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(RESEARCH ARTICLE)

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Isolation and characterization of alkaline amylase producers from the marine environment of Arabian sea coast

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Abstract

Alkaline amylase producers were isolated from the marine environment of Arabian Sea coast. A total of 10 isolates were obtained from the soil and water samples collected from the marine environment. Five isolates namely; BCSS, JBWS, RBWS, BCWS and ALSS showed good growth on alkaline M9 media with 10% NaCl having starch as the primary carbon source. Based on their maximal amylase activity, three microbial cultures (BCSS, JBWS, and RBWS) were selected for the study. The crude enzyme from these purified cultures was extracted and partial characterization was carried out. The partial characterization study involved the effect of metal ions and surfactants and determination of optimal pH and temperature and its stability studies. The amylase activity was optimum for all three cultures at 60 ^{0}C whereas, the optimum pH was found to be pH 7, pH 8 and pH 9 for RBWS, JBWS and BCSS. The pH and temperature stability studies showed maximum amylase activity at its optimal conditions for 5h (300 mins) in the order BCSS>RBWS>JBWS. The amylase activity was found to increase with sodium (BCSS), manganese (RBWS) and potassium (JBWS) at 1:1 dilution. The cultures were stable with the surfactants (Tween -20, SDS and Triton-X) at 1:10 dilution of 1% surfactant and enzyme. BCSS was the most stable at high temperature and pH among all the isolates with an optimal amylase activity at pH 9 and temperature of 60 ^{0}C. The isolate was identified to be *Bacillus nanhaiisediminis* NH3. These remarkable properties indicated its great potential in industrial applications.

Keywords: Alkaline; Amylase Activity; Stability; Characterization; BCSS

1. Introduction

Amylases are enzymes that catalyze the hydrolysis of glycosidic linkages in starch polymers. The source for obtaining the enzyme is from plants, animals and microbes. Amylase finds wide applications in textile, detergent, pharmaceutical, food and other fields. The enzymes that can retain its activities over a wide range of pH and temperature as well as in the presence of a denaturing agent are increasingly used for industrial applications because it reduces cost and increases substrate solubility. Such enzymes can be produced by the extremophiles [1]. The extremophiles include halotolerant, halophilic, alkalophilic, thermostable, thermophilic enzymes [2, 3] that are secreted by microbes which have an increasing demand in industries [4, 5]. These properties of an extremophile's α-amylase can be used in many harsh industrial processes where the concentrated salt solutions used would otherwise inhibit many enzymatic conversions [6]. Bacillus strains have been extensively used industrially to produce amylase. Numerous studies have been reported by different bacterial strains with diverse growth conditions and enzymatic production profile. They include *B. amyloliquifaciens* [7], *B. subtilis* [8], *B. licheniformis* [9, 10], *B. stearothermophilus* [11], *B. thermooleovorans* [12], *B. cereus* [13, 14], *B. barbaricus* [15] and *B. tequilensis* [16]. Thus, several studies have reported amylase producing bacteria. However, no study on amylase producing bacteria from marine samples from Arabian Sea coast has been

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reported. The current study reported the isolation and characterization of a microbial culture obtained from marine environment of Arabian Sea coast.

2. Methods

2.1. Sample collection

Soil and water samples were collected from different locations of Arabian Sea coast namely; Airoli creek, Thane creek (RetiBander), Belapur creek, Juhu beach and Malad creek. The samples were collected in sterile zip-lock polyethylene bags and brought to the laboratory for isolation of microbes. The samples were labeled as per their source and location of collection. Water samples were labeled as WS and soil samples as SS. Accordingly, they were labeled based on their location of sample collection; Airoli lake water and soil sample (ALWS; ALSS), RetiBandar water and soil sample (RBWS; RBSS), Belapur creek water and soil sample (BCWS; BCSS), Juhu Beach water and soil sample (JBWS; JBSS), Malad creek water sample (MCWS; MCSS).

2.2. Screening and isolation of amylase producers

The collected soil samples were diluted by serial dilution technique. The isolation was carried out by serial dilution method. The soil sample was weighed (1gm) and water sample (1ml) was suspended in 10ml of sterile saline and was mixed by vortexing. The pour plate technique was carried out for soil samples while spread plate technique was done using 100µl of the diluted samples from each dilution from 10^{-1} to 10^{-8}. The screening of alkaline amylase producers was done using M9 minimal media agar plate with starch as the primary source of carbon (pH 10) with 10% NaCl. After 24h of incubation, the growth was observed on the plates. Five isolates (BCSS, JBWS, RBWS, and BCWS AND ALSS) were found to be positive. Specific colonies were selected, cultured and were further purified by repeated streaking. They were stored at 4 ^{0}C and sub-cultured at 15 days interval.

2.3. Cultivation of the amylase producers

The bacterial isolates were cultivated in M9 minimal medium containing Na_2HPO_4 (6 g/L), KH_2PO_4 (3 g/L), NH_4Cl (1 g/L), $MgSO_4.7H_2O$ (0.24 g/L), $CaCl_2$ (0.24 g/L), NaCl (10%), Peptone (3 g/L), Soluble Starch (10g/L). The pH of the medium was adjusted to 10 with 10% Na_2CO_3. Cultures were grown for 24 hours at 37^{0}C in a shaker incubator at 121 rpm. Cell free supernatant was used as the crude enzyme for further studies.

