lake (Potheri) and highway road (Potheri) around Kancheepuram district.

Isolation of bacterial strains from soil samples

The samples were diluted using serial dilution technique, volume of about 0.1 ml from 10^{-7}, 10^{-8} and 10^{-9} dilutions of each soil samples were transferred aseptically onto starch agar plates using spread plate technique, which were marked as "L", "A" and "R" respectively and then the plates were incubated at 37°C for 24 hrs [11].

Screening of amylase producing colonies

The amylase producing colonies were screened using starch iodine test [12]. The isolates from each sample showing maximum zone of clearance and similar morphology were selected and utilized for the

further study [13]. Pure culture of each isolate were obtained through repeated sub-culturing and named as "L", "A" and "R" respectively.

Biochemical characterization

The isolates were identified by conducting various biochemical tests includes Gram staining, Methyl red test, VP test, Indole test, TSI test, Citrate test, Urease test, Gelatin test, Catalase test, Maltose test, Sucrose test and the results were analyzed through Bergey's manual of microbiology [14-15].

Amylase production

The isolates were made to produce amylase by aseptically transferring 1 ml of culture into a complex production media (starch – 1g/100 ml, yeast extract- 0.04 g/100 ml, diammonium hydrogen phosphate – 0.4 g/100 ml, potassium chloride – 0.1 g/100 ml and magnesium sulphatehepta hydrate – 0.05 g/100 ml) marked accordingly and incubated at 37°C in a shaking incubator.

Isolation of crude enzyme and quantification

The crude enzyme from each isolates were obtained at various incubation periods which include 12 hrs, 24 hrs and 36 hrs by centrifuging 10 ml of culture at 5000rpm for 20 min at 4°C [4]. It was quantified by the colorimetric method as described by Fisher and Stein. According to the procedure 1.0 ml of crude enzyme was taken in the test tube marked accordingly and 1.0 ml of substrate (soluble starch) was added into each test tubes. Then, the tubes were covered and incubated at 35°C for 15 minutes in water bath. Then 2.0 ml DNS reagents was added into each tube to stop the reaction and kept in boiling water bath for 5 minutes. After cooling to room temperature, the absorbance was read at 590 nm by colorimeter against the blank in which the reaction had been stopped without incubation (i. e) the 0 time blank. The amylase rate was quantified by inter plotting the standard graph of maltose and the results were expressed in terms of maltose liberated per ml of crude enzyme on 15 min of incubation with soluble starch substrate.

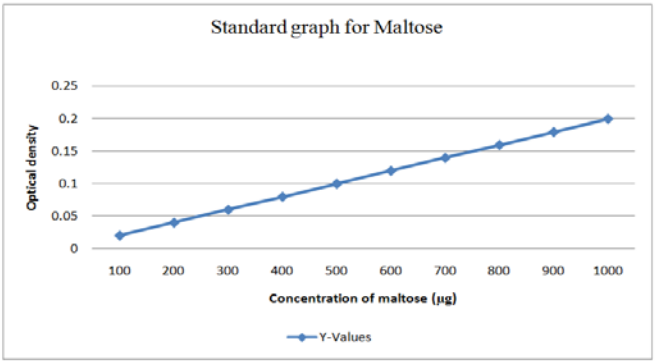

Fig. A: Standard graph for Maltose, which contains concentration of maltose on X-axis in terms of µg and optical density on Y-axis

RESULTS

Isolation of bacterial colonies producing amylase

The serially diluted samples were screened by inoculating 0.1 ml of the dilutions 10^{-7}, 10^{-8} and 10^{-9}on to the starch agar plates. Among twelve plates incubated at 37°C the microbial colonies were found in all the plates, as the dilution rate increases the number colonies present decreases.

The plates of dilutions 10^{-9} of "L", "A" and "R" respectively were screened for amylase production using starch-iodine test.

On the basis of the area of clearance, the strain showing maximum zone of clearance and with similar morphology was selected and utilized for the further study.

Colony morphology

The colonies isolated from the plates"L", "A" and "R" were small, round, creamy yellow without pigmentation and fast growing.

