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Isolation and identification of industrially important salt stable amylase producer

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Abstract

Efficient salt stable amylase producer was isolated by using starch agar medium containing 10 % NaCl (pH 7.2) from soil samples collected from over irrigated and water logged sites of Kaleshwar farming area in Nanded district of Maharashtra. The salt stable amylase producer was identified based on its morphological, microscopic and physiological characters, biochemical pattern and enzyme profile as *Halobacillus* sp. SSA1. Production of salt stable amylase was carried out in starch yeast extract medium containing 10 % NaCl. Production of salt stable amylase was recorded 2103.47 U/mL in cell free supernatant. The crude salt stable amylase has shown maximum catalytic efficiency at pH 7 and 4 ^{o}C temperature.

Keywords: salt stable amylase, *Halobacillus* sp., amylase activity, starch agar

1. Introduction

Amylases (alpha1,4-glucan-4-glucanohydrolases) break down starch into its more soluble forms such as oligosaccharides and monosaccharides. Amylases are used in various industries such as food, detergent, textile, distilling, brewing, baking, clinical, agricultural, medicinal and pharmaceutical industries and therefore considered as one the industrially

important enzymes. Amylases constitute approximately 25 % of world enzyme market (Souza, 2010).

At present many amylase producers have been isolated from mesophilic, thermophilic and many other sources and their extracellular amylases are characterized as well. However, very few reports are available regarding isolation and identification of salt stable amylase producers and their habitats. Therefore researchers are now targeting to isolate diverse salt stable amylase producers from various sources to fulfill the current industrial need. Characterization of amylases isolated from new sources is necessary to know their novel properties and to overcome with the limitations of existing amylases (Satyanarayana *et al*., 2005).

Therefore we have performed a set of experiments aimed at isolation and identification of salt stable amylase producer from soil samples collected from over irrigated area. Moreover we have also assessed effect of pH and temperature on salt stable amylase.

2. Materials and Methods

2.1. Isolation of salt stable amylase producer

Soil samples were collected from Kaleshwar farming area and these samples were dried, crushed and sieved. Further these samples were mixed in equal proportion to form a composite soil sample and pH was recorded. Aliquots of diluted soil samples were spread on starch agar (10 % NaCl, pH 7.2) plates. These plates were incubated at 30 ^{o}C for 24 h in an incubator (Kumar make, Mumbai). Morphologically distinct colonies were isolated and further spot inoculated on starch agar (10 % NaCl, pH 7.2) plates. These plates were incubated at the same temperature and incubation period. After incubation, Grams iodine solution was poured on the surface of agar and the plates were observed either for presence or absence of zone of clearances around the grown cultures. The efficient salt stable amylase

producer was selected based on the size of zone formed and further maintained on starch agar slants (Pathak *et al*., 2014; Pathak and Rathod, 2014).

2.2. Identification of salt stable amylase producer

Morphological characters viz. shape, size, margin, surface, elevation, consistency, colour and opacity and microscopic characters viz. cell shape, Gram nature and motility of the selected isolate were recorded. Gram staining was performed by using Gram staining kit (HiMedia, Mumbai) and motility test was performed by using hanging drop technique. Sugar utilization pattern of the selected isolate was recorded by performing carbohydrate fermentation tests in basal medium. The selected sugars were xylose, maltose, lactose and glucose. Indole test was performed by using tryptone broth. Methyl red and Voges-Proskauer tests were performed by using MR-VP broth. Citrate utilization test was performed by using Simmon's citrate agar. Catalase and oxidase tests were performed by using 3 % H_2O_2 and strips of 1 % tetramethyl-p-phenylenediamine dihydrochloride respectively (Polkade *et al.,* 2015). Protease production test was performed by using skimmed milk agar. 10 % NaCl was added in the aforementioned media. Optimum temperature, pH, incubation period and NaCl concentration required for the growth of selected isolate was determined. Selected isolate was identified by comparing its morphological, microscopic and physiological characters, biochemical pattern and enzyme profile with standard reference strain given in Bergey's manual of systematic bacteriology (Joshi *et al*., 2008; Tarlera *et al*., 2008; Sharma *et al*., 2009; Khairnar *et al*., 2012; Pathak and Sardar, 2012; Pathak *et al*., 2012; Hingole and Pathak, 2013; Kolekar *et al.,* 2013; Pathak and Rathod, 2013; Pathak *et al*., 2014; Sardar and Pathak, 2014; Rathod and Pathak, 2014a,b; Sharma *et al*., 2015; Dasteger *et al.,* 2015; Pathak and Rathod, 2015; Pathak et al. 2015a,b,c,d; Pathak and Gavali 2015, Sonalkar *et al.,* 2015; Pathak and Rathod, 2016).

