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Full Length Research Paper

Effect of starch containing organic substrates on alpha amylase production in *Bacillus* strains

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This study was conducted to identify and screen indigenous *Bacillus* strains from different soil samples for alpha amylase activity. Strains positive for the enzyme activity were *Bacillus subtilis, Bacillus mycoids, Bacillus licheniformis* and *Bacillus cereus*. These were further studied for amylase production in the presence of starch containing organic substrates such as powdered peels of potato, melon, banana, mango and apple. Solid state fermentations were carried out in mineral salt medium for 24 and 48 h at 37°C. Crude enzyme extract was used for amylase assay to determine the enzyme activity against standard curve of maltose. As a result, banana peel was found to be the best solid organic substrate for the highest alpha amylase production with 331.37, 241.45, 233.12 and 183.17 IU/ml/min in *B. subtilis*, *B. licheniformis*, *B. cereus* and *B. mycoids*, respectively in 24 h. Melon and potato were next to banana as nutrition, and the level of amylase production in all *Bacillus* strains with potato peels was comparable to melon peels with slight variations. All strains have shown minimum amylase production with apple or mango peels. A decline in the enzyme activity of the strains was observed after 24 h with all five substrates.

Key words: *Bacillus*, alpha amylase activity, organic substrates, solid state fermentation.

INTRODUCTION

Bacterial amylases are used in industry due to advantages such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimization. The increased demand for these enzymes in various industries has led to an enormous interest in developing enzymes with better properties such as raw starch degrading amylases suitable for industrial applications (Burhan et al., 2003). Alpha amylase (endo-1, 4-α-D-glucan glucohydrolase) is an extracellular enzyme extensively used in starch liquefaction, brewing, food, paper, textile, detergent and pharmaceutical industries (Teodoro and Martins, 2000).

Bacillus strains such as *Bacillus subtilis, Bacillus stearothermophilus, Bacillus licheniformis* and *Bacillus amyloliquefaciens* from soil samples are known as good producers of alpha-amylase for various applications (Pandey et al., 2000). Solid state fermentation (SSF) and submerged fermentation (SMF) can be used for the production of these amylases. Solid state fermentation holds tremendous potential for the production of alpha amylase. It can be of special interest in those processes where crude fermented product may be used directly as a source of enzyme (Pandey et al., 1999). Natural substrate or an inert substrate can be used as a solid support in the absence or near absence of free water in solid state fermentation (Pandey et al., 1999). The production of alpha amylase by SSF is limited to *Bacillus species* such as *B. subtilis, B. polymyxa, Bacillus mesentericus, Bacillus vulgarus, Bacillus coagulans, Bacillus megaterium* and *Bacillus licheniformis* (Lonsane and Ramesh, 1990). The production of bacterial alpha amylase using the SSF technique requires less fermentation time, 24 to 48 h, which leads to considerable reduction in the capital and recurring expenditure. In addition, the utilization of agro-industrial

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wastes, provide alternative substrates and also help in solving pollution problems, which otherwise may cause their disposal (Babu and Satyanarayana, 1995).

In some *Bacillus* species, starch and glycerol are known to increase enzyme production (Goyal et al., 2005). According to Hamilton et al. (1999), a *Bacillus sp*. gives a maximum raw starch digesting amylase in a medium containing 1% lactose and yeast extract. A synthetic analogue of maltose, alpha-methyl-D-glucoside, induced three-fold higher alpha amylase production than starch and maltose when supplemented to the medium as the only carbon source (Goto et al., 1998). Agricultural wastes are being used for both liquid and solid fermentations. These wastes consist of carbon and nitrogen sources necessary for the growth and metabolism of organisms. These nutrient sources include orange waste, pearl millet starch, potato, corn, tapioca, wheat and rice as flours (Sivaramakrishnan et al., 2006).

