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Partial Purification of Extracellular Amylase From Halotolerant Actinomycetes *Streptomyces brasiliensis* MML2028

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KEYWORDS

Amylase

Halophilic

NaCl

Streptomyces

Dinitro salicylic acid

ABSTRACT

Amylase is considered as an industrially important enzyme as it occupies the most important function in the food, paper, and pharmaceutical industries. The present study is concerned with the optimization, production and partial purification of halotolerant amylase from newly isolated *Streptomyces brasiliensis* MML2028, from Kelambakkam salt pan, Tamil Nadu, India. The primary screening was carried out by well diffusion assay to find the zone of lysis. The assay was observed for each media optimization by measuring the release of reducing sugar (RS) by the 3,5 dinitro salicylic acid (DNS) method and expressed in the international unit (UI). Ammonium sulphate precipitation was used to partially purify the enzyme and then lyophilized. SDS-PAGE was performed to identify the molecular weight. The production medium was optimized with 1% of the starch substrate, 3% of NaCl at 24 °C and pH 9, and incubation of 9 days. The total activity of the partially purified α-amylase was observed to be 1806.9U/mL. The partially purified enzyme was more active with 3% NaCl, pH 8, and 24 °C which is known to be a halotolerant alkaline α-amylase. The enzyme showed tolerance towards magnesium, manganese ions, Triton x-100, and urea. De-inking of α-amylase showed good results proving that the enzyme activity is more efficient. Hence, the alkaliphilic amylase from Halotolerant actinomycetes *S. Brasiliensis* MML2028 could be a better microbial source that can be used in many industries, especially in paper and textiles.

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1 Introduction

The thrust on industrially important enzymes has become more important due to their eco-friendly nature (Asrat and Girma 2018). The amylolytic enzymes function as a major part of the carbon cycle. They can be divided into three groups namely α-amylase, β-amylase, and glucoamylase, which are capable of hydrolyzing starch and glycogen. These enzymes cleave the starch and starch linked polymers to give up products. In the beginning, amylase was used to hydrolyze the α-1, 4- glucosidic bonds of amylose, amylopectin, glycogen, and their degradation products (Mohamed et al. 2021). Extreme environments are measured as a flourishing resource for extremophiles that can produce dynamic enzymes that have numerous industrial applications and are appropriate for harsh surroundings to evaluate their counterpart. Alpha amylase can be produced by micro and macro organisms (Simair et al. 2017) and is also used as an add-on in detergents owing to their elevated alkaline pH stability which is now needed for industries as the alkaline nature of groundwater (Asad et al. 2011). At present, there is a demand for alkaline amylase production in the worldwide market (Abdullah et al. 2014).

Extremophiles from the extreme environment can produce enzymes that are useful for the industrial process (Zhu et al. 2020). Alkaline environments have drawn attention to the isolation of alkaliphilic bacteria, which are capable of producing alkaline enzymes. The industrial demand for enzymes, with appropriate specificity and stability to pH, temperature, metal ions, surfactants, and organic solvents continues to stimulate the search for new enzyme sources. Enzymes with high activity and stability at higher temperatures are interesting for bioengineering and biotechnological applications (Arauza et al. 2009). Fabric, paper, food, and fermentation are the industries where amylases have a large range of applications (Haq et al. 2010).

The previous studies carried out at Biocontrol and Microbial Metabolites Lab, Centre for Advance Studies in the Botany Department of Madras University identified 30 halotolerant actinomycetes isolated from Kelambakkam salt pans that could produce a huge amount of industrially important extracellular enzymes. Therefore, the present study aimed to investigate these halotolerant actinomycetes for the production of amylase enzymes. So far, no report was available on amylase production from halotolerant actinomycetes *S. brasiliensis.* In addition to screening, partial purification and characterization of the amylase enzyme were also carried out. Furthermore, because of the increasing importance of amylase enzymes in biotechnological applications, the present study also indented to conclude the effect of pH, thermostability, and application of the partially purified amylase produced by the halotolerant actinomycetes *S. brasiliensis* MML 2028.

2 Materials and Methods

2.1 Preliminary screening for amylase production

In the current study, 30 halotolerant actinomycete isolates were screened for amylase production by plating the isolates on Nutrient agar amended with 1% of starch and incubated at 28°C for 4 days. After incubation, the plates were flooded with an indicator containing 2% of iodine and 1% potassium iodide and the zone of clearance was observed and measured the zone in diameter (mm). The maximum zone-produced culture was selected for further studies.

2.2 Identification of halotolerant actinomycete MML2028

The potential isolate with significant activity was further identified based on morphological, physiological, biochemical, and molecular characterization (Buchanan and Gibbons 1974).

2.2.1 Colony morphology

Halophilic actinomycetes MML2028 was grown on ISP-2 medium and incubated for eight days and the colony morphology, pigmentation, sporulation, etc. were recorded (Krishnakumar et al. 2015).

2.2.2 Cover-slip culture technique

This technique was performed to study the morphology of the actinomycetes. Sterile SCA medium was poured into Petri plates and 3-4 sterile square coverslips were inserted into the medium at an angle of 45°C. The broth culture of actinomycetes was slowly released over the coverslip at the intersection of the medium and the coverslip. The plates were incubated at 28°C for 4-8 days. The coverslips were removed and stained with Lactophenol cotton blue and then observed under a Light microscope (45X) (Jagan Mohan et al. 2014).

2.2.3 Determination of cell wall amino acid

A yeast extract medium was prepared and the culture was inoculated into the sterile medium. After 4 days of incubation, the culture was centrifuged and washed with methanol. The upper layer was removed and the precipitate was washed and it was then freeze-dried. From this, 20g of the freeze-dried pellet was dissolved in 5 mL of 6N HCl at 100°C for 18 hours and evaporated to remove HCl. After evaporation, the pellet was subjected to thin-layer chromatography (TLC). The solvent system contained methanol: H_2O: 10 M HCl: Pyridine in the ratio of 80:26:25:20 by volume (Becker et al.1964). The bands were viewed by spraying ninhydrin (0.1%) solution.

2.2.4 Molecular identification of Halotolerant actinomycetes

The DNA was extracted by the phenol-chloroform method. The extracted DNA sample was amplified by PCR technique consisting of initial denaturation (90s at 94ºC), 30 cycles (denaturation for 15s at 94ºC, annealing for 10s at 60°*C*; extension for 30s at 72ºC), and final extension for 4 min at 72ºC. Primers used were 5AGAGTTTGATCCTGGCTCAG-3 and 5'-ACGGCTACCTTGT TACGACTT-3'(Weisburg et al. 1991). Then the PCR product was subjected to sequencing. Nucleotide sequence data were obtained from DNA sequencing software of ABI 3730xl DNA analyzer (Model 373, Forster, CA, USA) (Monciardini et al. 2002; Li et al. 2016; Chen et al. 2016). Multiple sequence alignment was performed using CLUSTAL W and then the sequences were submitted to NCBI (GenBank).