2.3. Production of salt stable amylase

Volume of 3 mL fresh culture of selected isolate was inoculated in a 1 L conical flask containing 500 mL of starch yeast extract medium composed with starch 5 g/L, yeast extract 5 g/L, ammonium sulphate 2.5 g/L, NaCl 100 g/L, $MgSO_4 \cdot 7H_2O$ 0.2 g/L, KH_2PO_4 3 g/L and $CaCl_2 \cdot 2H_2O$ 0.25 g/L. The flask was incubated in orbital shaking incubator (CIS-BL 24, Remi Make, Mumbai) at 30 °C and 120 rpm agitation speed for 72 h (Pathak and Sardar, 2014; Pathak and Rathod, 2014).

2.4. Extraction of salt stable amylase and quantitative amylase assay

After completion of production period, whole fermented broth was centrifuged at 10,000 rpm for 10 min at 4 ^oC in a cooling centrifuge (BioEra, India). The pellets were discarded and cell free extract was considered as crude salt stable amylase and used for quantitative assay. Crude salt stable amylase (1 mL) was added to the test tube containing 1 mL of 1 % starch solution prepared in 0.2 M phosphate buffer pH 7.0 containing 10 % NaCl. This mixture was incubated at 30 °C for 10 min. The produced reducing sugar was measured using the dinitrosalicylic acid method (Ghorbel *et al.,* 2009). One unit activity of salt stable amylase was defined as the amount of enzyme required to liberate 1 µmol min^{-1} mL^{-1} of reducing sugar expressed as maltose equivalent, under the assay conditions (Pathak and Sardar, 2014).

2.5. Characterization of salt stable amylase

2.5.1. Effect of pH on crude salt stable amylase

Catalytic activities of crude salt stable amylase were recorded at pH 4, 7 and 9 using 0.2 M citrate buffer, phosphate buffer and Glycine-NaOH buffer respectively in standard assay conditions as described previously (Pathak and Rathod, 2014; Pathak and Sardar, 2014).

2.5.1. Effect of temperature on crude salt stable amylase

Catalytic activities of crude salt stable amylase were recorded at temperatures 4, 30 and 50 ^{o}C at standard assay conditions as described previously (Pathak and Rathod, 2014; Pathak and Sardar, 2014).

3. Results and Discussion

3.1. Isolation of salt stable amylase producer

pH of composite soil sample from Kaleshwar farming area was recorded as 7.3. Total 104 colonies were appeared on starch agar plates. Of these, 10 morphologically distinct colonies were designated as SSA1 to SSA10. After screening the isolate SSA1 has shown a largest zone of clearance on starch agar plate. Therefore, SSA1 was selected for identification and production of salt stable amylase.

3.2. Identification of salt stable amylase producer

Morphological, microscopic and biochemical characters, enzyme profile and physiological chracters of SSA1 are given in Table 1. SSA1 has shown luxurious growth at pH 8.0, temperature 30 ^{o}C, 48 h incubation period and 10 % NaCl concentration. Based on these characters selected salt stable amylase producer was identified as *Halobacillus* sp. SSA1.

Table 1: Morphological, microscopic and biochemical characters and enzyme profile of *Halobacillus* sp. SSA1

Morphological characters	*Halobacillus* sp. SSA1.	**Microscopic characters**	*Halobacillus* sp. SSA1.	**Biochemical characters**	*Halobacillus* sp. SSA1.
Shape	Round	Cell shape	Rod	Glucose	-
Size	2.5 mm	Cell motility	Motile	Maltose	+
Margin	Entire	Gram stain reaction	Positive	Lactose	+
Surface	Smooth	**Enzyme profile**	**SSA1**	Xylose	-
Elevation	Raised	Catalase	+	Indole test	+
Consistency	Non sticky	Oxidase	+	MR test	-
Colour	Off white	Amylase	++	VP test	-
Opacity	Opaque	Protease	-	Citrate test	-

3.3. Production of salt stable amylase

Production of crude salt stable amylase from *Halobacillus* sp. SSA1 was recorded 2103.47 U/mL after 72 h incubation period.