Media used in solid state fermentation are mainly based on wheat bran. Among different agricultural by-products, such as wheat bran, sunflower meal, cotton seed meal, soybean meal, rice husk, wheat bran was found to be the best medium for the production of alpha amylase (Haq et al., 2003). For the commercial production of enzyme as well as for its maximum activity, it is necessary to extract the maximum enzyme from the fermented medium. Phosphate buffer is found to be appropriate for the extraction of alpha amylase from fermented bran medium (Padmanabhan et al., 2008).

Bacillus are widely used for production of alpha-amylases and these bacteria need rich source of nutritional medium to grow, different fruit and vegetable peels usually considered a waste provide rich source of starch and nutrients for bacteria. Production of amylases from bacteria is beneficial for human population as their starch degrading ability can be exploited for preparation of special food items, easily digestible for infants, patients and elderly people. The objective of the present study was to isolate and identify *Bacillus* strains from a variety of local soil samples and to determine their level of alpha-amylase activity in the presence of potato, melon, banana, mango and apple powdered peels as starch containing organic substrates added to the mineral salt medium for solid state fermentation.

MATERIALS AND METHODS

Sampling

Samples of dry soil and mud were collected from different sites of Lahore, Sheikhupura, and Sharaqpur, Pakistan for isolation of the *Bacillus* species. These sites included garbage heap, industrial effluent area, canal side, flower bed, a swamp and a harvested wheat field. Samples were collected in plastic bags and stored in sterilized glass bottles.

Isolation of bacteria

One gram of each soil sample was added in 99 ml of sterile distilled water and stirred for 20 min. It was heated at 60°C for 60 min in water bath to make a soil suspension. Ten-fold serial dilutions of soil samples in sterile distilled water were prepared in duplicates up to 10^{-8} dilution and plated on 1.5% nutrient agar. Pour-plate method was used to isolate bacterial species from the soil samples (Fankhouser, 2005). Plates were incubated at 36±1°C for 24 to 48 h. A variety of single colony well separated on 10^{-6} dilution plates were picked randomly and streaked on fresh 1.5% nutrient agar plates. Incubation was done at 37°C for 24 h to get pure cultures of *Bacillus* species.

Identification and characterization of bacterial species

A variety of bacterial strains isolated by streak-plate and pour-plate methods were identified according to Holt et al. (1994) and characterized for their morphological features, Gram-staining behavior and biochemical activity. The biochemical activity was checked through catalase, oxidase, lactose utilization, glucose fermentation, hydrogen sulfide production and indole production tests. Chemicals and media used for this purpose were from MERCK and OXOID.

Screening of bacterial isolates through starch hydrolysis test

Starch agar medium (starch 10 g, beef extract 3 g, agar 12 g/L) was used for starch hydrolysis test of the isolates on petri plates. Pure single colony of each bacterial isolate was streaked on the culture media, which were allowed to grow for 24 to 48 h at 35 to 37°C. Starch agar plates were flooded with iodine solution for 10 min to select the amylolytic isolates. Blue black color on agar plate indicates a negative test. Positive reaction due to starch hydrolysis is indicated by a clear zone around the bacterial growth.

Solid state fermentation (SSF)

To prepare bacterial suspension, 50 ml of nutrient broth in 250 ml Erlenmeyer flasks was inoculated with loop full of each of the four starch hydrolyzing bacterial strains used in the present study and kept at 37°C in a rotatory shaker at 125 rpm for 24 h. Peels of potato, banana, melon, mango and apple were used as starch containing organic substrates. Peels were spread in steel trays, dried in oven (WISECUBE TM, DAIHAN Scientific. WON-050, Korea) at 60 to 70°C for 24 h, and were ground into powder. Solid state fermentations for alpha amylase synthesis were carried out in mineral salt medium with a composition ($Na_2HPO_4.2H_2O$ 1.10 g, $NaH_2PO_4.2H_2O$ 0.61 g, KCl 0.30 g, $MgSO_4.7H_2O$ 0.30 g in 100 ml distilled water at pH 7.0). Powdered peels (5 g) of each of the five substrates were moistened with 10 ml mineral salt medium to 50% moisture content (1 : 2) ratio in Erlenmeyer flasks, autoclaved (DAIHAN, Scientific) at 121°C and 15 lb/in^2 pressure for 15 min. The medium was cooled at 30°C and inoculated with 5% of each of the four bacterial cell suspensions (24 h old). The contents of the flasks were mixed thoroughly to ensure uniform distribution of the inoculum and incubated at 37°C for 24 to 48 h with occasional gentle shaking.

