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Amylase Production & Purification from Bacteria Isolated from a Waste Potato Dumpsite in District Farrukhabad U.P State India

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ABSTRACT

Starch is an abundant carbon source in nature, and a-amylase (1, 4-a-D-glucanohydrolase; EC 3.2.1.1), which hydrolyzes a-1, 4-glucosidic linkage in starch-related molecules, is one of several enzymes involved in starch degradation. Alpha amylase is a hydrolytic enzyme and in recent years, interest in its microbial production has increased dramatically due to its wide spread use in food, textile, baking and detergent industries. The soil samples were obtained (10gm) from Farrukhabad district, U. P. state, India. The samples were analyzed for bacteriological study. One gram of (1.0 gm) soil sample was inoculated in to a liquid soluble starch medium generated reducing sugar with a minimum concentration of 1.35 mg/ml after 48 hours of incubation. The soluble starch amylases were characterized and it was revealed that optimum temperature of activity was 40^0C. The optimum pH activity was observed between 7.5-8.5. The optimum substrate concentration for bacterial growth was 0.4% w/v with activity of 10.5IU/ml. The most effective precipitation of enzyme took place at 60% w/v salt concentration. Bacillus subtilis was found to be most frequently occurring amylolytic bacteria followed by Bacillus cerus and Bacillus megaterium. The mean zone of amylolytic activity for the isolates ranged between 1.8 mm for Bacillus subtilis and 0.9 mm for Bacillus cereus. The activity of the purified enzyme was 17 IU/ml. The purpose of the current investigation was isolation, production and purification of Bacillus species isolated from soil in order to study their suitability with regard to α-amylase production.

Key words: Amylase production from a waste potato, Gram positive Rod shaped, Bacillus species, Amylolytic activity, Zone diameters in millimeters.

INTRODUCTION

Amylases are hydrolyzing enzyme in function which causes hydrolysis of molecules. In biotechnology amylases are of the most important enzymes used [1]. The main use of enzymes includes hydrolysis of starch to yield glucose syrup, amylase-rich flour and in the formation of

dextrin during baking in food industries. Furthermore, in the textile industry, amylases are used for removal of starch sizing and as additives in detergents [2] However, the cost of producing this enzyme is high and the cost of procurement by developing countries can be even higher as a result of importation. Cheap and readily available agricultural waste such as Potato peels, which presently constitutes a menace to solid waste management, may be a rich source of amylolytic bacteria [3] . Therefore, the objectives of the study were to isolate, identify & purify amylolytic bacteria from potato waste dumpsites, and to perform partial characterization of the enzyme production and its properties with regard to the effect of substrate, temperature and pH.

Table No. 1 Amylase activity of *Bacillus* isolates

Isolate Codes	Probable Identity	Zones of amylase activity
R1	*Bacillus subtilis*	2.3
R2	*Bacillus cereus*	2.4
R3	*Bacillus subtilis*	1.8
R4	*Bacillus subtilis*	1.3
R5	*Bacillus cereus*	0.3
R6	*Bacillus subtilis*	2.8
R7	*Bacillus megaterium*	1
R8	*Bacillus megaterium*	2.3
R9	*Bacillus subtilis*	1.3

MATERIALS AND METHODS

Bacteria were isolated from the soil sample collected from potato waste dump site by the method described by [4]. Amylolytic activities of the Bacillus isolates were determined.[5] The widest diameter displaying isolate was selected for further study. The isolate showing maximum diameter of enzyme activity was propagated in broth supplemented with 1% (w/v) starch medium at incubator shaker at 150 rpm, 37^0c for 24hrs. After incubation time resultant Broth was centrifuged at 10000, rpm for 10 min. and the supernatant was collected as the source of crude enzyme. The assay was carried out by using soluble starch as substrate few drops of DNS 3-5, Dinitro salicylic Acid reagent were added and the absorbance was measured at wavelength of 540 nm. Enzyme activity was defined the micro mole of product (maltose) released by 1ml of enzyme extract in 1 minute. Optimal temperature and thermo stability was measured by above described method temperature ranging from 10^0C-70^0C. Optimum pH of enzyme was determined by using 50mM tris-HCl Buffer pH-8**.** pH stability was determined by incubating the enzyme in a water bath at 40^0C and the activity was then measured as described above. Purification of enzyme was carried out by precipitating the enzymes present in the crude sample. A range of Ammonium Sulphate $(NH_4)_2SO_4$ salt was used from 30%-85% to precipitate the enzyme. DNS assay determines the best concentration for precipitation of amylase enzyme. The salt concentrations where we find precipitation of enzyme were pooled out and the others were discarded. Thus we obtain fairly purified amylase enzyme. Now finally ion exchange chromatography is performed to purify the amylase enzyme. Bed for the ion exchange chromatography is made by glass wool & DEAE is used as the exchanger. An elution profile is created by using tris-HCl buffer pH-8 of different concentrations ranging from 50mM-1000mM. A DNS assay is carried out of all the elutes and the fractions showing positive readings were again pooled out and further DNS assay was carried out. Thus we obtained the activity of the purified enzyme.