2. 3 Selection of amylase production medium

Six different optimized media for actinomycetes from various articles were screened for enzyme production, which included: Amylase production medium 1 (APM 1) (Chao-Hsun and Wen-Hsiung 2004), Amylase production medium 2 (APM 2) (Poornima et al. 2008), Amylase production medium 3 (APM 3) (Ray and Kar 2009), Amylase production medium 4 (APM 4) (Prabavathy et al. 2006), Amylase production medium 5 (APM 5) (Suman and Ramesh, 2010) and Amylase production medium 6 (APM 6) (Stamford et al. 2001). The culture was grown in different production media (APM 1 - 6) and incubated at 24°C for eight days, after incubation the enzyme activity was visualized by a zone of lysis by adding the indicator. Protein concentration was determined by a dye-binding method of Bradford using Bovine Serum Albumin as the standard protein **(**Bradford 1976**).**

2.3.1 DNS assay method

The culture supernatant was used for amylase activity and the activity was estimated by a modified DNS assay method. The assay was carried out at 50°C using 1% starch as a substrate. The substrate was prepared in 100 mM Glycine NaOH buffer (pH10.5). About 0.5 ml of starch buffer solution was pre-incubated at room temperature for 5min. The reaction was initiated by adding 1 ml of the enzyme. After incubation for 60 min at 5°C, the reaction was terminated by adding 2 ml of 1% dinitrosalicylic acid (DNS) reagent. The content was incubated for 15 min at 50°C. Further, 1 ml of 40% Rochell's salt solution (Potassium sodium tartrate) was added and the sample absorbance was read at 540 nm. One unit of enzyme activity was defined as 1µg of glucose released per min (Ashabil et al. 2008).

$$\text{Enzyme activity} = \frac{\text{Amount of glucose liberated } \left(\frac{\text{mg}}{\text{ml}}\right) \text{ x Total assay volume}}{\text{Volume of enzyme x Time of incubation x Volume in cuvette}}$$

2.4 Optimization of the amylase production medium for increased enzyme production

The culture was grown in different time intervals (1 to 9 days), different starch concentrations (0.5% to 3.5%), different temperatures (4°C to 55°C), different NaCl concentrations (3% to 7 %), and different pH levels (pH 5 to11) to get a suitable medium for maximum amylase production. The enzyme activity was visualized as a zone of clearance by adding the indicator and measure zone in diameter (mm) (Balakrishnan et al. 2021)

2.5 Mass production in optimized amylase production medium

The culture MML2028 was grown in an optimized production medium at 28°C for 8 days. Then, the culture broth was centrifuged at 10,000 rpm for 20min and the supernatant containing the crude enzyme was collected.

2.6 Partial purification of amylase produced by Halotolerant actinomycete MML2028

All the steps in partial purification were carried out at 4°C. The actinomycetes culture supernatant liquid containing the extracellular enzyme was lyophilized to perform the further assay. The lyophilized supernatant was added with ammonium sulphate (Green and Hughes 1955) with nonstop overnight stirring and removed into the following saturation ranges: 0–20, 20–40, 40–60, and 60–80%. Then it was centrifuged at 12,000*g* for 15 min and the pellet was collected. The pellet was dissolved in 0.1 M citrate-phosphate buffer, pH 5.0. Dialysis was carried out with the same buffer for 12 hours many times to remove the salt content (Plumer 1978).

2.7 Quantitative and Qualitative assay of a partially purified amylase

The lyophilized sample was qualitatively assayed for amylase production. The amylase production medium was poured into Petri plates and after solidifications, 8 mm diameter wells were made using a cork borer, 100µL of culture filtrate, and 100 µL of dialyzed protein sample were inoculated in a well. After 24 h of incubation, the enzyme activity was visualized as a clear zone with the addition of an indicator. The zone was determined in diameter (mm). Protein and DNS assays were performed on the lyophilized sample.

2.8 Effect of pH, temperature, salt concentration, and metal ions on amylase activity and stability

To find the temperature stability of the partially purified enzyme was pre-incubated at 16°C to 55°C up to 45 min at the optimum pH and the remaining activity was determined under standard enzyme assay conditions. For pH stability, the enzyme was pre-incubated at pH between 4 to 10 at 24°C for one and two hours. NaCl concentration up to 3 to 7% was used according to the standard enzyme assay conditions.

The effects of metal ions ($CaCl_2$, $MgSo_4$, $CoCl_2$, $MnSo_4$, $ZnS0_4$) and chemical reagents including chelating agents and inhibitors (EDTA, Triton, Beta Mercaptoethanol, Urea, SDS) on partially purified enzyme activity were studied by pre-incubating the enzyme in the presence of substances with a concentration of 3 to 5 mM for 45 min at 24°C, and then continue the assay in the presence of the same substances at the optimum temperature. The melts ions used for this study were in chloride form.

2.9 SDS - Polyacrylamide gel electrophoresis

The protein content of the lyophilized crude was examined by SDS-PAGE. The sample was resuspended in 1 ml of Phosphate buffer (0.2M, pH 7) and used as the source of protein. Sodium dodecyl sulphate is a very strong anionic detergent. It is an amphiphatic molecule that consists of a non-polar hydrophilic region and a strong polar anionic group. In the presence of SDS and reducing agents such as β-mercaptoethanol, oligomeric proteins are dissociated into their constituent polypeptide chains. These polypeptide chains were shown to migrate in SDS gels of the correct porosity according to their molecular weights. The size of polypeptide chains of given proteins can be determined by comparing its electrophoresis mobility in SDS gels with mobility marker proteins of known molecular weights (Laemmli 1970).

2.10 Application for Amylase

2.10.1 Deinking with Alpha-Amylase

Ink jet-printed paper from an HP printer was pulped by soaking in hot water for 2 hours and macerated in a domestic mixer after adding 0.1 % Tween 80, a non-ionic surfactant. The macerated pulp was oven-dried at 50°C and stored in a sterile container under refrigeration. Immediately before use, the pulp was soaked in water for 30 min. The pulp was sterilized at a consistency of 3-6% (3-6 g pulp in 100 ml of half-strength seawater) by autoclaving. After cooling, it was incubated with the enzyme at room temperature. After 3 to 4 days the decolorized pulp was washed thoroughly with tap water. The washed pulp was filtered to obtain the pulp in the form of hand sheets. These sheets were pressed flat between two stainless steel plates and oven-dried at 50°C for 12 h. They were gently pressed with a steam iron to get uniform thickness (Saxena and Singh Chauhan 2017)

2.10.2 Desizing with α-amylase

A stiff piece of grey fabric having maximum starch was used in the present study. An equal size (5×5 inch) fabric piece was weighed on an electric balance. The cloth strip was then dipped in 100 ml of enzymatic solution (pH 6.5) and then placed in an incubator at 60°C for 1 hr. The cloth strip was washed with tap water and then oven-dried. After drying, the cloth strip was weighed again (Chimata et al. 2011). The % removal of starch was calculated by applying the following formula:

$$\% = \frac{\text{Wt. of starch removed by enzyme}}{\text{Total starch present on the fabric strip}} \text{ x } 100$$

2.11 Statistical analysis

The amylase activity was done in a triplicate manner, and data existing in figures and tables are the mean of three experiments. Statistical assessment for important differences between average values was performed using one-way ANOVA software.

3 Results

3.1 Preliminary screening of amylase enzyme

Cultures for screening amylase activity were obtained from the culture collection of Biocontrol and Microbial Metabolite Lab (MML2001 to MML2030). Among them, halotolerant actinomycete MML2028 showed good amylolytic activity by producing a maximum clear zone of clearance (Table 1).