Crude enzyme extraction

After 24 to 48 h of incubation, fermented carrier was taken, eluted with 20 ml 0.02 M phosphate buffer, pH 7.0, shaken properly at 175 rpm for 60 min and filtered with muslin cloth. The filtrate was centrifuged at 9000 rpm for 15 min at room temperature in UNIVERSAL 320R HETTICH ZENTRIFUGEN. The culture filtrate was used as a crude enzyme extract for enzyme assay.

Table 1. Colony characteristics and Gram staining of bacterial strains.

S/N	Bacterial strain	Color	Opacity	Elevation	Margin	Gram Staining	Shape
1	*Bacillus subtilis*	Creamy off white	Opaque	Umbonate	Entire, Undulate	Gram Positive	Rod
2	*Bacillus mycoids*	White	Translucent	Flat	Rhizoid, Branching	Gram Positive	Rod
3	*Bacillus licheniformis*	Yellow white	Opaque	Hilly	Irregular Spreading	Gram Positive	Rod
4	*Bacillus cereus*	Creamy white	Opaque	Raised	Irregular, Spreading	Gram Positive	Rod

Table 2. Biochemical properties of the bacterial strains.

S/N	Bacterial strain	Biochemical tests						
		Catalase	Oxidase	Lactose	Starch hydrolysis	Glucose	Indole	H_2S production
1	*Bacillus subtilis*	+	+	-	+	+	-	-
2	*Bacillus mycoids*	+	+	-	+	+	-	-
3	*Bacillus licheniformis*	+	+	-	+	+	-	-
4	*Bacillus cereus*	+	+	-	+	+	-	-

Enzyme assay

Maltose was used as a standard reference curve for determining the amylase activity. One mg/ml (w/v) of maltose stock solution was used for preparation of the standard curve. From this stock solution, 10 appropriate dilutions with concentrations of 0.2 to 2.0 mg/ml were prepared. To 1 ml of each dilution, 1 ml of 3, 5 dinitrosalicylic acid reagent (DNS 1 g, sodium potassium tartarate tetrahydrate 30 g, 2N NaOH 40 ml, distilled water 100 ml) was added and blank was prepared by adding 1 ml of DNS in 1 ml of distilled water. These test tubes were placed in boiling water bath for 5 min, cooled at room temperature. After cooling, the contents of the tube were diluted up to 20 ml and the absorbance was measured at 540 nm in spectrophotometer (UV-visible 3000, ORI, Germany). Standard curve was prepared by plotting absorbance on y-axis and maltose concentration on x-axis.

Alpha amylase was assayed according to the method of Rick and Stegbauer (1974). Crude enzyme extract (1 ml) was added to the test tube containing 1 ml of 1 % starch solution prepared in 0.02 M phosphate buffer pH 7.0. This mixture was incubated at 25°C for 10 min. Two ml of DNS was added to the test tube and placed in water bath for 5 min. The contents of the test tubes were cooled and diluted up to 20 ml with distilled water. The absorbance of the reaction mixture was determined at 540 nm in spectrophotometer against maltose as standard. The amylase activity was determined in IU/ml/min by applying the standard formula.

$$\text{Amylase activity (IU/ml/min)} = \frac{\text{Amount of sugar released x 1000}}{\text{Molecular weight of maltose x Time of incubation}}$$

RESULTS

Among the 10 isolated bacterial strains, four *Bacillus* strains were identified and characterized as *B. subtilis, B. mycoids, B. licheniformis* and *B. cereus* indicating that *Bacilli* were more prevalent. Their Gram staining behavior, colony characteristics and biochemical activities are given in Tables 1 and 2. These *Bacillus* strains, formed clear zones with very light orange color around colonies after addition of iodine solution on starch agar plates, thus indicating the presence of bacterial amylases that could degrade starch in this area. *Neisseria subflava*, *Micrococcus varians* were also positive for alpha-amylase activity.