2.4. Amylase Assay

The amylase assay was done by using 3,5-di-nitro salicylic acid (DNSA) method following the method of Miller [17]. The reaction mix containing 0.5ml of 1% soluble starch as the substrate, 0.5ml of the crude enzyme was incubated for 30 mins at 37 ^{0}C with 0.1M phosphate buffer. Appropriate controls were maintained. The reaction was stopped by addition of 1ml DNSA and heated in boiling water for 10min. the absorbance was read at 540nm after cooling. One unit of amylase is defined as the amount of enzyme releasing 1µg of reducing sugars per ml per minute under the standard assay conditions in 30mins at 37 ^{0}C. A standard calibration curve was plotted to calculate the concentration of the product formed followed by the amylase activity.

2.5. Effect of temperature and Study of Thermostability

The effect of temperature on production of amylase was studied by incubating the buffered substrate (1% starch in 0.1 M phosphate buffer pH 7.0) with crude enzyme extracted from the three isolates namely; BCSS, JBWS, RBWS at various temperatures 30 ^{0}C, 37 ^{0}C, 45 ^{0}C, 60 ^{0}C, 80 ^{0}C and 100 ^{0}C. After 30 mins of incubation, the amylase assay was done under the standard assay conditions [18]. The optimum temperature was determined. The thermal stability of amylase was determined by measuring the residual enzyme activity after incubating the buffered substrate (1% starch) with an aliquot of the crude enzyme extracted from the three isolates BCSS, JBWS, RBWS and were kept at three different temperatures viz; 60 ^{0}C, 80 ^{0}C and 100 ^{0}C at different time interval of 30 mins, 60 mins, 90 mins, 120 mins, 180 mins, 240 mins and 300 mins.

2.6. Effect of pH and Study of pH stability

The effect of pH on production of amylase was studied by incubating the crude enzyme extracted from the three isolates namely; BCSS, JBWS, RBWS with substrate (1% starch) prepared in 0.1 M buffers of varying pH; phosphate buffer (pH 6 and pH 7), Tris buffer (pH 8), glycine NaOH buffer (pH 9 and 10) and $NaHCO_3$-NaOH (pH 11). The pH stability was determined by pre-incubating the buffered substrate (1% starch) with amylase extracted from the three isolates BCSS, JBWS, RBWS and were kept with various buffers at pH 6, pH 7, pH 8, pH 9, pH 10 and pH 11. The enzyme activity was

carried out under the standard assay conditions after an incubation of 30 mins, 60 mins, 120 mins, 180 mins, 240 mins and 300 mins [10].

2.7. Effect of metal ions and Surfactants

The effect of different metal salts of such as NaCl, $CaCl_2$, $CuCl_2$, $MnCl_2$, $MgCl_2$, $HgCl_2$ and KCl (5mM concentration each) on the activity of the crude amylase enzyme was measured under the standard assay conditions. Prior to the enzyme assay, the enzyme was pre-incubated for 30 mins in a solution containing respective metal ion solution and crude enzyme in three different sets of the ratio 1:1, 1:2 and 1:10. Similarly, the effect of surfactants on amylolytic activity was estimated in presence of different surfactants like SDS, Tween-20 and Triton-X-100 of 1% concentration each. Prior to the enzyme activity assay, the enzyme was pre-incubated for 30 mins in respective solutions with surfactant: enzyme ratio being 1:1, 1:2 and 1:10 respectively.

2.8. Characterization and Identification of amylase producer

The morphological and molecular characterization was done for the alkaline amylase producer. The morphological characterization included the size, shape, colour, elevation, consistency, motility, gram nature. The molecular characterization was carried out by growing an overnight culture of BCSS isolate at 60 ^{0}C and used for the preparation of genomic DNA. The DNA was isolated using Qiagen DNeasy Extraction kit according to the manufacturer's manual instructions. PCR reaction was carried using the primer pair 27F and 1492R with an annealing temperature of 57°C. PCR was carried out for 30 cycles at 94 ^{0}C for 1min, 57 °C for 1min and 72 ^{0}C for 2min. PCR product was purified using geneO-Spin PCR purification kit and examined for its purity using agarose gel electrophoresis. The PCR product was sequenced using ABI PRISM BigDye Terminator V3.1 kit (Applied Biosystems, USA) at geneOmbio Technologies, Pune.

The bacterium was characterized and identified by 16S rRNA gene sequencing. The 16S rRNA gene sequence was aligned and compared with sequences in GenBank (National Centre for Biotechnology Information NCBI) using the basic local alignment search tool (BLAST) (http://blast.ncbi.nlm.nih.gov/) was used to access the DNA similarities. The sequence was submitted to the GenBank.

2.9. Wash analysis

The cotton cloth pieces (2cm X 2cm) were soiled with blood, chocolate, tomato ketchup and coffee and were dried in the incubator at 60 ^{0}C for 60 mins. The soiled cotton cloth was then subjected to wash treatments with commercial detergent (Tide) diluted in tap water at 7mg/ml, supplemented with crude enzyme. The control was maintained with 7mg/ml of detergent only. Two soiled cloth pieces were taken in separate flasks; flask with tap water and commercial detergent at final concentration of 7mg/ml and flask with tap water, commercial detergent and crude enzyme of *Bacillus nanhaiisediminis* NH3. Each flask was incubated at room temperature with constant stirring (150rpm) for 60 mins. After incubation, cloth pieces were rinsed with tap water and dried. Visual examination of various pieces showed the effect of crude enzyme in the removal of stains [19,20].

3. Results

3.1. Screening and Morphological characterization

Screening of alkaline amylase producers was carried out using M9 media plates having starch as their primary carbon source at pH 10. Five out of ten isolates showed growth on the selective M9 media plates with 10% NaCl. They were isolated, sub-cultured on the selective M9 medium until purified colonies were obtained. The five samples which presented positive results were RBWS, BCWS, JBWS, BCSS and ALSS. Fig. 1 indicates maximum amylase production by BCSS after 72h.