Table 1 Preliminary screening for amylase production

S. N	Culture	Results	S. N	Culture	Result
1.	MML2001	++	16.	MML2016	+
2.	MML2002	_	17.	MML2017	++
3.	MML2003	++	18.	MML2018	+
4.	MML2004	_	19.	MML2019	-
5.	MML2005	_	20.	MML2020	-
6.	MML2006	++	21.	MML2021	+
7.	MML2007	_	22.	MML2022	+
8.	MML2008	++	23.	MML2023	+
9.	MML2009	_	24.	MML2024	++
10.	MML2010	_	25.	MML2025	++
11.	MML2011	_	26.	MML2026	-
12.	MML2012	+	27.	MML2027	-
13.	MML2013	_	28.	MML2028	+++
14.	MML2014	+	29.	MML2029	+
15.	MML2015	+	30.	MML2030	-

3.2 Identification of haloterant actinomyceteMML2028

3.2.1 Colony morphology

Colonies were raised, wrinkled, opaque, pink coloured, earthy odour, and non-motile. These morphological characters are found to coincide with the characters of *Streptomyces* sp. based on Bergey's manual of systematic bacteriology (Table 2).

Table 2 Colony morphology of halotolerant actinomycetes MML2028

Colony Morphology	Characteristics
Elevation	Raised
Surface	Wrinkled
Density	Opaque
Size	Very Long Rods
Shape	Rods
Pigment and Odor	Pink, Earthy
Spore and Sporangia	Positive
Mycelium	Aerial
Spore Motility	Non Motile
Spore Surface	Smooth and Hairy

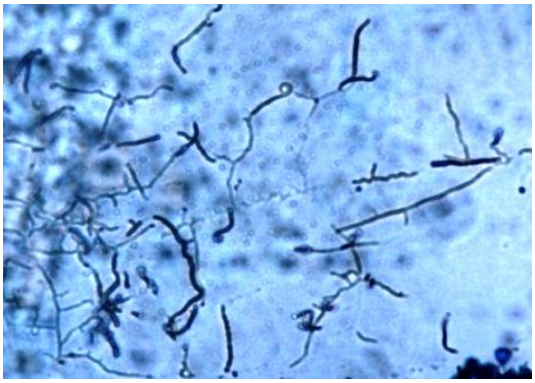


Figure 1 Morphological view of MML 2028 on Cover-slip culture technique

3.2.2 Cover-slip culture technique

Coverslip culture of MML2028 was observed periodically under a phase contrast microscope from day 2 onwards up to seven days. The development of dense substrate mycelium was observed after 2 days, which later gave rise to highly branched aerial mycelium with a typical spiral branching pattern (Figure 1).

3.2.3 Determination of cell wall amino acid

The cell wall amino acid analysis of MML2028on TLC revealed the presence of LL-diaminopimelic acid (LL-DAP), which was visualized as a yellow spot with R_f value of 0.71. The same R_f value was also determined for the LL-DAP of the reference strain, *S. avermitilis*. In addition to the above yellow LL-DAP spot, glycine was observed as a pink spot (cell wall type I) (Figure 2).

3.2.4 Molecular identification of halophilic actinomycetes MML 2028

DNA was isolated and it was amplified by PCR technique. The PCR products were sequenced and the sequences were compared with 16S rRNA gene sequences available in the NCBI database by BLASTn search. The sequence showed 97% of similarity to *S. brasiliensis.* The GEN bank accession Number is KF542679.

3.3 Selection of amylase production medium

Among the six amylase production medium used, APM1 was found as an efficient production medium for the production of amylase enzyme, protein estimation, and DNS enzyme assay was recorded and tabulated (Figure 3a to f).

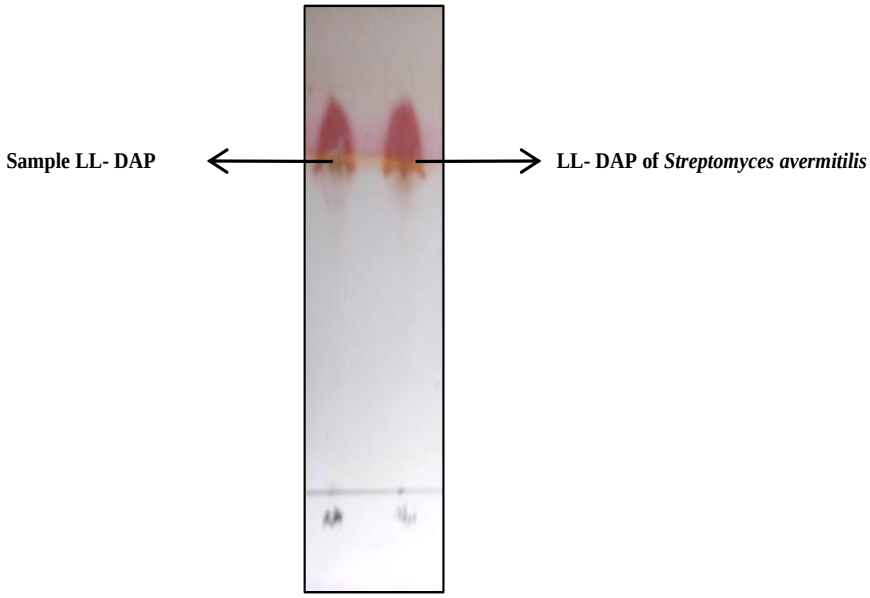


Figure 2 Determination of cell wall amino acid

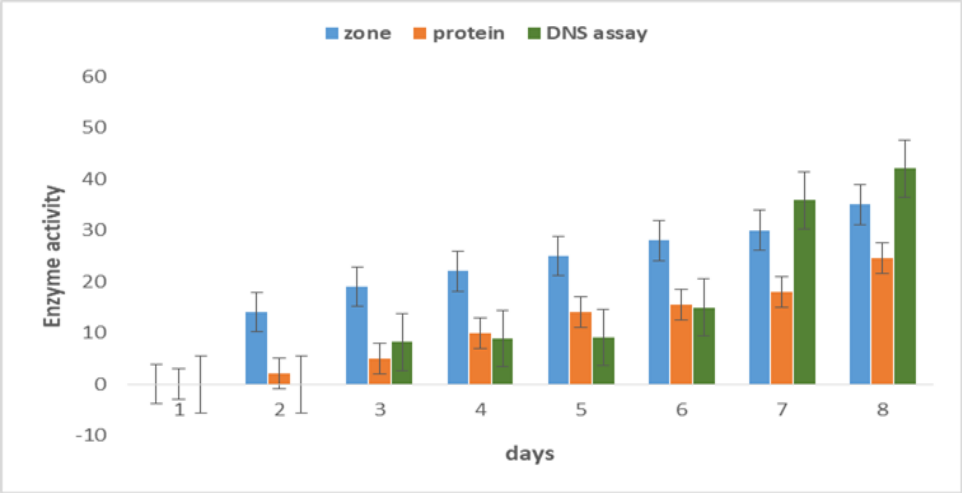


Figure 3a Screening of Halotolerant *S. brasiliensis* MML2028 in APM 1 for amylase production