Effect of starch containing organic substrates such as potato, melon, banana, mango and apple powdered peels on alpha-amylase activity of *Bacillus* strains has been studied. Different levels of crude amylase activity were obtained against maltose standard curve (Figure 1). The absorbance values of crude enzyme extracts obtained from 24 and 48 h old bacterial cultures in the presence of five substrates at 540 nm, and the respective enzyme units are given in Table 3.

Highest alpha-amylase activity obtained with 5 g of banana peels for all *Bacillus* strains range from 183.17 to 331.37 IU/ml/min in 24 h of SSF at pH 7 and 37°C, *B. subtilis* producing 331.37 IU/ml/min with banana in mineral salt medium. The enzyme activity was 155.41 to 227.57 IU/ml/min for potato in three *Bacillus* strains, except *B. mycoids* showing less activity with 44.40 IU/ml/min in 24 h. The amylase activity of the *Bacillus* strains in the presence of melon was 116.56 to 220.35 IU/ml/min in 24 h. The enzyme units obtained with apple in 24 h for four *Bacillus* strains range from 13.87 to 21.09 IU/ml/min.

The enzyme units ranged from 12.76 to 21.09 IU/ml/min in the presence of mango in 24 h. Figures 2 and 3 show the effect of the organic substrates on alpha-amylase activity in 24 and 48 h old bacterial cultures with a decline in this activity in 48 h.

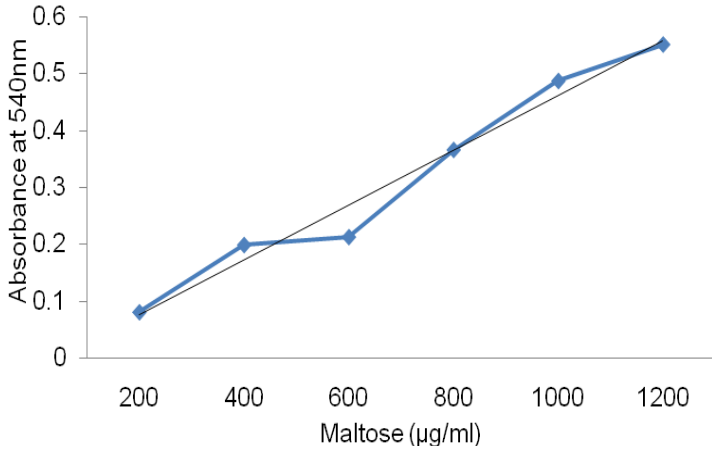

Figure 1. Standard curve of maltose to determine the amount of maltose released during starch hydrolysis by bacterial alpha amylase.

Table 3. Alpha amylase activity of *Bacillus* strains using starch containing organic substrates.

S/N	*Bacillus* strain	Powdered peels of organic substrates (5 g)	Absorbance of crude enzyme extract at 540 nm (A)		Alpha amylase activity (IU/ml/min)	
			24 h	48 h	24 h	48 h
1	*Bacillus subtilis*	Potato	0.164	0.159	219.24	210.92
		Melon	0.165	0.133	220.35	179.28
		Banana	0.22	0.184	331.37	244.22
		Mango	0.019	0.011	21.09	11.65
		Apple	0.019	0.012	21.09	12.76
2	*Bacillus mycoids*	Potato	0.026	0.019	44.40	23.86
		Melon	0.085	0.079	116.56	111.01
		Banana	0.138	0.107	183.17	144.31
		Mango	0.015	0.01	16.65	11.10
		Apple	0.013	0.011	13.87	11.65
3	*Bacillus licheniformis*	Potato	0.114	0.085	155.41	116.56
		Melon	0.150	0.117	199.82	160.96
		Banana	0.186	0.163	241.45	219.24
		Mango	0.011	0.011	16.65	11.65
		Apple	0.018	0.01	18.87	11.10
4	*Bacillus cereus*	Potato	0.170	0.075	227.57	105.46
		Melon	0.103	0.08	135.99	113.78
		Banana	0.180	0.169	233.12	222.02
		Mango	0.012	0.01	12.76	11.10
		Apple	0.018	0.01	18.87	11.10