Morphological characterization of these isolates was carried out by studying the colony characteristics that included the size, shape, colour, elevation, consistency, motility, gram nature and presence of spores (Table 1). All the isolates were found to be gram positive rods with spore bearers.

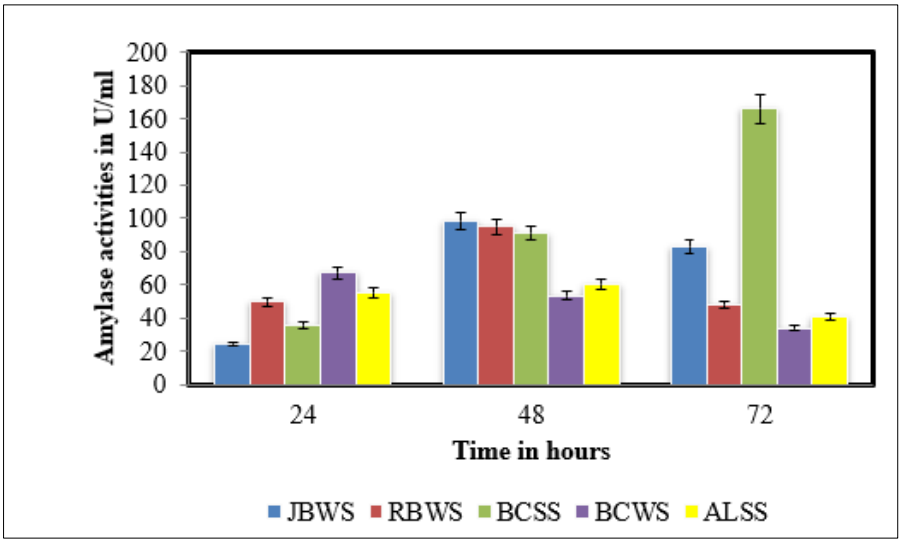

Figure 1 Amylase activity of five samples: JBWS, RCWS, BCCS, and BCWS, ALSS isolated from marine source after an incubation period of 24 h, 48 h and 72 hrs

Table 1 Morphological characterization of isolated cultures

Isolate	Size (mm)	Texture	Colour	Shape	Elevation	Motility	Gram staining	Endospore staining
RBWS	1	Slimy	White	Circular	Flat	+	gm (+ve) short rods	+
BCWS	3	Slimy	Cream	Circular	Flat	–	gm (+ve) cocci	+
JBWS	3	Smooth	White	Circular	Flat	–	gm (+ve) short rods	+
BCSS	1	Slimy	Cream	Circular	Flat	–	gm (+ve) short rods	+
ALSS	1	Slimy	Cream	Circular	Flat	+	gm (+ve) short rods	+

+ positive results; – negative results

3.2. Effect of temperature

Enzyme activity of crude enzyme produced from the isolates RBWS, JBWS and BCSS was checked at 30 ^{0}C, 37 ^{0}C, 45 ^{0}C, 60 ^{0}C, 80 ^{0}C and 100 ^{0}C.

Fig. 2 showed a steady growth in amylase activity for all three isolates upto 60 ^{0}C and then a steady decline in their respective amylase activities. Therefore, the optimal temperature for all the three isolates was found to be 60 ^{0}C. BCSS showed the highest amylase activity at 60 ^{0}C in comparison to JBWS and RBWS.

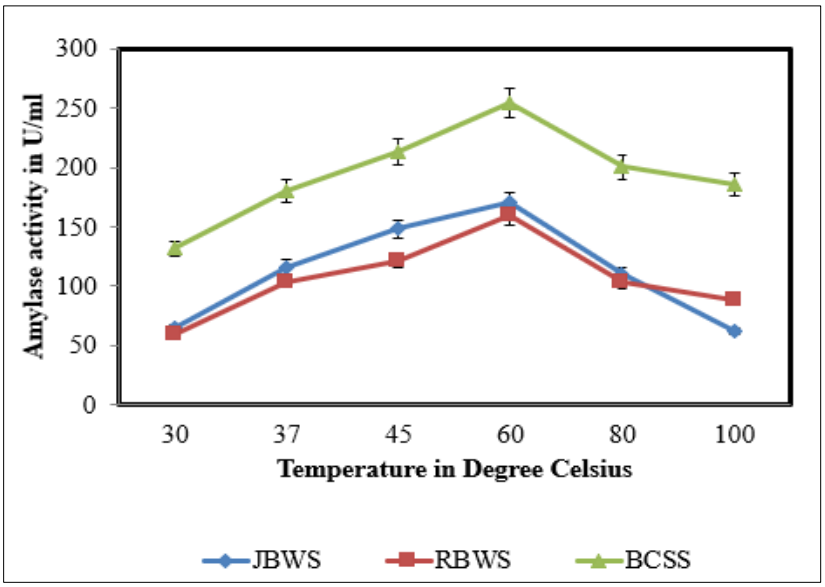

Figure 2 Detemination of optimum temperature of amylase for JBWS, RBWS and BCCS

3.3. Study of Thermostability

Temperature stability studies were carried out for the crude amylase extracted from the three isolates BCSS, JBWS and RBWS at 60 ^{0}C, 80 ^{0}C and 100 ^{0}C at an interval of 30 mins, 60 mins, 90 mins, 120 mins, 180 mins, 240 mins and 300 mins. Of all the three isolates BCSS showed maximum amylase activity of 230 U/ml at 60 ^{0}C, 218 U/ml at 80 ^{0}C and 100 ^{0}C respectively (Fig. 3). BCSS was found to withstand the high temperatures only for an interval of 90 mins at 100 ^{0}C and 80 ^{0}C, but it showed maximum activity of 254 U/ml at 60 ^{0}C for an interval of 300 mins. Whereas, RBWS and JBWS was found to be stable at 60 ^{0}C for a period of 90 min and its activity decreased above 60 ^{0}C.