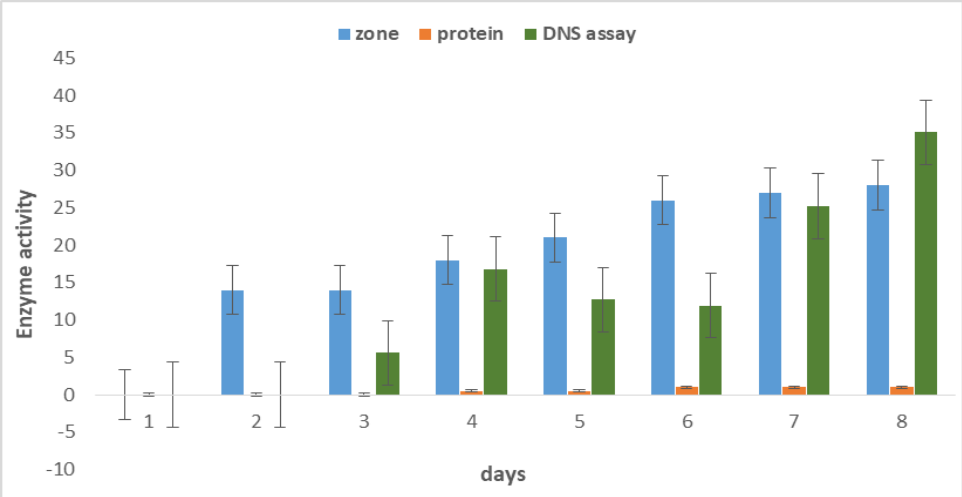


Figure 3b Screening of Halotolerant *S. brasiliensis* MML2028 in APM 2 for amylase production

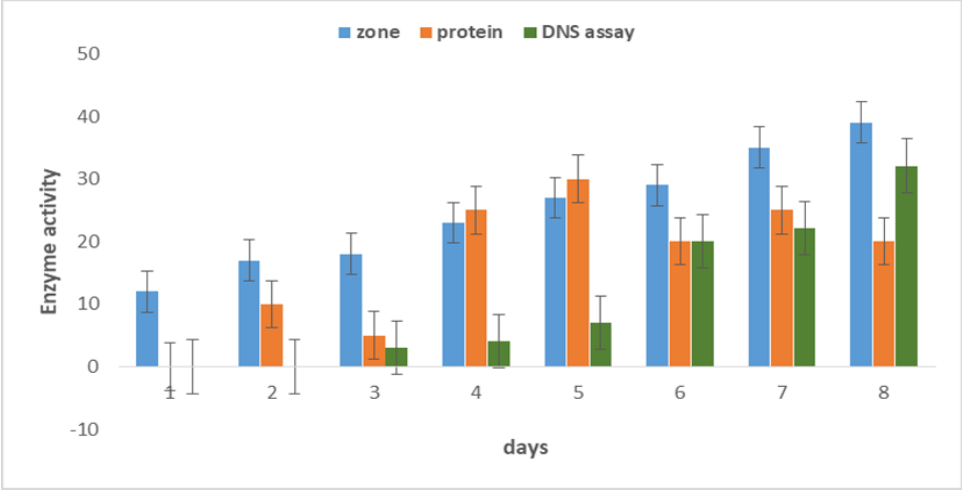


Figure 3c Screening of Halotolerant *S. brasiliensis* MML2028 in APM 3 for amylase production

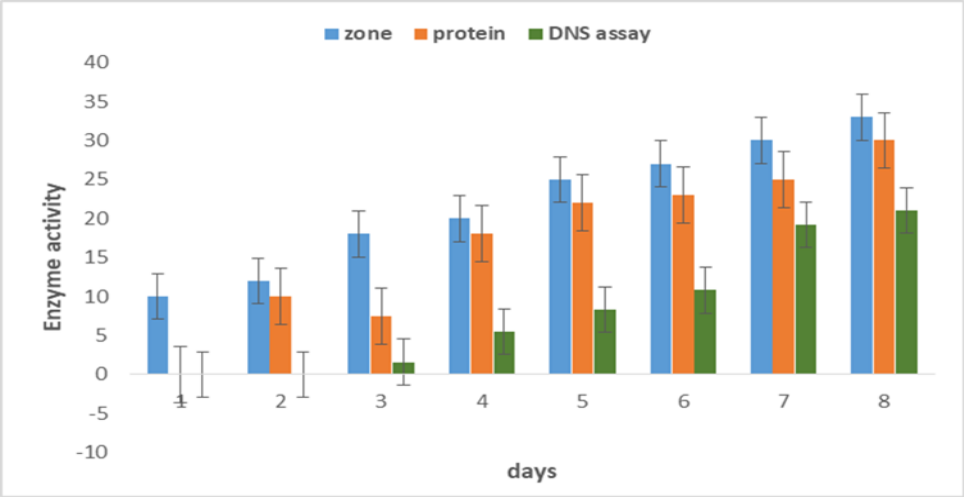


Figure 3d Screening of Halotolerant *S. brasiliensis* MML2028 in APM 4 for amylase production

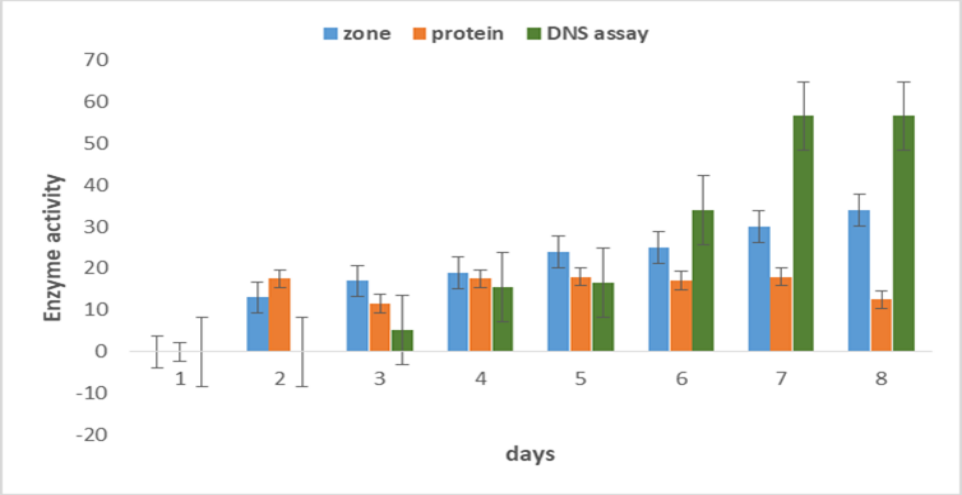


Figure 3e Screening of Halotolerant *S. brasiliensis* MML2028 in APM 5 for amylase production

