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PRODUCTION OF AMYLASE ENZYME BY ISOLATED MICROORGANISMS AND IT'S APPLICATION

Akansha Karnwal[1], Varsha Nigam[2]

[1] Quality Control Microbiologist, Tidal Laboratories Pvt.Ltd., Dist. Bilaspur (Himachal Pradesh) INDIA

[2] Deptt of Botany,Guru Nanak Khalsa College,Yamuna Nagar -13500(Haryana) INDIA

*Corresponding Author Email: akanshakarnwal28@gmail.com

ABSTRACT

The present work comprises the amylase enzyme production by isolated amylase producing microorganism and its industrial application. To isolate amylase producing strain, three samples were collected from different sources, soil sample, rotted potato and spoiled food waste. All isolated colonies were screened by activity zone techniques with iodine solution. Out of 12 bacterial strains, only 5 bacterial colonies showed positive results for amylase production. Amylase enzyme activity of bacterial strain-2 was found maximum (1.48IU/ml) at pH 6 and the activity decreased with increase in incubation period. Amylase enzyme used for starch degradation studies from bacterial strain 2 showed better results than commercial enzyme at same dose at pH 6.5 at 50^{0}C. Enzyme treated substrate (ararote) showed better properties when compared to control ararote.

KEY WORDS

Amylase, Microorganism, starch degradation.

INTRODUCTION

Amylase is an enzyme that breaks starch down into sugar. Amylase is present in human saliva, where it begins the chemical process of digestion. Foods that contain much starch but little sugar, such as rice and potato, taste slightly sweet as they are chewed because amylase turns some of their starch into sugar in the mouth. Plants and some bacteria also produce amylase. As diastase, amylase was the first enzyme to be discovered and isolated (by Anselme Payen in 1833)[1]. All amylases are glycoside hydrolases and act on α-1,4-glycosidic bonds. Amylases are significant enzymes for their specific use in the industrial starch conversion process. Amylolytic enzymes act on starch and related oligo- and polysaccharides. In the food industry amylolytic enzymes have a large scale of applications, such as the production of glucose syrups, high fructose corn syrups, maltose syrup, reduction of viscosity of sugar syrups, reduction of turbidity to produce clarified fruit juice for longer shelf-life, solubilisation and saccharification of starch in the brewing industry. The baking industry uses amylases to delay the staling of bread and other baked products; the paper industry uses amylases for the reduction of starch viscosity to achieve the appropriate coating of paper. Amylase enzyme is used in the textile industry for warp sizing of textile fibers, and used as a digestive aid in the pharmaceutical industry.

Amylases are a group of important enzymes which are mainly employed in the starch processing industries for the hydrolysis of polysaccharides like starch into simple sugars (Akpan et al., 1999; Mitchell and Lonsane, 1990; Damien et al., 2010). Amylases accounts for about 30% of the world's enzyme production (Vander et al., 2002; Rita et al., 2009).

Amylase enzyme for biofilm removal and degradation of extracellular polymeric substances (EPS) produced by Pseudomonas fluorescens bacteria.(Phyllis Molobela,T. Eugene Cloeteand Mervyn Beukes, 2010). The amylase of Bacillus amyloliquefaciens was the first liquefying α-amylase used on a large scale. Later, a more heat stable enzyme from Bacillus licheniformis was introduced commercially (Madsen et al., 1973).

Fungi belonging to the genus Aspergillus have been most commonly employed for the production of a-amylase. Production of enzymes by solid-state fermentation (SSF) using these moulds turned a cost-effective production technique. (S.Shivaramakrishnan, 2006) Among the various types of amylases produced, commercially thermostable amylases are gaining much more advantages in comparison to other types (Popovic et al., 2009).

Other microorganisms producing appreciable amount of different amount of amylase enzyme are Escherichia sp. Micrococcus sp. Pseudomonas spp. Proteus sp., Serratia sp. Candida, Cephalosporium, Mucor, Penicillium Neurospora e.t.c

In recent years the potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms (Akpan et al., 1999; Pandey et al., 2000; Abu et al., 2005). Amylases are important enzymes employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents (Akpan et al., 1999; Mitchell and Lonsane, 1990).