DISCUSSION

Selection of a suitable solid substrate and its level are important factors for solid state fermentation. Potato, banana, melon, mango and apple powdered peels added to mineral salt medium for alpha-amylase production have an important role in providing nutrients for microbial growth. The ultimate benefit of utilizing agro-industrial

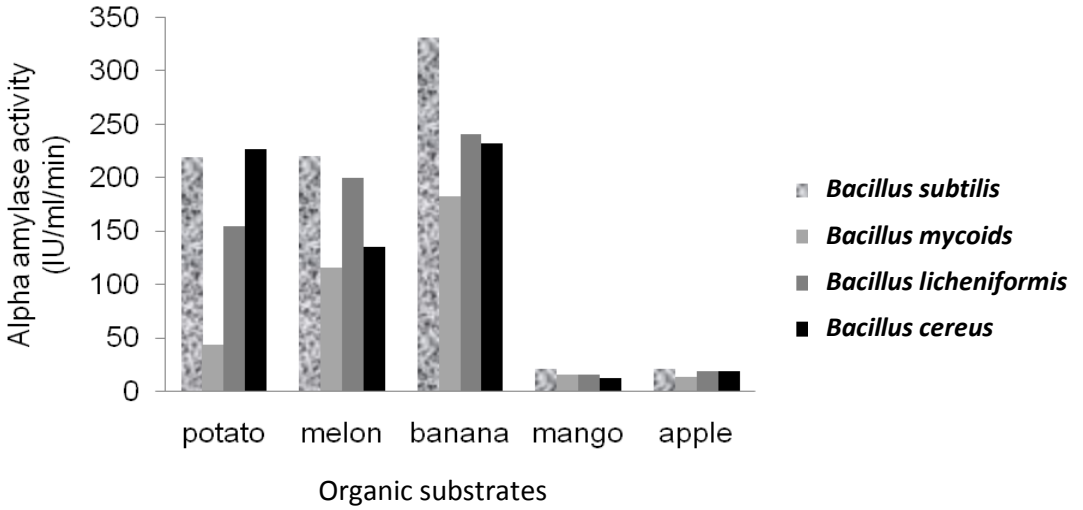

Figure 2. Effect of starch containing organic substrates on alpha amylase activity in 24 h old cultures of *Bacillus* strains in mineral salt medium.