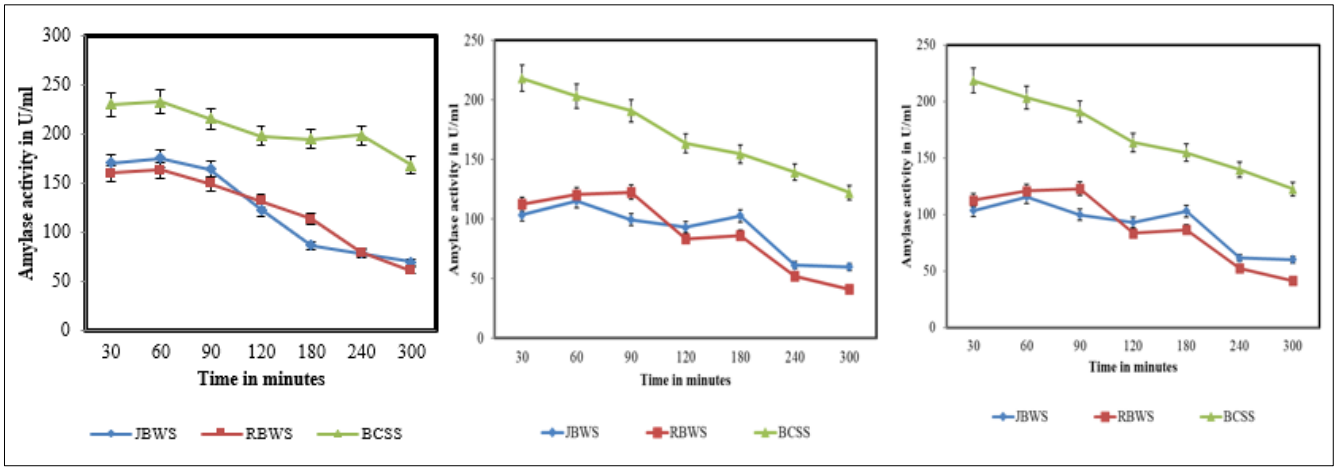

Figure 3 Thermostability study of for amylase activity of JBWS, RBWS and BCCS within a time range of 30 to 300 mins at a temperature of (a) 60 **^{0}C** (b) 80 **^{0}C** (c) 100 **^{0}C**

3.4. Effect of pH

Enzyme activity of crude enzyme produced from the isolates RBWS, JBWS and BCSS was checked at pH 6, 7, 8, 9, 10 and 11. Fig. 4 indicates a steady increase in the amylase activity for BCSS and was seen to be optimal at pH 9. Comparatively RBWS and JBWS showed the optimum pH to be 7 and 8 respectively.

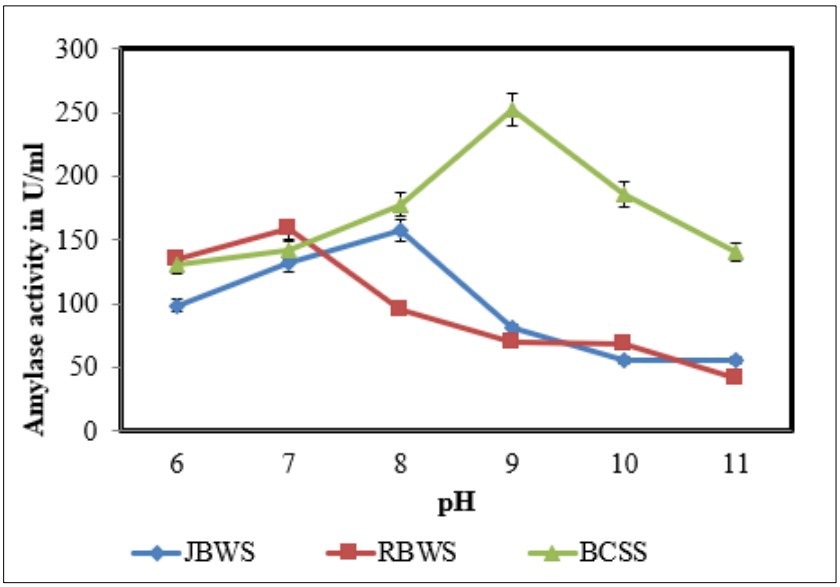

Figure 4 Determination of optimum pH of amylase for JBWS, RBWS and BCCS

3.5. Study of pH stability

pH stability of the crude enzyme extracted from the three isolates BCSS, JBWS and RBWS was observed for pH 6, pH 7, pH 8, pH 9, pH 10 and pH 11. This test was conducted for a time interval of five hours and the enzyme activity was checked at 30, 60, 120, 180, 240 and 300 mins.