MATERIAL AND METHODS

For the production of amylase from microbial sources, samples from different habitats like soil sample, rotted potato and spoiled food waste were collected and used for isolation studies. Soil and rotted potato were collected from CPPRI Campus, Saharanpur and spoiled food waste from city canteen CPPI, Saharanpur. The culture media used Nutrient Agar Medium (NAM), Isolation Medium-I, Isolation Medium- II, Starch agar medium, Nutrient broth, Media- A. Growth measurement was analyzed by using two methods, spectrophotometric method and oven dry method. Isolation of amylase producing bacteria from different source was done by serial dilution method 1gm sample was added in 9 ml of sterile distilled water and homogeneous suspension was obtained, aseptically transferred 1ml sample of above dilution into 9ml sterile distilled water blanks and shake well to obtain 10^{-5} and 10^{-6} dilution. 1 ml of each dilution was pipette out and spread into the isolating media (Nutrient agar for bacteria only). After this the inoculated plates were incubated at 34^0C for 5-6 days.

Purification & Screening of isolated strains for amylase enzyme is done. During primary and secondary screening iodine solution, Nutrient Agar Medium, Starch Agar Medium were used. Amylase producing microorganisms were primarily screened by activity zone technique with iodine solution.

The pure cultures were inoculated at the centre of the sterile media poured plates and were incubated at 37^0C for 24 hrs for bacterial strains. After incubation, 1% iodine solution was over layered on the agar plates and the observation was made to note the substrate utilized zone around the colony.

The isolated pure strains were screened for the production of extra cellular amylase production using starch agar medium.

Amylase activity was assayed by measuring the reducing sugar formed by the enzymatic hydrolysis of soluble starch. The reaction mixture containing 1 ml of1% (w/v) soluble starch in citrate phosphate buffer (pH 6.5) and 1 ml culture extract enzyme was incubated at 40^0 C for 30 min. The reaction was stopped by addition of 2 ml of dinitrosalicylic acid (DNS) reagents. The reaction mixture was heated for 5 min in boiling water bath and absorbance was read at 540 nm to estimate reducing sugars released. The activity of amylase enzyme was determined as IU/ ml Enzyme activity was calculated from the amount of reduced sugar produced in 30 minutes, by following formula-

$$\text{Enzyme activity IU/ML} = \frac{\text{Amount of reducing sugar} \times 1000 \times \text{dilution factor}}{\text{Molecular weight of glucose} \times \text{time} \times \text{enzyme volume}}$$

RESULTS AND DISCUSSION

In the present study results of screening showed positive results which are indicated by hydrolytic zone (**Fig 1**), and revealed that out of 12 cultures, 5 cultures (strain-1, strain-2, strain-3, strain-4 and strain-5) showed positive results (**Table 1**), these 5 strains selected on the basis of maximum hydrolytic zone from screening, were further studied for temperature, pH like parameters which effect on amylase production.

Fig1: Bacterial culture showing hydrolytic zone.

Table 1: Screening of amylase producing microorganism.

Media	Sample	Dilution Factor	Plates	Colonies showing starch hydrolytic zone	Strain name
Media 1	Soil	10^{-5}	I	+1 (Bacteria)	Strain-1
			II	-	
			I	-	-
		10^{-6}	II	-	
	Spoiled Food Waste	10^{-5}	I	+1 (Bacteria)	Strain-2
			II	-	
			I	-	-
		10^{-6}	II	-	
	Rotted Potatoes	10^{-5}	I	+1 (Bacteria)	Strain-3
			II	+1 (Bacteria)	Strain-4
			I	+1 (Bacteria)	Strain-5
		10^{-6}	II	-	

Results of temperature effect on the growth of selected bacterial strain-1 is 1.48 at 32^0c after incubation of 72 hrs (**Table 2**), bacterial strain-2 showed maximum growth 1.46 at 37^0c after 72 hrs incubation period (**Fig 2**). Results of bacterial strain- 3 showed 1.54 at 34^0c after 72 hrs incubation period (**Table 3**) and bacterial strain-4 showed maximum growth at 1.63 at 37^0c after 72 hrs incubation period (**Fig 3**).