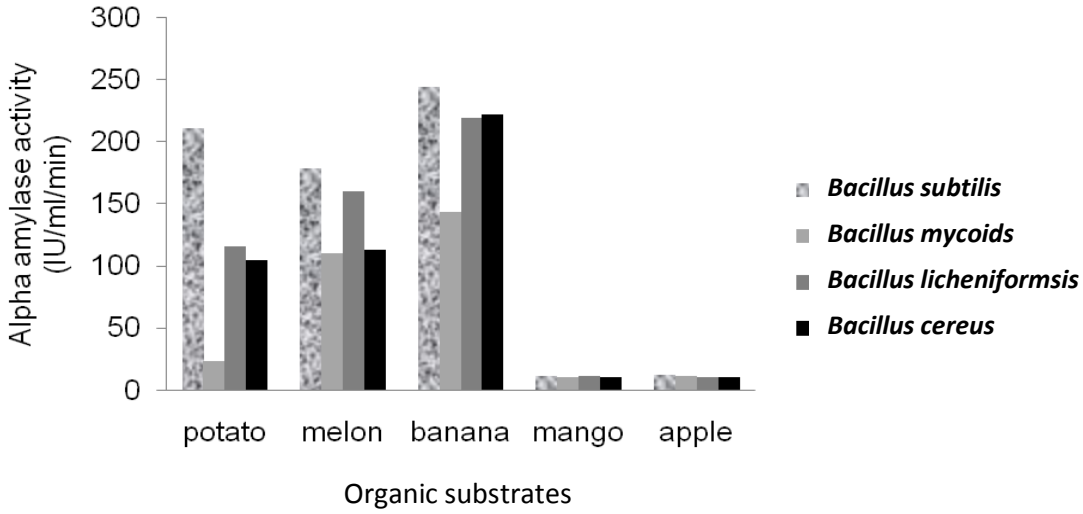

Figure 3. Effect of starch containing organic substrates on alpha amylase activity in 48 h old cultures of *Bacillus* strains in mineral salt medium.

waste is to reduce pollution problems for human beings, which otherwise need to be disposed off thus adding to environmental pollution.

Maximum alpha-amylase production has also been reported by *Bacillus subtilis* utilizing 10 g banana stalk as substrate in mineral salt medium (Krishna and Chandrasekaran, 1996). However, in present study, 5 g banana peel supported high enzyme activity in the same strain. Banana husk was found to be the best substrate for alpha-amylase production in *B. subtilis* through solid state fermentation in 72 h and 37°C, melon giving lesser enzyme activity according to Ozdemir et al. (2009). The maximum activity of alpha-amylase obtained by Kokab et al. (2003) was 9.06 IU/ml/min in 24 h of solid state fermentation of *B. subtilis* at pH 7 and 35°C, utilizing banana peel as a substrate.

Potato peel was found to be the second best substrate after wheat bran for alpha amylase activity from *Paenibacillus amylolyticus* as reported by Haq et al. (2012). According to Kanwal et al. (2004), 3.09 U/ml of alpha-amylase in crude extract prepared from 100 g soft thalamus of apple have been obtained. This difference shows that bacterial amylase is being produced in higher amount than that extracted directly from the plant source.

Banana, melon and potato are more effective substrates in this study, as they appear to provide sufficient necessary nutrients for *Bacilli* to grow and synthesize high levels of alpha-amylases. However, the level of enzyme activity obtained with apple and mango is minimum and comparable between these two substrates.

Response of bacteria towards these substrates with reference to enzyme production is important. Thermophilic *B. subtilis* and *B. licheniformis* have been reported to show maximum alpha-amylase activity in mineral salt medium containing potato peel as a solid substrate according to Shukla and Kar (2006). However, maximum enzyme synthesis is observed in local *B. subtilis* at 37°C in 24 h with all substrates, except potato, which gives slightly higher activity in *B. cereus*. Riaz et al. (2003) have obtained maximum amylase activity with 535 IU/ml/min from a mutant strain of *B. subtilis* incubated at 40°C for 48 h in basal medium through continuous shaking of flasks. *B. licheniformis* is synthesizing more amylase with banana and melon than with other substrates, 241.45 IU/ml/min and 199.82 IU/ml/min in 24 h, respectively. This is less than the enzyme units obtained by Aiyer (2004), 350 IU/ml amylase in the presence of potato starch and 396 IU/ml with potato soluble starch from *B. licheniformis* through submerged fermentation at pH 9 in 24 h and 37°C. As compared to *B. subtilis* and *B. licheniformis, B. cereus* has shown less amylase production with all substrates, except with potato. In an investigation by Vijayabaskar et al. (2012), *B. cereus* has shown maximum amylase activity of 232.65 U/ml with sugarcane bagasse*. B. mycoids* has shown the least enzyme activity with all substrates. Amylase production in submerged fermentation by *Bacillus* spp. has been optimized by Vidyalakshmi et al. (2009). The maximum activity of enzyme with 11 U/ml was obtained at 35°C and pH 7 in 10 h. However, in the reported *Bacillus* strains, addition of starch rich organic substrates to mineral salt medium for solid state fermentations has shown the enhanced enzyme activity at 37°C and pH 7 in 24 h.