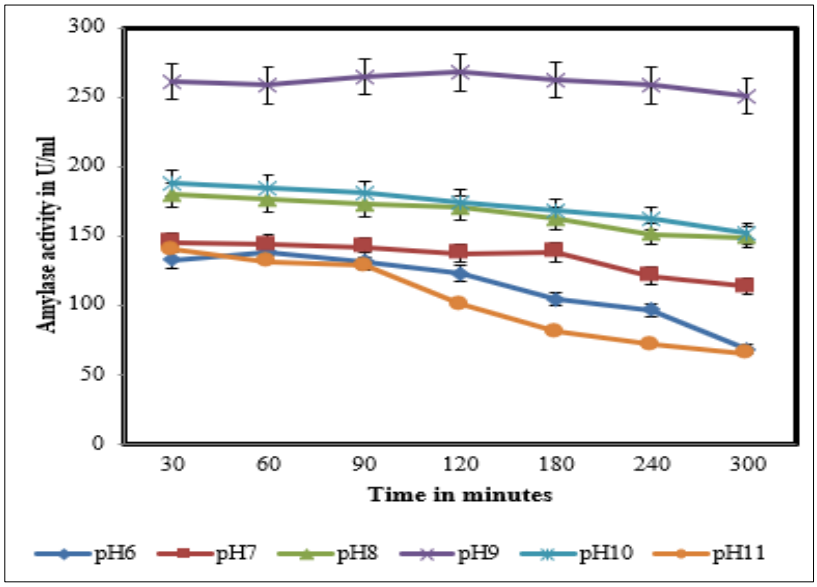

Figure 5 pH-stability study at different pH of 6,7,8,9,10 and 11 for time range of 30 to 300 mins for amylase activity of BCCS

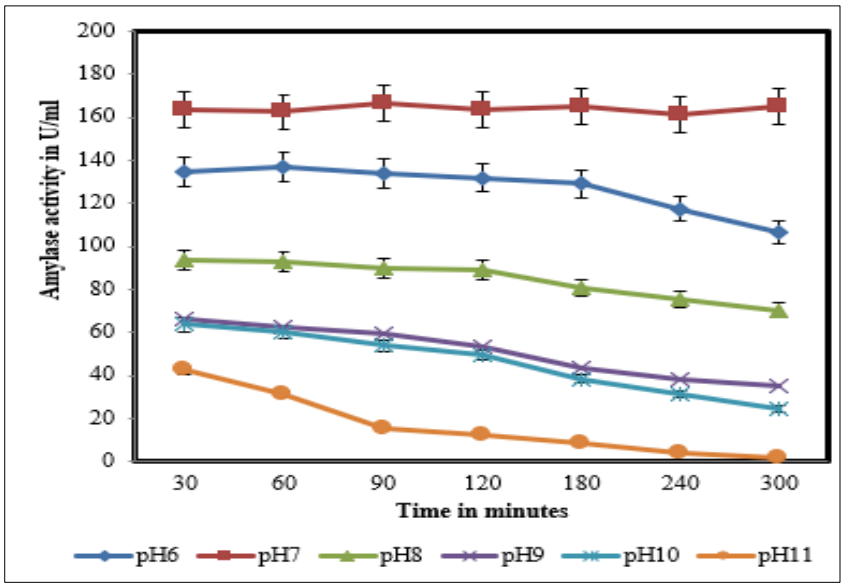

Figure 6 pH-stability study at different pH of 6,7,8,9,10 and 11 for time range of 30 to 300 mins for amylase activity of RBWS

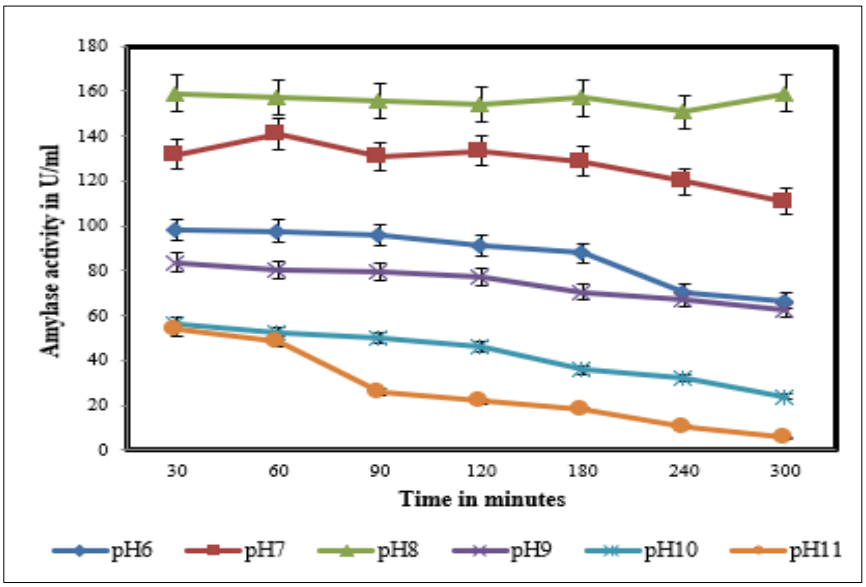

Figure 7 pH-stability study at different pH of 6,7,8,9,10 and 11 for time range of 30 to 300 mins for amylase activity of JBWS

BCSS showed the enzyme activity to be highly active and stable at pH 9 with amylase activity of 250 U/ml for 300mins (Fig.5). Its activity was the highest compared to both RBWS (Fig. 6) and JBWS (Fig. 7). It showed least stability at pH 6, pH 8, pH 10and pH 11, the enzyme activity declined with an increase in time interval. Compared with JBWS and RBWS, BCSS showed stability over a wide range of pH and higher enzyme activity.

3.6. Effect of metal ions

Effect of metal ions were checked by letting the enzyme hydrolyze starch in the presence of different metal salts which include NaCl, $CaCl_2$, $CuCl_2$, $MnCl_2$, $MgCl_2$, $HgCl_2$ and KCl each of 5 mM concentration. Prior to the enzyme activity assay

the enzyme was incubated for 30 mins in respective metal ion solutions with metal ion solution: enzyme ratio being A as 1:1, B as 1:2 and C as 1:10.