Biochemical characterization

Comparative analysis

Through the morphological and biochemical characterization the isolates "L", "A" and "R" were identified as *Proteus sp* based on Bergey's manual of microbiology. On analysis of the crude enzyme obtained from production media, the *Proteus sp* from highway soil (R) had maximum production rate on 12 hrs of incubation, which was quantified to 650µg/ml of maltose/15mis and its production was decreased on further incubation period as 500µg/ml of maltose/15mis on 24 hrs and 400µg/ml of maltose/15mis on 36 hrs. On contrast, the *Proteus sp* obtained from sewage dumped lake soil (L) had minimum amylase production rate on 12 hrs of incubation, which was quantified to 200µg/ml of maltose/15 min and its production rate was increased with the incubation period as 400µg/ml of maltose/15mis on 24 hrs and 700µg/ml of maltose/15mis on 36 hrs. The *Proteus sp* obtained from agricultural soil (A) had a stable amylase production rate on 12 hrs, 24 hrs and 36 hrs of incubation, which was quantified to 250µg /ml of maltose/15 mins.

Table 1: Biochemical characterization of "L" "A" and "R"

S. No.	Biochemical tests	Results		
		L	A	R
1.	Gram staining	–	–	–
2.	Methyl RED test	+	+	+
3.	Indole test	+	+	+
4.	Voges proskauer test	–	–	–
5.	Citrate utilization test	–	–	–
6.	Triple sugar iron test	+	+	+
7.	Urease test	+	+	+
8.	Gelatin test	–	–	+
9.	Catalase test	+	+	+
10.	Sucrose	+	+	–
11.	Maltose	+	+	+

Note: "+" indicates positive result and "–" indicates negative result

Table 2: Highway road (R) sample, absorbance values from DNS methods and their corresponding concentration of maltose liberated which is obtained by inter plotting the standard graph

Time	Abs_{590}	Conc. Of maltose (µg/ml)
12 hrs	0.13	650
24 hrs	0.10	500
36 hrs	0.08	400

Table 3: Sewage dumped lake soil (L) sample, absorbance values from DNS methods and their corresponding concentration of maltose liberated which is obtained by inter plotting the standard graph.

Time	Abs_{590}	Conc. Of maltose (µg/ml)
12 hrs	0.04	200
24 hrs	0.08	400
36 hrs	0.14	700

Table 4: Agricultural soil (A) sample, absorbance values from DNS methods and their corresponding concentration of maltose liberated which is obtained by inter-plotting the standard graph.

Time	Abs_{590}	Conc. Of maltose (µg/ml)
12 hrs	0.05	250
24 hrs	0.05	250
36 hrs	0.05	250

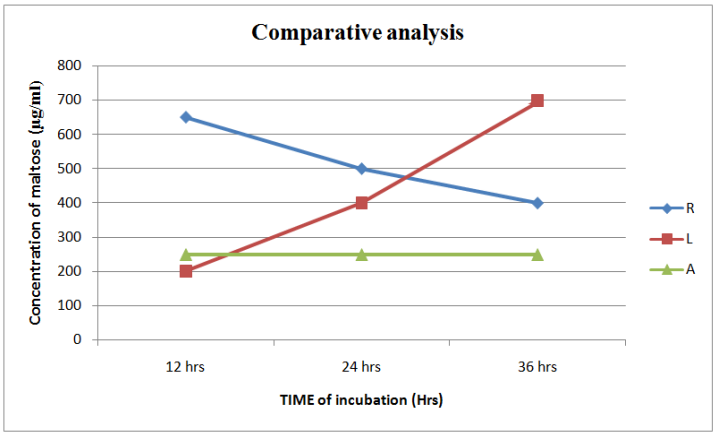

Fig. B: Comparative analysis of amylase production on different incubation period, blue line-amylase production rate by *Proteus sp* from highway road soil (R), red line- amylase production rate by *Proteus sp* from sewage dumped lake soil and green line- amylase production rate by *Proteus sp* from agricultural soil

DISCUSSION

Proteus sp was isolated from different soil habitat. In the first step of isolation process the sewage dumped lake soil had a large number of bacterial colonies when compared with agricultural and highway road soil. The number of colonies in highway road was comparatively low, perhaps environmental pollution and dryness of the soil. The amylase producing strains were more in the sewage dumped lake soil, it was a competent source for isolating amylase producing bacterial strains.