Incubation time and temperature affects the characteristics of the culture, growth rate and enzyme production. The cell cultures harvested at 24 h have shown maximum amylase production but decreased thereafter as already reported by Asghar et al. (2002). A gradual decrease was seen in alpha-amylase production from 24 to 48 h for all *Bacillus* strains. Maximum enzyme production at 24 h could be due to the fact that the microorganisms were in their exponential phase. Later, in stationary phase, nutrients were depleted and secondary metabolites were produced. This might have resulted in denaturation of the enzyme due to interaction with other components in the medium (Ramesh and Lonsane, 1987; Ozdemir et al., 2009), thus resulting in a lower yield of enzyme after 24 h in the present study. Variations in factors such as temperature, pH, incubation period, carbon sources acting as inducers, nitrogen sources, phosphate, metal ions, moisture with regard to SSF could influence the alpha-amylase activity resulting in less or even a higher yield than what is reported here.

Conclusion

Banana peel powder was found to be the best starch containing organic substrate in mineral salt medium used for solid state fermentations at pH 7 and 37°C in 24 h due to the maximum alpha-amylase production in all *Bacillus* strains, *B. subtilis* giving the highest activity with this substrate. The enzyme units from the indigenous *Bacillus* strains in the presence of banana are either comparable or higher than what has already been reported in some research studies. Melon and potato are next to banana in terms of the source of nutrition for alpha-amylase production with more or less comparable units. The minimum amylase activity has been obtained from all strains in the presence of apple and mango peels. *B. subtilis* has shown maximum level of alpha-amylase activity with all substrates, except potato, which has given slightly higher activity in *Bacillus cereus*. There is a gradual decline in the enzyme activity after 24 h for all *Bacillus* strains.

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REFERENCES

Aiyer PVD (2004). Effect of C: N ratio on alpha amylase production by *Bacillus licheniformis* SPT 27. Afr. J. Biotechnol. 3(10): 519-522.

Asghar M, Aisha F, Asad MJ, Saleem Y (2002). Production of α-amylase through solid substrate fermentation of banana stalks by *Bacillus subtilis.* Pak. J. Agric. Sci. 39(4):307-311.

Babu KR, Satyanarayana T (1995). α-Amylase production by thermophilic *Bacillus coagulans* in solid-state fermentation. Process Biochem. 30:305-309.

Burhan A, Nisa U, Omer C, Ashabil A, Osman G (2003). Enzymatic properties of a novel thermostable, thermophilic, alkaline and chelator resistant amylase from an alkaliphilic *Bacillus sp.* isolate ANT 6. Process Biochem. 38:1397-1403.

Fankhouser DB, (2005). Pour plate technique for bacterial enumeration. www.biology.clc.uc.edu, Accessed: February 12, 2010.

Goto CE, Barbosa, EP, Kistner LCL, Moreira FG, Lenartovicz V, Peralta RM (1998). Production of amylase by *Aspergillus fumigatus* utilizing α- methyl-D-glucoside, a synthetic analogue of maltose, as substrate. FEMS Microbiol. Lett. 16:139-143.

Goyal N, Gupta JK, Soni SK (2005). A novel raw starch digesting thermostable α-amylase from *Bacillus* sp. I-3 and its use in the direct hydrolysis of raw potato starch. Enzy. Microbiol. Technol. 37:723-734.

Hamilton LM, Fogarty WM, Kelly CT (1999). Purification and properties of the raw starch degrading α-amylase of *Bacillus* sp. IMD 434. Biotechnol. Lett. 2:111-115.

Haq I, Ashraf H, Iqbal J, Qadeer MA (2003). Production of alpha amylase by *Bacillus licheniformis* using an economical medium. Bioresour. Technol. 87:57-61.