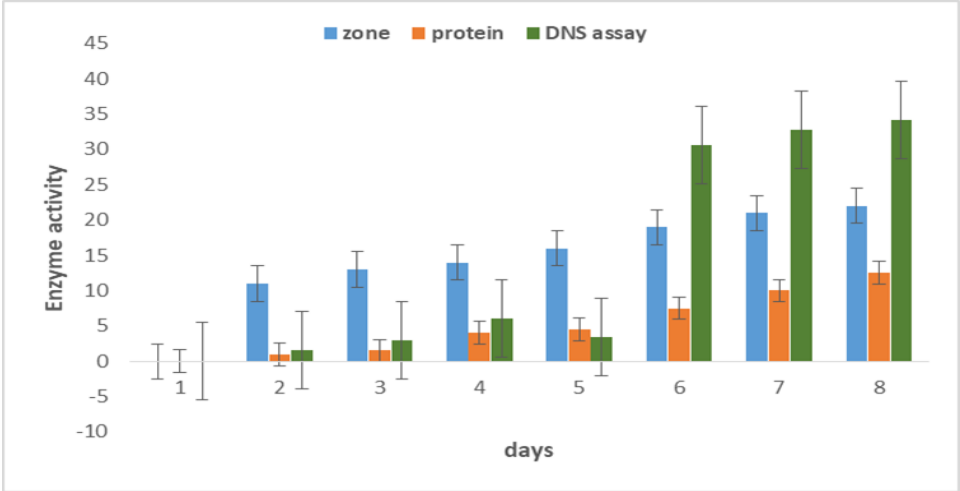


Figure 3f Screening of Halotolerant *S. brasiliensis* MML2028 in APM 6 for amylase production

3.4 Optimization of amylase production medium for increased enzyme production

Maximum production of amylase was recorded in 1% starch concentration and 21 mm in well diffusion assay. In different levels of pH, the maximum zone was visualized at pH 9 with 36mm. In the case of different temperatures (4°C, 16°C, 24°C, 37°C, 45°Cand 55°C) the optimum temperature for enzyme production was observed 24°C and the maximum zone of clearance was observed to be 31mm. Moreover, the enzyme production was found to be maximum at 3% NaCl, with a 30 mm zone. The culture was grown in different time intervals (1 to 9 days) in an APM1 medium to determine the suitable age for maximum amylase production. The maximum growth and amylase production were observed at 8 days (Figures 4a to d).

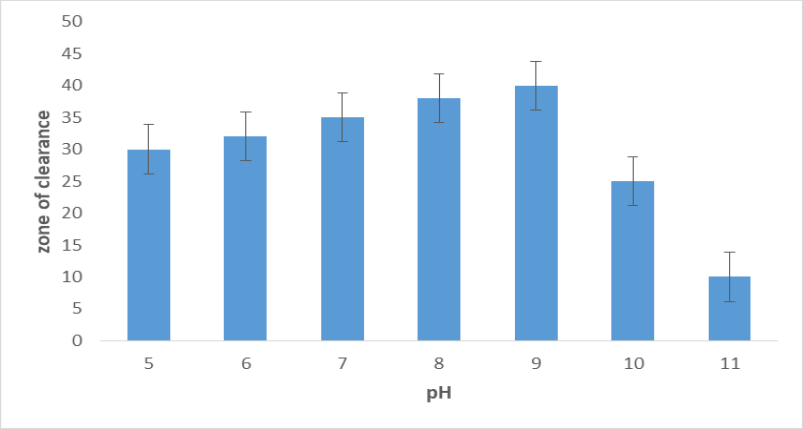


Figure 4a Optimization of different Starch concentrations

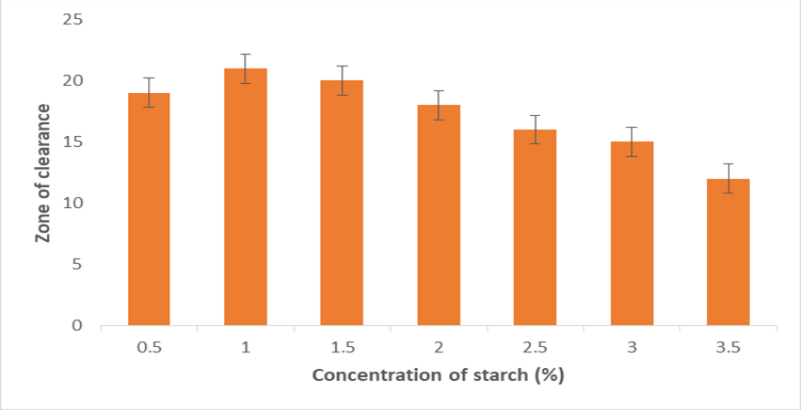


Figure 4b Optimization of different pH

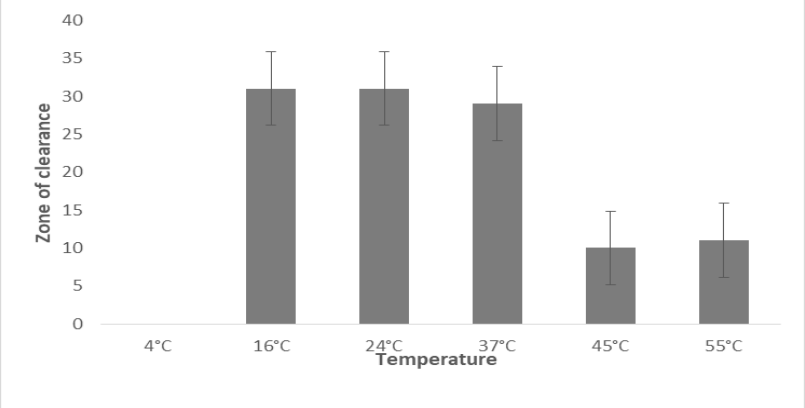


Figure 4c Optimization of different temperatures

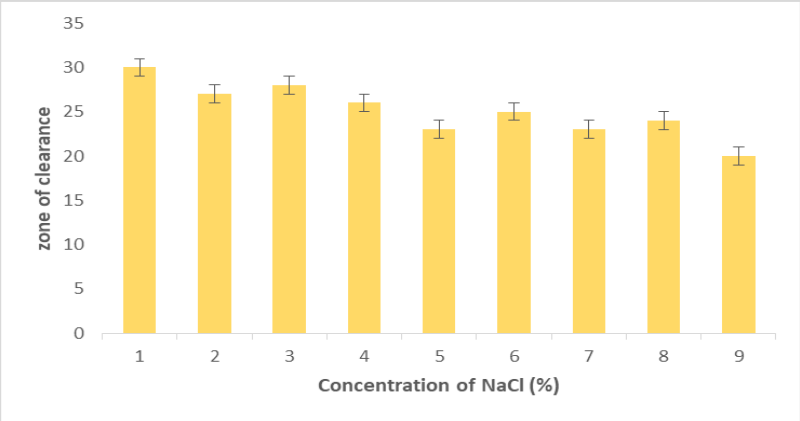


Figure 4d Optimization of different NaCl

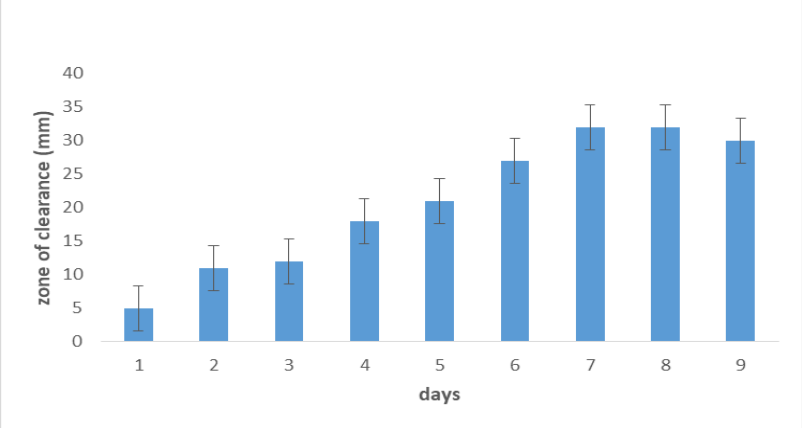


Figure 4e Optimization of different days

3.5 Mass production in optimized amylase production medium

Optimized APM1 medium was prepared for 5 liters and autoclaved. Then the medium was inoculated with 100 ml of 3 days old seed culture of *S. brasiliensis* MML2028and kept in a rotary shaker at 24°Cfor 8 days.