RESULTS AND DISCUSSION

Nine strains of *Bacillus* species were isolated and identified from the Waste potato dumpsites. The morphological and biochemical characterization revealed the presence of *Bacillus subtilis* (R1, R3, R4, R6 and R9), *Bacillus* cereus (R2, and R5), and *Bacillus megaterium* (R7, and R8). All the *Bacillus* isolates were Gram-positive, rod-shaped, spore formers and hydrolyzers of starch. The amylolytic activity of *Bacillus* isolates according to their halos is presented in Table No. 1. *Bacillus subtilis* (R6) had the highest halo (2.8 mm). Similar observations were made by [4] from amylolytic haloes produced by different Bacillus sps. isolated from starchy soil. Thus, the strain R6 identified as *Bacillus subtilis*, which showed the highest amylolytic halo, was selected for further analysis. The isolated *B. subtilis* had the highest frequency (55.5%), while *B. cereus* had the lowest frequency (22.2%). In this study, the activity of amylase produced increased from 0 to 48 h with a reducing sugar content of 1.35 mg/ml (Figure 2). The temperature stability result of amylase obtained from *Bacillus subtilis* (R 6) is shown in (Figure 3). The figure revealed that the enzyme remained stable at 20 and 50°C. The enzyme stability declined at temperatures above 50°C. The maximum activity was displayed at 40°C with a enzymatic activity 9.5 IU/ml The enzyme stability trend, as reported in the present study, agrees with the behavior of amylases from *Bacillus* spp.[6] The optimum pH were 7.5 and 8.5 with activity of 9.5 and 9.0 IU/ml, respectively (Figure 2). The maximum activities at a pH range of 4.8 and 9.2 for an amylase obtained for *B. licheniformis* isolated from a cassava processing waste [7]. The bacterial growth increased with increasing the substrate concentration and was maximum at 0.4% w/v with activity 10.5 IU/ml, on further increasing the substrate concentration the growth declined (Figure 4). Ammonium Sulphate was used to preciptitate amylase enzyme for purification purpose and the best salt concentration was 60% w/v. At other salt concentrations significant precipitation was not found. On performing the Ion Exchange Chromatography an elution profile is created (Table No.3). All the elutes showing significant readings were pooled out and the activity of the purified enzyme came out to be 17IU/ml.

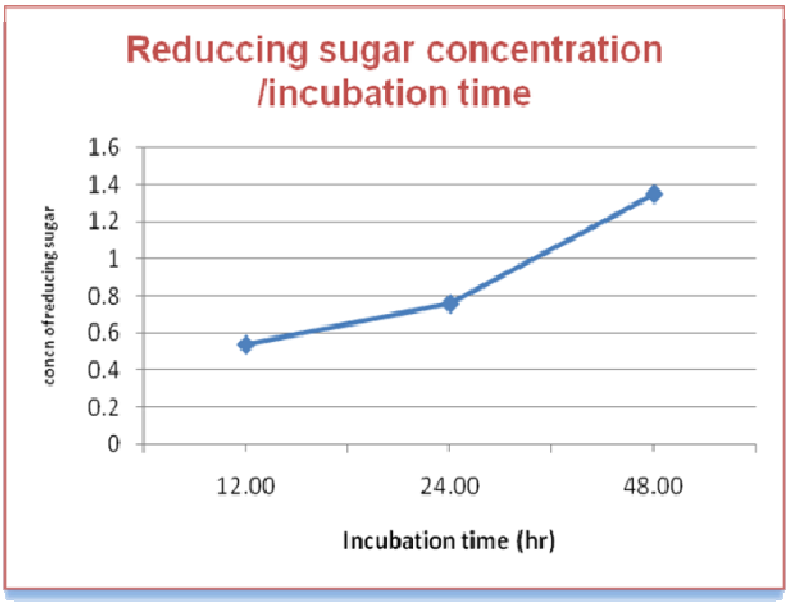

Figure 1 Changes in reducing sugar content

Table No.2 Amylase activity of Bacillus isolates

Bacterial isolate	Frequency (%)
Bacillus subtilis	55.5
Bacillus cereus	22.2
Bacillus megaterium	33.3