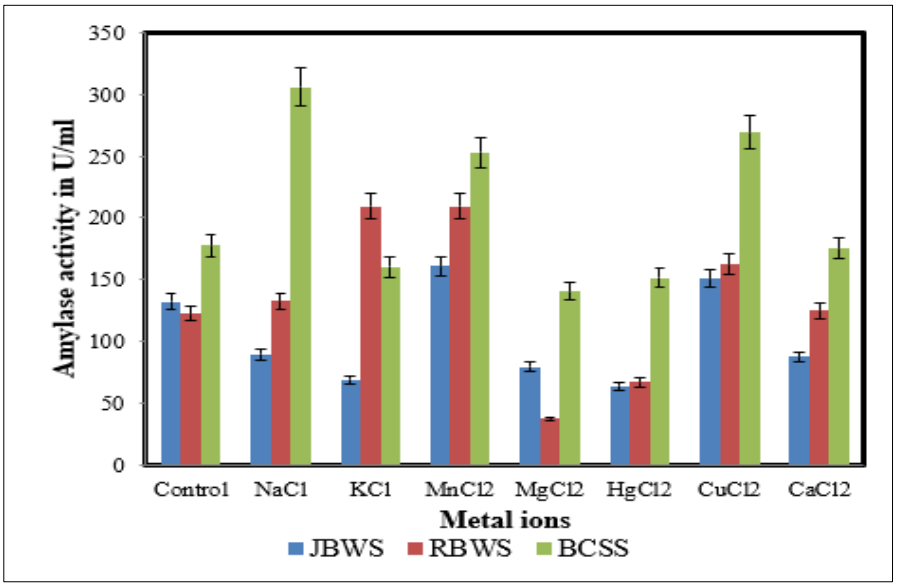

Figure 8 Effect of different metal salts (NaCI. CaCI2, CuCI2, MnCI2, MgCI2 and HgCI2 and KCI) on amylase activity of JBWS, RBWS and BCCS with a concentration of 1:1 ratio of 5 mM metal ion solution and amylase

Fig. 8 and Fig. 9 indicated increasing amylase activity at 1:1 and 1:2 dilution of 300 U/ml for BCSS which was enhanced greatly by the presence of Na^{+}, Mn^{2+} and Cu^{2+} metal ions, while it was minimal with K^{+}, Mg^{2+} and Hg^{2+}. RBWS activity was enhanced in the presence of K^{+}, Mn^{2+} and Cu^{2+} ions and was minimal with Mg^{2+} and Hg^{2+} ions. While, only Mn^{2+} and Cu^{2+} enhanced the activity for JBWS and showed minimal activity with all other metal ions (Fig. 10).

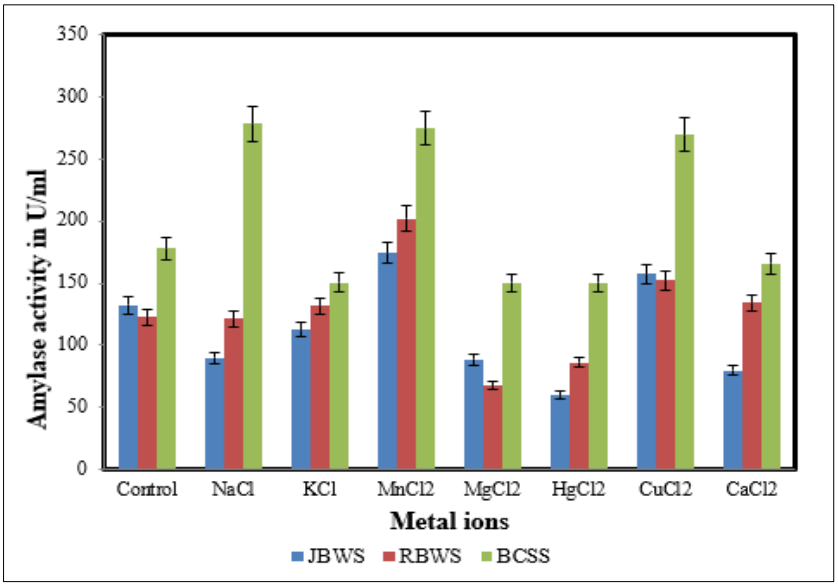

Figure 9 Effect of different metal salts (NaCI, CaCI2, CuCI2, MnCI2, MgCI2, HgCI2 and KCI) on amylase of JBWS, RBWS and BCCS with a concentration of 1:2 ratio of 5 mM metal ion solution and amylase