3.6 Partial purification

After 8 days the culture was harvested, centrifuged at 10,000 rpm for 15 min and then the supernatant was collected. The protein was precipitated using 70% ammonium sulphate. Once again, the content was centrifuged and then the pellet was collected and dissolved in 0.2 M phosphate buffer (pH 7.5). The above sample was dialyzed and this partially purified sample was lyophilized which yielded 1.387 g of enzyme sample which was analyzed quantitatively and qualitatively.

3.7 Quantitative and Qualitative assay of a partially purified amylase

The partially purified sample was analyzed by well diffusion method by addition of the sample (50, 100, 150 µl) in the starch abundant medium. The clear zone was visualized by the addition of iodine (indicator) measuring up to 4 mm, 10 mm, and 20 mm respectively as shown in Figure 5. The protein content was estimated by Bradford's method **(**Bradford 1976**).** The reaction mixture consisted of 1 ml sample + 5 ml of CBB-G250 which showed 35 µg/ml. The amount of protein was calculated using BSA standard graph (Figure 5). The lyophilized sample was quantified by DNS (di nitro salicylic acid) assay method as detailed below.

Total activity =

$$\frac{\text{Amount of glucose liberated} \times \text{total assay volume}}{\text{Volume of enzyme} \times \text{Time of incubation} \times \text{volume in cuvette}} = \frac{1.508 \times 5}{0.5 \times 60 \times 2}$$

= 1809.6 U/mL

Parameter	Optical density (O.D)
Live (enzyme + substrate)	1.508
Heat killed (enzyme killed+ substrate)	1.267
Enzyme blank	0.007
Substrate blank	0.029

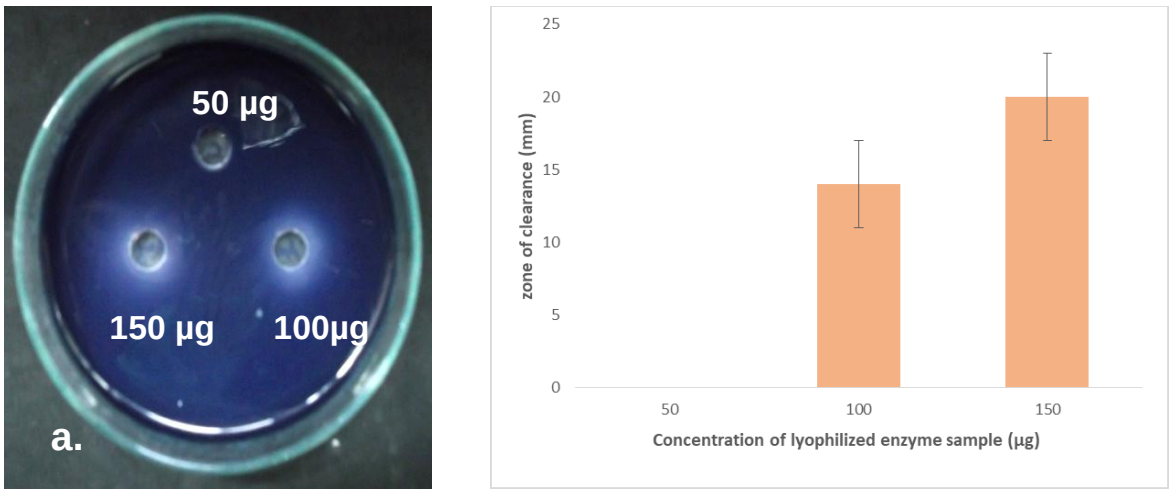


Figure 5 Well diffusion assay of partially purified amylase enzyme

3.8 Effect of pH, temperature, salt concentration, and metal ions on amylase activity and stability

The optimum pH was determined using four different buffer systems. Through the protein and plate assays, the activity and stability of the enzyme was found to be maximum at pH 8. For the thermal stability estimation, the enzyme was pre-incubated at a temperature between 4°C and 55°C from 15 min to 60 min at the optimum pH, and it was found that the enzyme was stable up to 24°C. The optimal salt concentration for maximum activity was 3% of NaCl. Although the enzyme activity increased in the presence of 3% and 5% of NaCl, the activity was observed to be lost in the presence of 7% NaCl. The enzyme was noted to be stable in Mg^{2+} and Zn^{2+}ions and was lost with metals like Fe^{3+}, Co^{2+}, $Ca^{2+,}$ and Mn^{2+}. Among the tested inhibitors, EDTA and SDS (5 mM) was the most inhibitory in which the enzyme lost its activity (Figure 6a to 6e).