3.4. Characterization of salt stable amylase

3.4.1. Effect of pH on crude salt stable amylase

Crude salt stable amylase from *Halobacillus* sp. SSA1 exhibited maximum catalytic efficiency at pH 7 (2100 U/mL) followed by at pH 9 (1922.57 U/mL) and pH 4 (1165.19 U/mL).

3.4.2. Effect of temperature on crude salt stable amylase

Crude salt stable amylase from *Halobacillus* sp. SSA1 exhibited maximum catalytic efficiency at 4 ^{o}C (1980.83 U/mL) temperature followed by at 30 ^{o}C (1957.70 U/mL) and 50 ^{o}C (1776.92 U/mL).

4. Conclusions

Efficient salt stable amylase producer was isolated and identified as *Halobacillus* sp. SSA1. Remarkable production of salt stable amylase (2103.47 U/mL) was recorded from *Halobacillus* sp. SSA1. Beside pH 7, crude SSA1 salt stable amylase has also shown remarkable catalytic efficiency at alkaline pH 9. SSA1 amylase was catalytic active at low temperature as well. Therefore salt stable amylase from *Halobacillus* sp. SSA1 can be used in different biotechnological industries where hydrolysis of starch is carried out at low temperature, alkaline pH and extreme salt concentration.

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6. References

Dastager, S. G., Mawlankar, R., Mual, P., Verma, A., Krishnamurthi, S., Joseph, N., & Shouche, Y. S. (2015). Bacillus encimensis sp. nov. isolated from marine sediment. *International journal of systematic and evolutionary microbiology*, *65*(5), 1421-1425.

Gavali, J., & Pathak, A. (2015). Isolation And Identification Of Cold Active Amylase Producer From Gastro Intestinal Tract Of Channa Striata.*Current Trends in Aquaculture*, *53*.

Ghorbel, R. E., Maktouf, S., Massoud, E. B., Bejar, S., & Chaabouni, S. E. (2009). New thermostable amylase from *Bacillus cohnii* US147 with a broad pH applicability. *Applied biochemistry and biotechnology*, *157*(1), 50-60.

Joshi, A. A., Kanekar, P. P., Kelkar, A. S., Shouche, Y. S., Vani, A. A., Borgave, S. B., & Sarnaik, S. S. (2008). Cultivable bacterial diversity of alkaline Lonar Lake, India. *Microbial Ecology*, *55*(2), 163-172.

Khairnar, R. S., Mahabole, M. P., & Pathak, A. P. (2012). Nanoactivator mediated modifications in thermostable amylase from *Bacillus licheniformis*. *Indian J Biochem. Biophy*.49, 468-471.

Kolekar, Y. M., Pawar, S. P., Adav, S. S., Zheng, L., Li, W., Shouche, Y. S., Dastager, S. G., Kodam, K. M. (2013). *Alishewanella solinquinati* sp. nov., isolated from soil contaminated with textile dyes. *Current microbiology*, 67(4), 454-459.

Pathak A. P., Lohagave A. G., Rathod M. G. (2015a). Exploration of paper industry effluent for isolation of efficient starchy material degrader to promote bioremediation. *Int. J. Adv. Pharm. Biol. Chem*. 4(4) 729-736.

Pathak A.P., Gavali J., Gunjakar P (2015) Isolation and Characterization of Psychrophilic Carotenoid producers from gastrointestinal tract of *Rohtee vigorsi*. *Int. J. Adv. Pharm. Biol. Chem*, 4(4): 821-824.

Pathak A.P., Jethaliya C.S., Sarsar M.S., Jadhav S.R. (2015d) Isolation and characterisation of potential amylase producing strain from the agriculture waste. *Int. J. Adv. Pharm. Biol. Chem*, 4(4): 829-832.

Pathak A.P., Kamble G.T., Jadhav S.R., Sarsar M.S. (2015c) Isolation and biochemical Characterization of potential thermostable Lipase producer from industrial effluent of oil, dairy and paper industry. *Int. J. Adv. Pharm. Biol. Chem*, 4(4): 825-828.

Pathak A.P., Kulkarni, A., Sardar, A.G. & Karuppayil, S.M.. (2012) Enhanced growth of *Vigna aconitifolia* mediated by *Pseudomonas spp*., an efficient phosphate solubilizer. Life sciences leaflets 377-380.