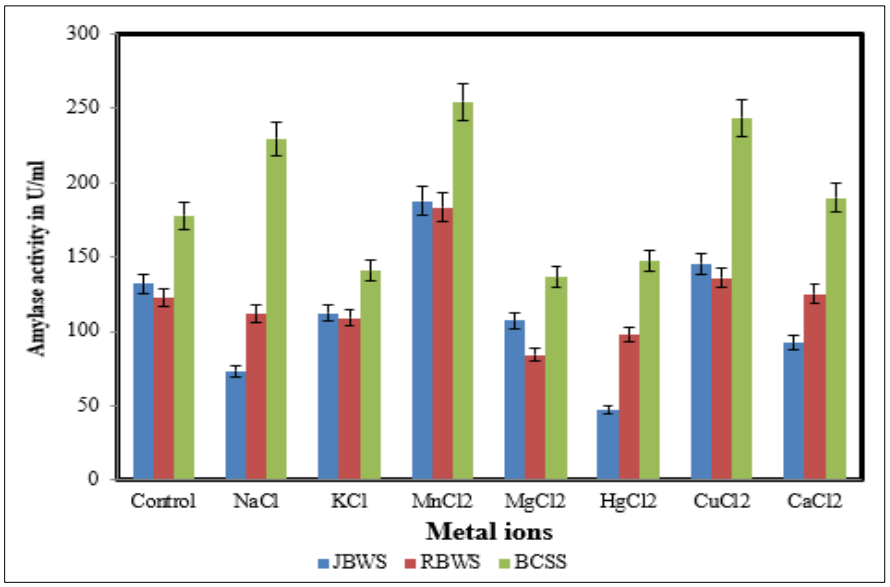

Figure 10 Effect of different metal salts (NaCI, CaCI2, CuCI2, MnCI2, MgCI2, HgCI2 and KCI) on amylase activity JBWS, RBWS and BCCS with a concentration of 1:10 ratio of 5 mM metal ion solution and amylase

3.7. Effect of surfactants

Effect of surfactants was checked by letting the enzyme hydrolyze starch in the presence of different surfactants which include SDS, Tween-20 and Triton-X-100 of 1% concentration. Prior to the enzyme activity assay the enzyme was incubated for 30 mins in respective surfactant solutions. The surfactant solution: enzyme ratio being as 1:1, 1:2 and 1:10 (Fig.11). The effect of surfactant indicated that 1: 10 ratio of 1% Tween -20 and amylase showed increased activity of 220 U/ml followed by Triton-X and SDS. However, BCSS showed maximum enzyme activity in presence of all the types of surfactants in comparison to JBWS and RBWS.

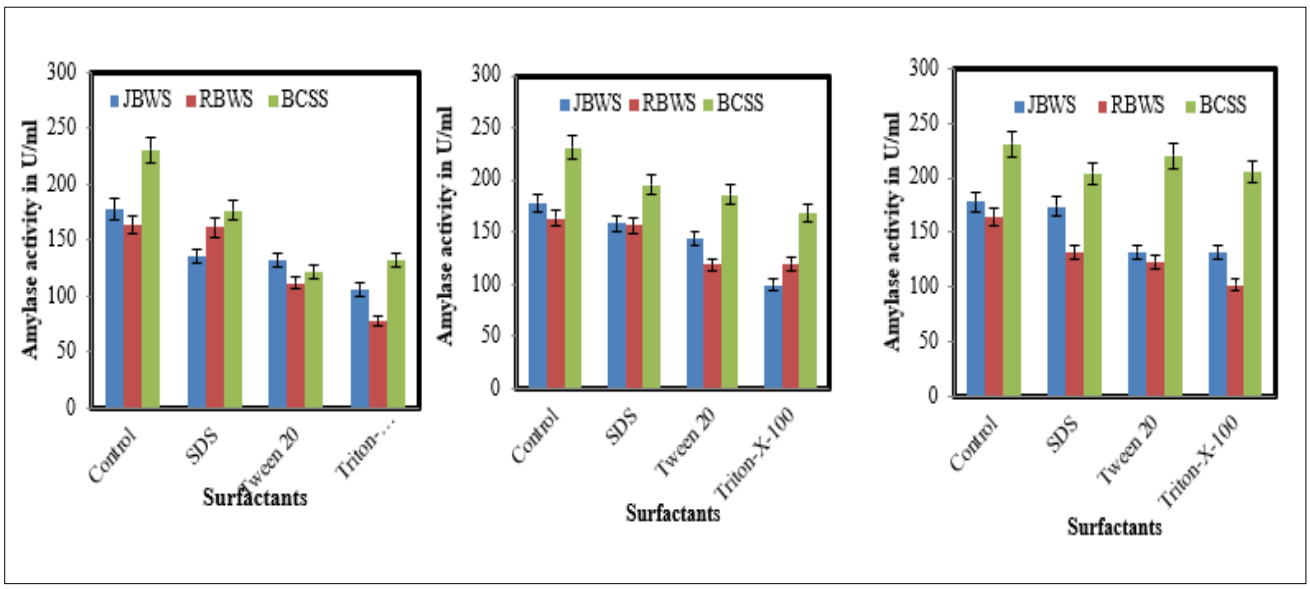

Figure 11 Effect of three different surfactants (SDS, Tween-20 and Triton-X) on amylase activity of JBWS, RBWS and BCCS with a concentration of 1:1, 1:2 and 1: 10 ratio of 1 % surfactant solution and amylase

3.8. Molecular Identification

Molecular identification (16S rRNA sequence) of BCSS showed 99% similarity with *Bacillus nanhaiisediminis* NH3. Table 2 indicates the result obtained from BLAST. The sequence was submitted to the GenBank database and can be accessed under GenBank in National Center for Biotechnology Information (NCBI) with an accession number KU165762.

Table 2 BLAST for BCSS

Isolate	Description	Max Score	Total Score	Query cover	E value	Ident	Accession [a]	Accession no. [b]
BCSS	*Bacillus nanhaiisediminis* NH3 16S ribosomal RNA gene, partial sequence	1983	1983	100%	0.0	99%	NR_117286.1	KU165762

[a]GenBank accession no. of closest relative strains on NCBI website; [b]GenBank accession no. of our strain deposited on NCBI website

3.9. Wash Analysis

Stain removal ability of crude enzyme was assessed using cotton cloths stained with blood, chocolate, tomato ketchup and coffee. The stained cloth pieces were subjected to wash treatments at 37 ^{0}C with constant stirring for 60 mins with commercial solid detergent (Tide) of concentration 7mg/ml, supplemented with crude enzyme. After the incubation, cloth pieces were dried and compared to the stained cloth pieces washed with only detergent. As shown in Fig. 12, the wash performance was limited with detergent whereas; the treatment of these stains with detergent supplemented with crude amylase gave a better stain removal ability with respect to all type of stains.