Haq I, Hameed U, Mahmood, Z, Javed, MM (2012). Solid state fermentation for the production of α-amylase by *Paenibacillus amylolyticus*. Pak. J. Bot. 44:341-346.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994). Bergey's Manual of Determinative Bacteriology, 9th Ed. Williams and Wilkins company, USA, pp 149, 233, 254, 562.

Kanwal B, Zia MA, Yaseen M, Rahman K, Sheikh MA (2004). Purification and characterization of α-amylase from apple (*Malus pumila*). Int. J. Agric. Biol. 6(2):233-236.

Kokab S, Asghar M, Rehman K, Asad MJ, Adedyo O (2003). Bio-processing of banana peel for α-Amylase production by *Bacillus subtilis.* Int. J. Agric. Biol. 5(1):36-39.

Krishna C, Chandrasakaran M (1996). Banana wastes as substrates for alpha amylase production by *Bacillus subtilis* (CBTK-106) under solid state fermentation. Appl. Microbiol. Biotechnol. 46:106-111.

Lonsane BK, Ramesh MV (1990). Production of bacterial thermostable α-Amylase by solid-state fermentation: A potential tool for achieving economy in enzyme production and starch hydrolysis. Adv. Appl. Microbiol. 35:1-56.

Ozdemir S, Guven K, Baysal Z, Uyar F (2009). Screening of various organic substrates and the development of a suitable low-cost fermentation medium for the production of α-amylase by *Bacillus subtilis.* Food Technol. Biotechnol. 47(4):364-369.

Padmanabhan S, Ramakrishna M, Lonsane BK, Krishnaiah MM (2008). Alpha amylase produced by *Bacillus licheniformis* M27 in solid state fermentation system. Lett. Appl. Microbiol. 15(6):235-238.

Pandey A, Azmi W, Singh J, Banerjee UC (1999). Types of fermentation and factors affecting it. In: Joshi VK, Pandey A, (eds) Biotechnology: Food fermentation, Educational Publishers, New Delhi pp. 383-426.

Pandey A, Nigam P, Soccol CR, Soccol V, Singh D, Mohan R (2000). Advances in microbial amylases (review article). Biotechnol. Appl. Biochem. 31:135-152.

Pandey A, Selvakumar P, Soccol, CR, Nigam P (1999). Solid state fermentation for the production of industrial enzymes. Curr. Sci. 77(1):149-162.

Ramesh MV, Lonsane BK (1987). A novel bacterial thermostable alpha–amylase system produced under solid state fermentation. Biotechnol. Lett. 9:501-504.

Riaz N, Haq I, Qadeer MA (2003). Characterization of α-amylase by *Bacillus subtilis.* Int. J. Agric. Biol. 5(3):249-252.

Rick W, Stegbauer HP (1974). Alpha amylase measurement of reducing groups. In H.U. Bergmeyer (ed) Methods of enzymatic analysis, 2nd Ed. Verlag Chemie, Weinheim pp. 885-915.

Shukla J, Kar R (2006). Potato peel as a solid substrate for the thermostable alpha amylase production by thermophilic *Bacillus* isolates. World J. Microbiol. Biotechnol. 22:417-422.

Sivaramakrishnan S, Gangadharan D, Nampoothiri KM, Soccol CR, Pandey A (2006). α-amylases from microbial sources- an overview on recent developments. Food Technol. Biotechnol. 44(2):173–184.

Teodoro CED, Martins MLL (2000). Culture conditions for the production of thermostable amylase by *Bacillus sp.* Brazil. J. Microbiol. 31:298-302.

Vidyalakshmi R, Paranthaman R, Indhumathi J (2009). Amylase production on submerged fermentation by *Bacillus* spp. World J. Chem. 4(1):89-91.

Vijayabaskar P, Jayalakshmi D, Shankar T (2012). Amylase production by moderately halophilic *Bacillus cereus* in solid state fermentation. Afr. J. Microbiol. Res. 6(23):4918-4926.