Pathak, A. P., & Rathod, M. G. (2013). Production and Characterization of Alkaline Protease by *Bacillus pasteurii*: a Lonar Soda Lake Isolate. *Innovative Research in Chemistry*, *1*(1), 22-26.

Pathak, A. P., & Rathod, M. G. (2014). Exploration of Unkeshwar hot springs in Maharashtra for thermostable amylase producer. *Res. Rev. Biosci*, *8*(7), 269-276.

Pathak, A. P., & Rathod, M. G. (2015). Cultivable bacterial diversity of terrestrial thermal spring of Unkeshwar, India. *Journal of Biochemical Technology*, *5*(4), 814-818.

Pathak, A. P., & Sardar, A. G. (2012). Isolation and characterization of carotenoid producing Haloarchaea from solar saltern of Mulund, Mumbai, India. *Indian J. Natural Products Resources*, *3*, 483-488.

Pathak, A. P., & Sardar, A. G. (2014). Isolation and characterization of salt stable protease producing archaea from marine solar saltern of Mulund, Mumbai. *Indian J. Mar. Sci*, *43*(3), 412-417.

Pathak, A. P., Lodge, N., Gavali, J. T., & Rathod, M. G. (2015). Isolation and characterization of cold-active protease producer from ice factory samples. *Int. J. Adv. Pharm. Biol. Chem*, *4*, 751-754.

Pathak, A. P., Rathod, M. G., & Rampurkar, V. V. (2014). An eco-friendly approach for thermostable amylase production using Bacillus firmus APP6: a hot spring isolate, Asiatic J. *Biotech. Res*, *4*(4), 101-105.

Pathak, A. P., Sardar, A. G., & Janaj, P. C. (2014). Exploring the salted fish for salt stable amylase producing bacteria. *Indian J. Mar. Sci.*, *43*, 10 (in press).

Pathak, A.P. & Rathod, M.G. (2016) Assessment of diverse thermostable alkaline lipase producers from Unkeshwar hot spring of Maharashtra, India. *Concept. Pure Appl. Sci. 3*(1), 1-9.

Polkade, A. V., Ramana, V. V., Joshi, A., Pardesi, L., & Shouche, Y. S. (2015). Rufibacter immobilis sp. nov., isolated from a high-altitude saline lake. *International journal of systematic and evolutionary microbiology*, *65*(5), 1592-1597.

Rathod, M. G., & Pathak, A. P. (2014a). Isolation and identification of alkaline protease producer from selected alkaline habitat. *International Journal of Innovative Biological Research*, *3*(1), 1-6.

Rathod, M. G., & Pathak, A. P. (2014b). Wealth from waste: Optimized alkaline protease production from agro-industrial residues by Bacillus alcalophilus LW8 and its biotechnological applications. *Journal of Taibah University for Science*, *8*(4), 307-314.

Sardar, A. G., & Pathak, A. P. (2014). Exploring the microbiota of solar saltern of Mulund, Mumbai, India. *Indian J. Mar. Sci.*, *43*, 634-641.

Satyanarayana, T., Raghukumar, C., & Shivaji, S. (2005). Extremophilic microbes: Diversity and perspectives. *Current science*, *89*(1), 78-90.

Sharma, A., Pandey, A., Shouche, Y. S., Kumar, B., & Kulkarni, G. (2009). Characterization and identification of Geobacillus spp. isolated from Soldhar hot spring site of Garhwal Himalaya, India. *Journal of basic microbiology*,*49*(2), 187-194.

Sharma, B., Agrawal, R., Singhania, R. R., Satlewal, A., Mathur, A., Tuli, D., & Adsul, M. (2015). Untreated wheat straw: Potential source for diverse cellulolytic enzyme secretion by *Penicillium janthinellum* EMS-UV-8 mutant. *Bioresource technology*, *196*, 518-524.

Sonalkar, V. V., Mawlankar, R., Ramana, V. V., Joseph, N., Shouche, Y. S., & Dastager, S. G. (2015). *Bacillus filamentosus* sp. nov., isolated from sediment sample. *Antonie van Leeuwenhoek*, *107*(2), 433-441.

Souza, P. M. D. (2010). Application of microbial α-amylase in industry-A review. *Brazilian Journal of Microbiology*, *41*(4), 850-861.

Tarlera, S., Jangid, K., Ivester, A. H., Whitman, W. B., & Williams, M. A. (2008). Microbial community succession and bacterial diversity in soils during 77 000 years of ecosystem development. *FEMS microbiology ecology*, *64*(1), 129-140.