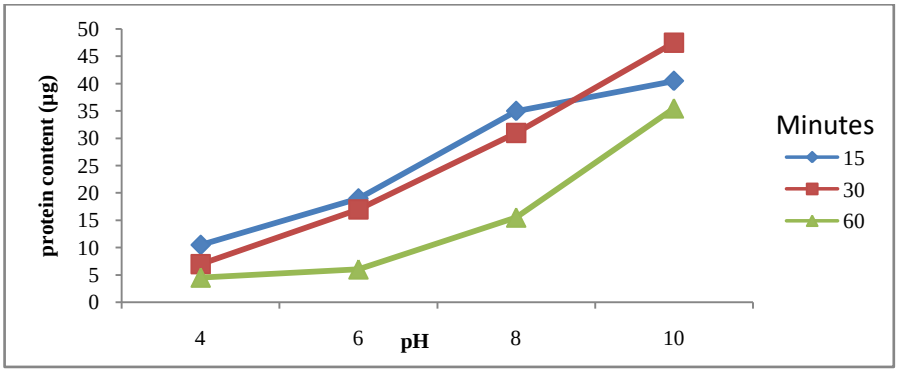


Figure 6a Effect of pH on activity and stability of amylase enzyme

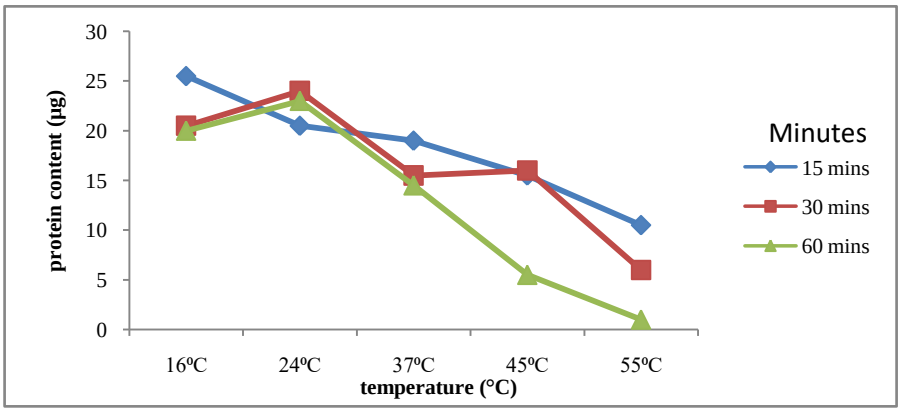


Figure 6b Effect of temperature on activity and stability of amylase enzyme

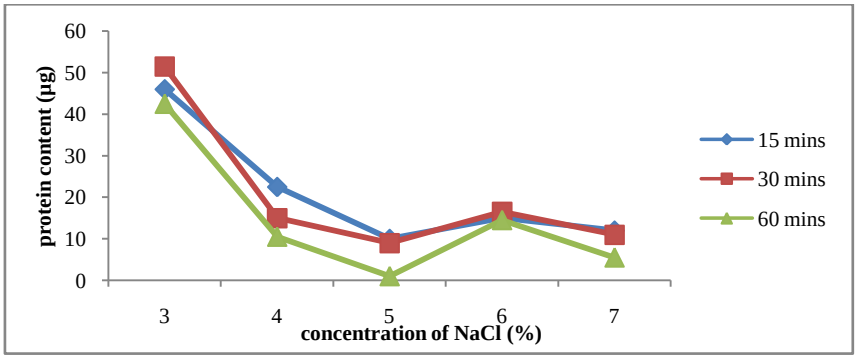


Figure 6c Effect of NaCl on activity and stability of amylase enzyme

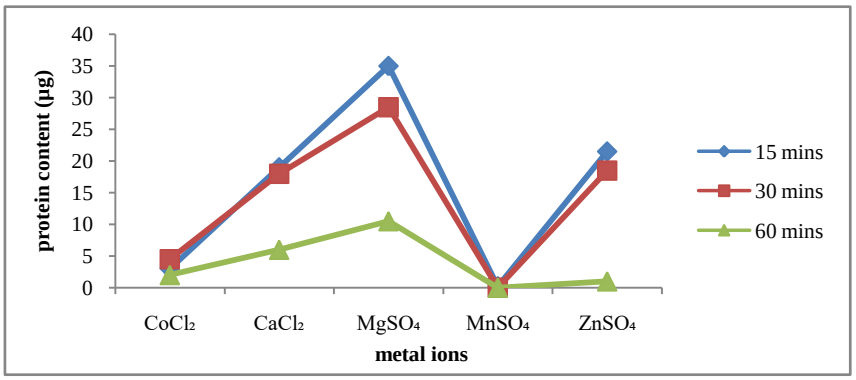


Figure 6d Effect of metal ions on activity and stability of amylase enzyme

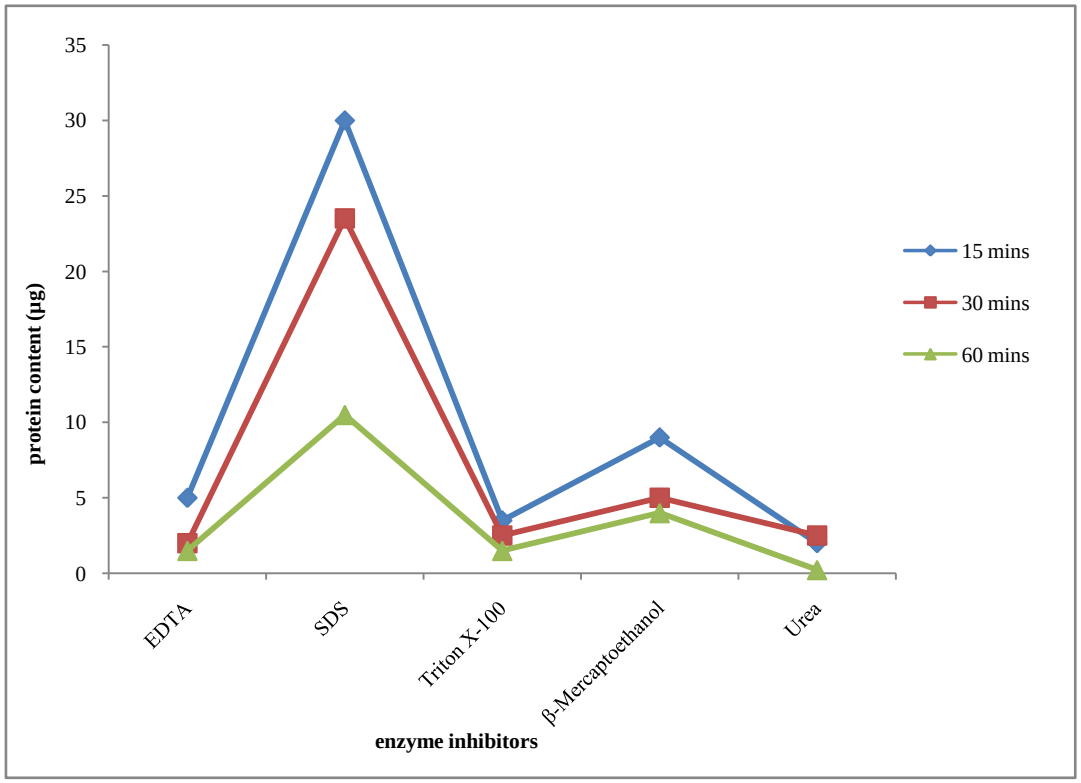
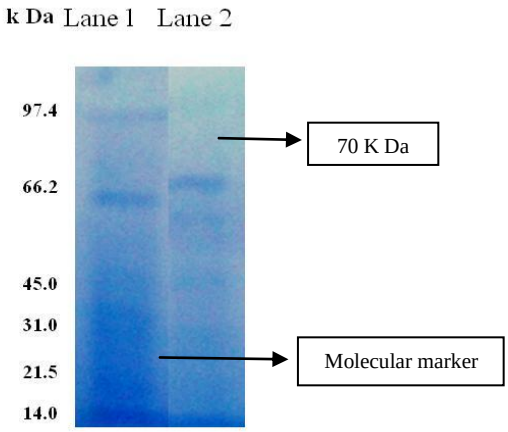


Figure 6e Effect of metal ions on activity and stability of amylase enzyme



Lane 1 - Protein marker; Lane 2 - Enzyme sample

Figure 7 Protein analysis of amylase by SDS – PAGE

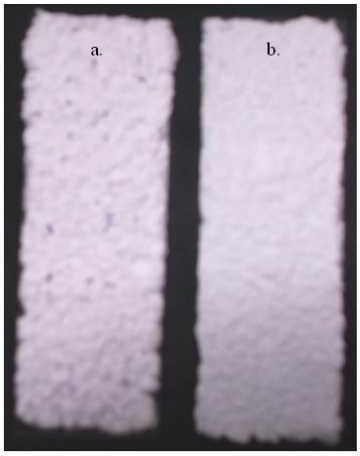
3.9 SDS - polyacrylamide gel electrophoresis

Proteins after the partial purification followed by lyophilization were separated using 12% acrylamide in SDS - polyacrylamide gel electrophoresis. Protein bands were visualized by staining in CBB-R250 which revealed a prominent band estimated to be 70 kDa (Figure 7).