Table 2: Effect of Temperature on growth of selected bacterial strain -1

S.No	INCUBATION TIME (hrs)	GROWTH AT DIFFERENT TEMPERATURES (Absorbance at 600nm)			
		32^0c	34^0c	37^0c	42^0c
1	12	0.984	1.007	1.033	0.685
2	24	1.453	1.320	1.365	0.984
3	48	1.477	1.134	1.462	1.003
4	72	1.489	1.261	1.467	1.042

Table 3: Effect of Temperature on growth of selected bacterial strain 2

S.No	INCUBATION TIME (hrs)	GROWTH AT DIFFERENT TEMPERATURES (Absorbance at 600nm)			
		32^0c	34^0c	37^0c	42^0c
1	12	0.692	0.657	1.043	0.836
2	24	0.709	0.721	1.263	1.371
3	48	1.005	0.937	1.309	1.422
4	72	1.093	1.146	1.462	1.437

Fig 2: Graphical analysis of effect of temperature on bacterial strain-2

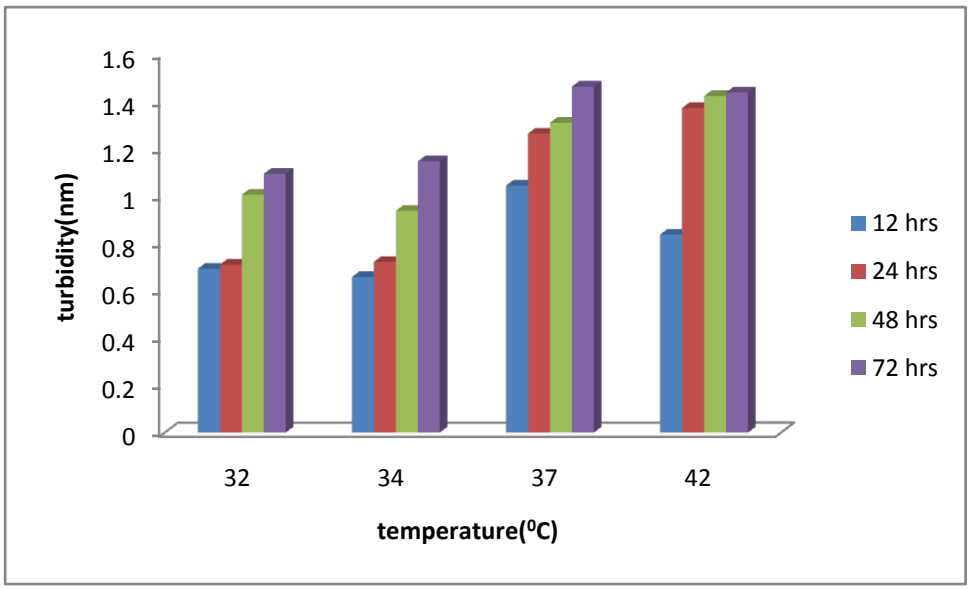
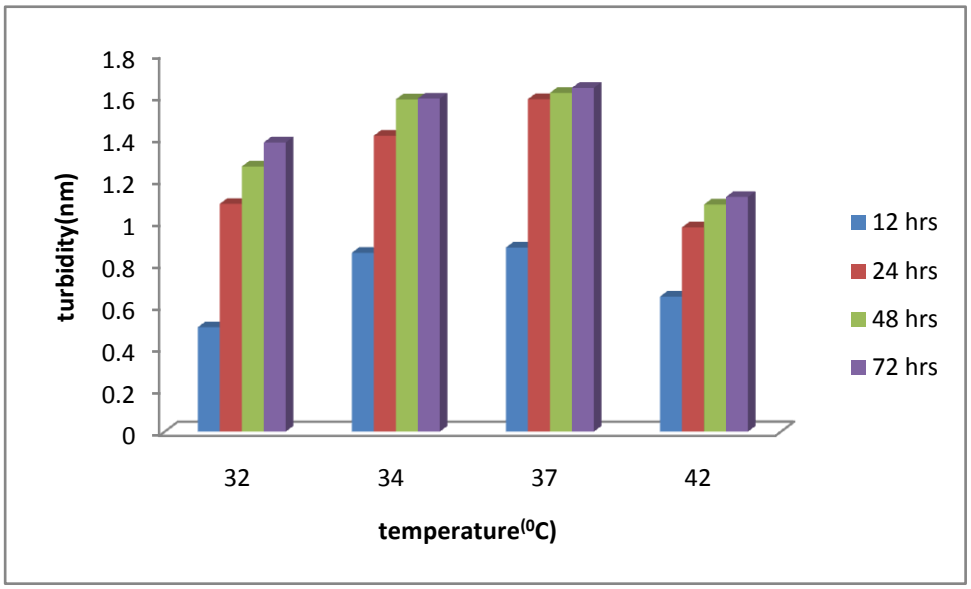