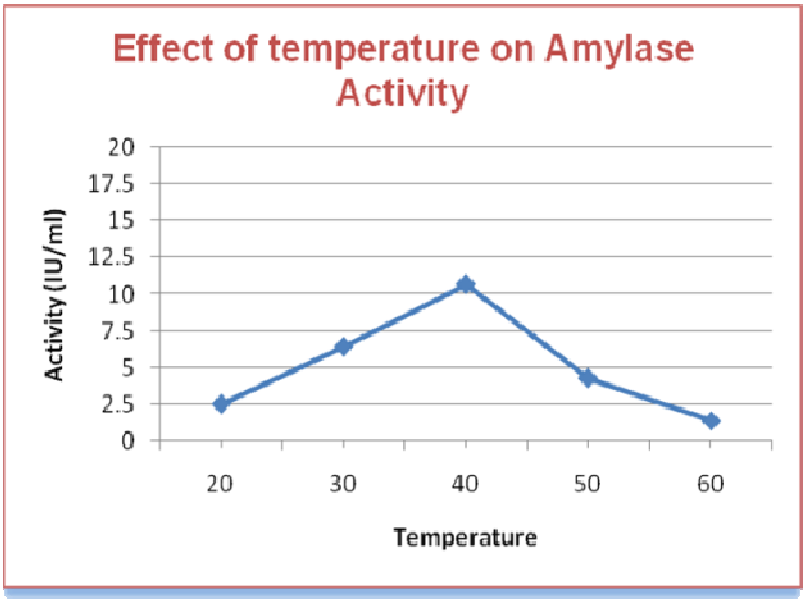

Figure 2 Effect of temperature on Amylase Activity

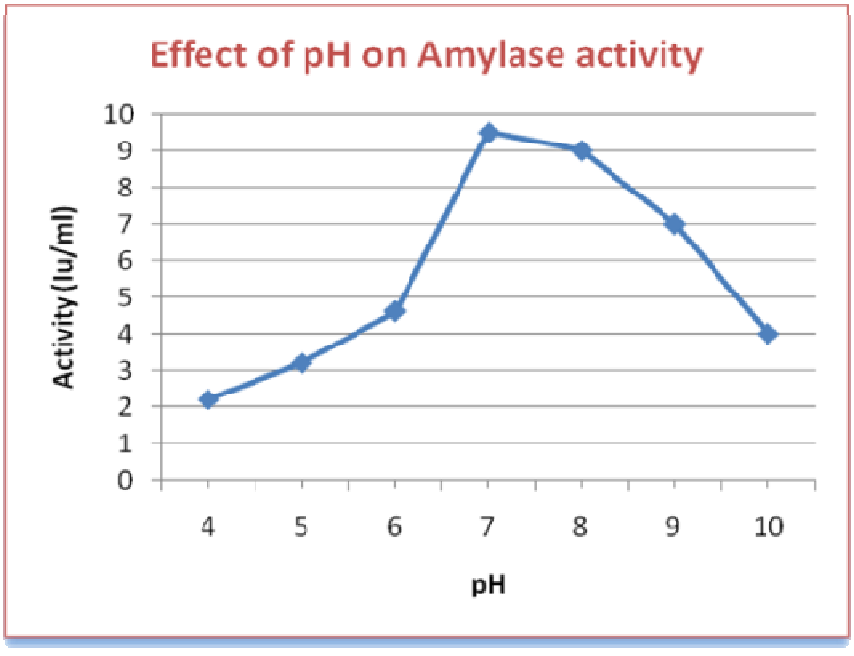

Figure 3 Effect of pH on Amylase Activity

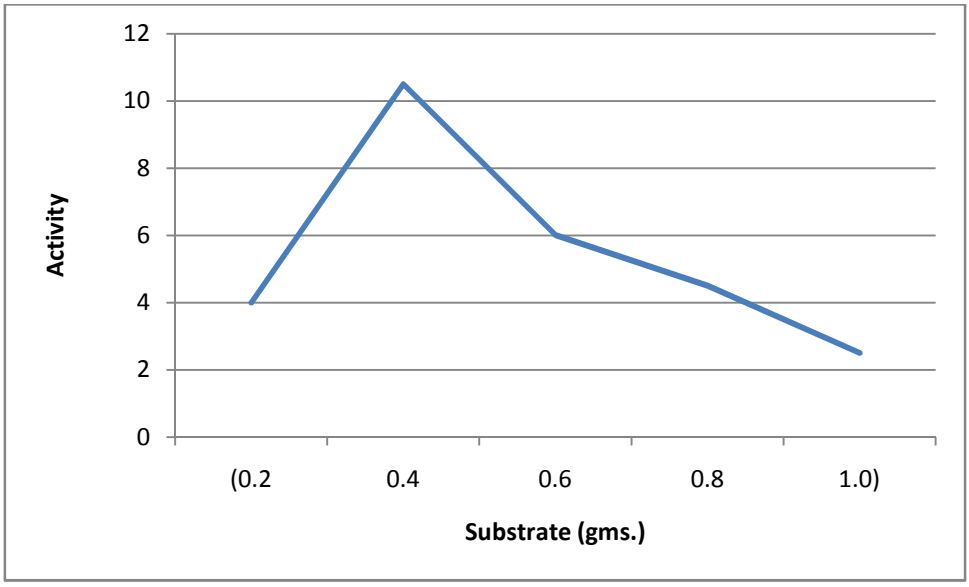

Figure 4 Effect of substrate concentration on bacterial growth.

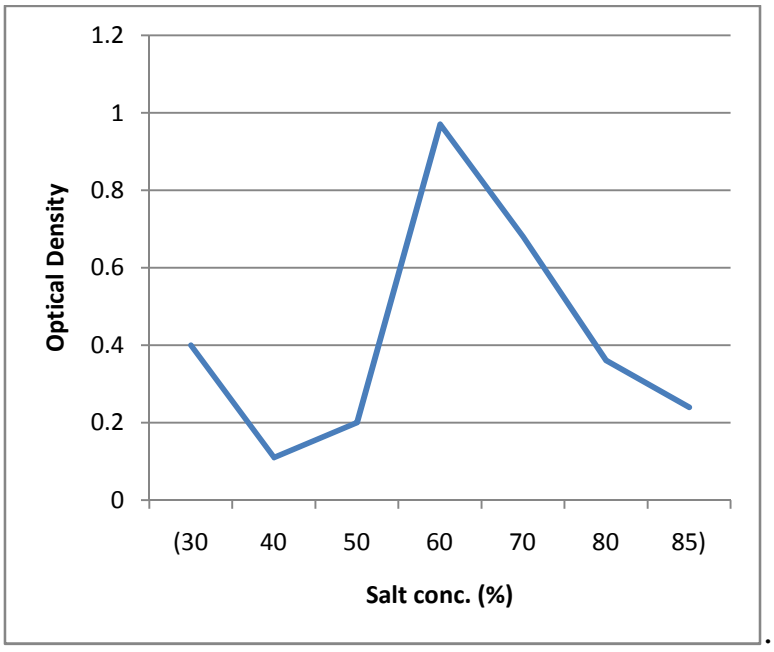

Figure 5 Effect of salt concentration on enzyme precipitation

Table No.3 Elution profile created after ion exchange chromatography

Buffer conc.	Fraction no.	DNS ASSAY O.D (540nm)
unbound	1	0.03
washed	2	-0.01
	3	-0.0
	4	0.01
25mM	5	0.04
	6	0.05
	7	0.05
50Mm	8	0.03
	9	0.04
	10	0.01
75mM	11	0.06
	12	0.02
	13	0.05
100mM	14	0.05
	15	0.05
	16	0.04
200mM	17	0.04
	18	0.01
	19	0.04
300mM	20	0.02
	21	0.02
	22	0.01
400mM	23	0.09
	24	0.02
	25	0.04
500mM	26	0.02
	27	0.06
	28	0.0
600mM	29	0.02
	30	-0.0
	31	0.03
700mM	32	0.0
	33	0.03
	34	0.01
800mM	35	0.01
	36	0.0
	37	0.02
900mM	38	0.0
	39	0.03
	40	0.0
1000mM	41	0.01
	42	0.0
	43	0.0

CONCLUSION

The soluble starch amylases were isolated, characterized & purified. It was revealed that optimum temperature of activity was 40^0C. The optimum pH activity was observed between 7.5-8.5. Optimum time for production of bacterial population was 48hrs. Optimum substrate concectration of bacterial growth was 0.4%w/v. Finally the activity of the purified enzyme was found to be 17 IU/ml. *Bacillus subtilis* was found to be most frequently occurring amylolytic bacteria followed by *Bacillus cerus* and *Bacillus megaterium*. The mean zone of amylolytic activity for the isolates ranged between 1.8 mm for *Bacillus subtilis* and 0.9 mm for *Bacillus cerus.*

This study revealed that potato waste harbours amylolytic *Bacillus* species and that the amylase produced by these bacteria may in future, be used to treat this agricultural waste material.

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