3.10 Applications of Amylase

3.10.1 Deinking

The amylase treated paper showed far better results while compared with the control. The effective removal of ink from the paper pulp was clearly visualized. The enzyme is good enough to be applied in paper industry for deinking (Figure 8a).

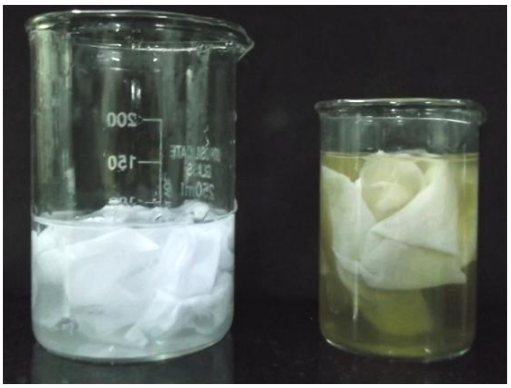


a. control, b. enzyme treated paper

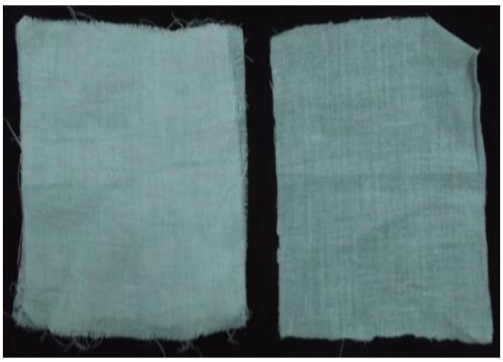
Figure 8a Deinking of paper

3.10.2 Desizing

The desizing of starch from the fabric by α-amylase was calculated as 81%. This shows the effective removal of starch by the enzyme and is hence considered to have an effective role in the textile industry (Figure 8b).



a. Starch de-sizing in water



a. Control; b. Test

Figure 8b Starch de-sizing in amylase enzyme

4 Discussions

In this work, an attempt has been made to produce stable amylase from halotolerant actinomycetes, isolated from the soil sample collected from the Kelambakkam salt pan. *S. brasiliensis* MML2028 was found to be the best producer of amylase enzyme, among the 30 actinomycetes cultures. *Streptomyces sp.* was reported to grow well on starch casein agar (SCA) as reported by earlier researchers (Laidi et al. 2008; Valsalam et al. 2019). Hence, SCA media enriched with 30 days old 100% natural seawater was used for the isolation and subculturing of actinomycetes (Bhanu et al. 2021).

The morphology of the colonies was elevated with wrinkled surface and solid density was similar to other studies on *Streptomyces sp.* (Djebbah et al. 2021). *Streptomyces* is characterized by dry, tiny compact, soft to powdery colonies constantly attached to the medium forming aerial and substrate mycelium with an earthy smell (Sathi et al. 2001; Yanti et al. 2019). The developed mycelia are colourless to white, chalky red, or grey olive (Oskay et al. 2004). The conversion of spore colour from gray to dark brown is a trait of the genus *Streptomyces*. The unique property of the genus *Streptomyces* is the formation of spira and retinaculliaperti spores (Li et al. 2016). The colour of the *Streptomyces* is due to pigment production. Some of the pigments are phenazines, phenoxazinones, and prodiginines as reported by Abdur Rahman et al. (2000). These were sporangia and spore-forming in nature and spores were non-motile with sporulating nature in the surfaces (You et al. 2005).

The cell wall amino acid assay also confirmed the test organism as *Streptomyces.* Similarly, strain AE-19 contains LL-Diaminopmelic (LL-DAP) and glycine on the cell wall. This indicates that the *Streptomyces* belongs to chemo type-I. *Streptomyces*, *Streptoverticillium*, *Chainia*, *Actinopycnidium*, *Actinosporangium*, *Elyptrosporangium*, *Microellbosporia*, *Sporichthya*, and *Intrasporangium* are examples of the chemotype-I cell wall (Lechevalier and Lechevalier 1970). Polyphasic taxonomy for the identification of the culture revealed that it was *S.brasiliensis* which can able to tolerate and grow in NaCl.

The six optimized amylase production media screened for enhanced production of amylase showed good results, whereas, amylase production in medium 1 showed constant enzyme production even in an alkaline pH. Similarly, Singh et al. (2017) used three production media for amylase production. Roy et al. (2012) observed the maximum amylase activity at pH 6 while Demirkan et al. (2017**)** reported the maximum enzyme activity at pH 7. Sodhi et al. (2005) found that the enzyme activity was higher at pH 6.5. However, in the current study, the maximum activity was reported at pH 9 and these results are in agreement with the findings of Saxena et al. (2007). Since alkaline amylases are considered to be efficient, especially in the detergent industry, the amylase produced by this alkaline medium is considered to be an alkali-stable amylase, and therefore, this production medium was chosen for mass production.

Molecular weights (MW) of α-amylases differ between 10 to 210 kDa. The least MW was found = 10 kDa in *Bacillus caldolyticus* (Grootegoed et al. 1973) while the highest MW was recorded 210 kDa in *Chloroflexus aurantiacus* (Ratanakhanokchai et al. 1992). Further, the molecular weight of the partially purified amylase enzyme was 70 KDa. Similarly, 70 K Da was reported in *T. harzianum* amylase A3 (Mohamed et al. 2011).

The temperature for the actinomycete of the present study was optimized as 24°C which indicates that the enzyme is not thermostable, likewise, the strain AE-19 showed maximum amylase activity at the temperature of 45°C which further indicates that the enzyme is not thermostable. In contrast, Stamford et al. (2001); Nipkow et al. (1989); Kundu (2006) reported that the as optimum temperature for the production of amylase was found to be 70°C, 50°C, and 60°C respectively. This discloses that the optimum conditions for amylase enzyme production differ from species to species (Eman et al. 2018).

The partial purification of the enzyme includes ammonium sulphate precipitation and Dialysis in phosphate buffer pH 7.5. The dialyzed sample was lyophilized for future use. The maximum enzyme activity was found to be 1809.6 U/mLwas observed in AMP1 medium. Similarly, the maximum production was found 2000 U/mL in soil microorganisms (Vidyalakshmi et al. 2009; Singh et al. 2017).

The partially purified enzyme is studied for its effect on different concentrations of NaCl, temperature, pH, metal ions, and inhibitors, and their results proved stability with NaCl at 3%, temperature at 24°C, and pH 8. The enzyme was stable in Urea and Triton – X. Similar results were reported by Roy et al. (2014).

Conclusion

In the present study, it was concluded that the soil samples of the Kelambakkam salt pan are a good source of materials for the isolation of potential actinomycetes. It also revealed that the tentatively identified species, *S. brasiliensis* MML2028 isolated from the soil possesses good α-amylase activity. The study has also standardized the growth parameters of the actinomycetes for maximum enzyme production, which can be effectively used in the large-scale production of enzymes for commercial purposes. The halotolerant and alkaliphilic quality of the amylase enzyme can be used in many industries, especially in paper and textiles.

Conflict of interest

The author and the co-authors declare no conflict of interest.

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