Table 3: Effect of Temperature on growth of selected bacterial strain 3

S.No	INCUBATION TIME (hrs)	GROWTH AT DIFFERENT TEMPERATURES (Absorbance at 600nm)			
		32^0c	34^0c	37^0c	42^0c
1	12	0.752	0.863	0.742	0.614
2	24	1.050	1.251	1.115	0.997
3	48	1.084	1.324	1.228	1.062
4	72	1.121	1.541	1.527	1.085

Fig 3: Graphical analysis of effect of temperature on bacterial strain-4



In the present study, effect of pH on bacterial growth showed maximum enzyme production 1.38 at pH 6.2 (**Table 4**).

Table 4: Effect of pH on growth & enzyme production by selected bacterial strain 2

S.No.	Initial pH	Final pH	Growth (g)	Activity (IU/ml)
1)	4	4.7	0.41	0.13
2)	5	5.3	0.73	0.27
3)	6	6.2	1.28	1.38
4)	7	6.8	1.22	1.32
5)	8	7.8	0.81	1.04
6)	9	8.4	0.44	0.77

Table5. Effect of various substrates (Potato infusion and Ararote) and amylase production of selected bacterial strain2.

S. No.	Incubation period (Days)	Final pH		Turbidity at 600nm		Activity (IU/ml.)	
		Potato Infusion	Ararote	Potato Infusion	Ararote	Potato Infusion	Ararote
1.	1	6.08	6.21	0.78	0.83	0.921	0.32
2.	2	6.24	6.17	1.32	1.04	1.216	0.58
3.	3	6.14	6.19	1.47	1.15	1.482	0.63
4.	4	6.18	6,08	1.51	1.19	1.304	0.47

In the present work both natural and industrial substrate (potato infusion and ararote) were used by incorporating them in the medium and results showed, maximum enzyme production in potato infusion (1.48 IU/ml) after three days of incubation period at pH 6.

Amylase is used for modification of starch for paper coating in pulp and paper industry. The sizing of paper is performed to protect the paper against mechanical damage during processing and also improve the quality of the finish paper.

The viscosity of natural starch is too high for paper sizing and this can be altered by partially degrading the polymer with α-amylase.

Table 6: Starch modification of ararote by amylase enzyme at 50^{0}C

S.No.	Sample	Enzyme dose (IU/g)	Viscosity at 32^{0}C, (cp)
1.	Control Starch	--	1.69
2.	Commercial enzyme treated Starch	50	1.47
3.	Amylase produced enzyme treated Starch	50	1.38

Table 7: Starch modification of ararote by amylase enzyme at 70^{0}C

S.No.	Sample	Enzyme dose (IU/g)	Viscosity at 32^{0}C, (cp)
1.	Control Starch	--	1.63
2.	Commercial enzyme treated Starch	50	1.34
3.	Amylase produced enzyme treated Starch	50	1.29

Amylase enzyme used for starch degradation studied from bacterial strain-2 showed better results 1.38 at 50 IU/g enzyme dose than commercial enzyme 1.47 at same dose at pH 6.5 at 50^{0}C (**Table 6**). Enzyme treated ararote showed better properties when compared to control ararote and it also showed that with the increase in temperature from 50^{0}C to 70 ^{0}C amylase produced enzyme treated starch showed 1.29 viscosity at 50 IU/g enzyme does than commercial enzyme 1.34 at same dose at pH 6.5 at 50^{0}C (**Table 7**).

CONCLUSION

The present study showed that the isolated amylolytic bacterial strain- 2 produced appreciable amount of amylase enzyme. Further, the study also revealed that amylase enzyme produced by the isolated amylolytic bacterial bacterial strain -2 can be used for industrial application like starch modification with better efficiency with the increase in temperature.

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***Corresponding Author:**
AKANSHA KARNWAL*
Quality Control Microbiologist,
Tidal Laboratories Pvt.Ltd.,
Dist. Bilaspur
(Himachal Pradesh) INDIA.