The difference in the enzyme production may be due to its difference in its habitat or it may be due to its difference in strains of *Proteus sp*. The strains can be identified by advanced techniques like *ribotyping* technique in which the DNA sequence encodes for the rRNA of the strains are isolated, sequence and checked for its similarity in standard databases like NCBI. Purification of the enzyme and its physical characterization at different temperature and pH to be further analyzed.

CONCLUSION

Felicitous incubation period for the strain from sewage dumped lake soil was 36 hrs and 12 hrs for the strain from highway road soil. Sewage dumped lake soil was an adequate source for isolation of amylase producing bacteria than other sources.

CONFLICT OF INTERESTS

Declared None

REFERENCE

1. Shipra Das, Surendra Singh, Vinni Sharma, Manohar Lal Soni. Biotechnological applications of industrially important amylase enzyme. Int J Pharm Bio Sci 2011;2:1.
2. Shivaji Bole, Anindita Maji, Ankita Dey, Ashutosh Acharya, Siddharth Dubey, Rakeshlal. Isolation, purification and characterization of amylase from airborne-bacteria. World J Pharm Pharm Sci 2013;2:5878-87.
3. Bhaskara Rao KV, Ashwini K, Gaurav Kumar, Karthik L. Optimization, production and partial purification of extracellular α-amylase from *Bacillus sp*. Appl Sci 2011;3(1):33-4.
4. Shyam Sunder Alariya, Sonia Sethi, Sakhsam Gupta, B Lal Gupta. Amylase activity of a starch degrading bacteria isolated from soil. Appl Sci 2013;5(1):15-24.
5. Swetha Sivaramakrishnan, Dhanya Gangadharan, Kesavan Madhavan Nampoothiri, Carlos Ricardo Soccol, Ashok Pandey.

α-Amylases from microbial sources–an overview on recent developments. Food Technol Biotechnol 2006;44(2):173–84.

6. Pranav Kumar, Usha Mina. Life sciences funadamentals and practice. Pathfinder Publication; 2013. p. 84-106.
7. Rani Gupta, Paresh Gigras, Harapriya Mohapatra, Vineet Kumar Goswami, Bhavna Chauhan. Microbial α-Amylases: A Biotechnological Perspective. Process Biochemistry; 2003. p. 1-18.
8. R Vidyalakshmi, R Paranthamannd, J Indhumathi. Amylase production on submerged fermentation by Bacillus sp. World J Chem 2009;4(1):89-91.
9. Rwarinda U Angelo, S Rangabhashiyam. Amylase production from bacteria isolated in the soil receiving kitchen wastes. Int J Pharm Chem Sci 2013;2:1.
10. Promita Deb, Saimon Ahmad Talukdar, Kaniz Mohsina, Palash Kumar Sarker, Sm Abu Sayem. Production and partial characterization of extracellular amylase enzyme from bacillus amyloliquefacien. Springerplus 2013;2:154.
11. Bharat Pokhrel, Priyesh Wanjare, Suman Singh, Purushotham B, Kumara Swamy M. Isolation, screening and characterization of promising α-amylase producing bacteria from sewage enriched soil. Int J Adv Biotechnol Res 2013;4(2):286-90.
12. Kunal Madhav, Satish Verma, Ritu Tanta. Isolation of amylase producing bacillus species, from soil sample of different regions in Dehradun and to check the effect of ph and temperature on their amylase activity. J Pharm Biomed Sci 2011;12.
13. Arvinder Kaur, Manjeet Kaur, Manohar Lal Samyal, Zabeer Ahmed. Isolation, characterization and identification of bacterial strain producing amylase. J Microbiol Biotech 2012;2(4):573-9.
14. Musliu Abdulkadirand Salawudeen Waliyu. Screening and isolation of the soil bacteria for ability to produce antibiotics. Eur J Appl Sci 2012;4(5):211-5.
15. Sasmita Mishra, Niranjan Behera. Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. Afr J Biotechnol 2008;7(18):3326-33.