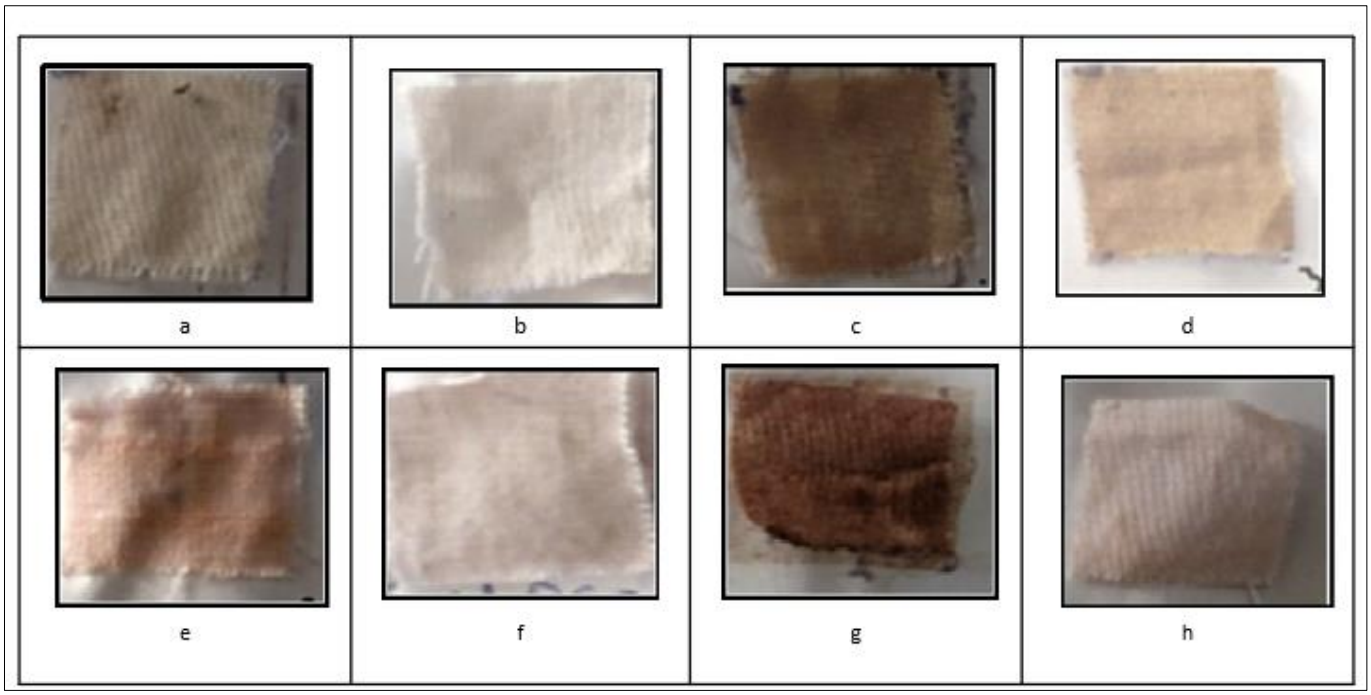

Figure 12 Wash performance of alkaline amylase from *B. nanhiisediminis* in combination with commercial detergent (7 mg/ml) on soiled 400mm two cotton fabric (a) blood stain washed with detergent (b) blood stain treated with enzyme and detergent (c) chocolate stain washed with detergent (d) chocolate stain treated with enzyme and detergent (e) ketchup stain washed with detergent (f) ketchup stain treated with enzyme and detergent (g) coffee stain washed with detergent (h) coffee stain treated with enzyme and detergent

4. Discussion

In the present study, isolation of bacteria was done from Arabian Sea coast. Screening of alkaliphilic amylase producers was carried out using M9 selective media for amylase producers. The pH of the culture medium was adjusted to pH 10 and 10% NaCl was added to it. Enzyme production was carried out by inoculating the pure cultures of the isolated bacteria in the M9 selective medium and incubation time was optimized for the crude enzyme. Out of the five isolates, three isolates were selected depending on their amylolytic activity namely; BCSS, JBWS and RBWS.

Enzyme characterization was carried out for parameters like temperature and its stability, pH and its stability. BCSS, JBWS and RBWS showed optimal temperature at 60 ^{0}C. Increase in temperature results in higher kinetic energy of the molecules resulting in more molecular collisions and interactions for the reaction to proceed faster. It can influence the rate of reaction constantly either by facilitating it or diminishing due to thermal denaturation of the enzyme [21]. BCSS was found to be thermostable for a period of 300mins and it was found to be stable within a range of 60 ^{0}C – 100 ^{0}C in comparison to JBWS and RBWS. Enzyme thermostability is an intrinsic property, determined by the primary structure of the protein. However, external environmental factors including cations, substrates, co-enzymes, modulators, polyols often increase enzyme thermostability. Previous studies showed that alkaline amylase was active within the temperature range of 30 ^{0}C to 100 ^{0}C with different optimal temperatures. The amylase producer *Bacillus tequilensis* RG-01 [16] and *Bacillus subtilis* AS-S01a [22] showed an optimal temperature of 55 ^{0}C. Another group found that the optimal temperature of amylase from *Bacillus flexus* was 60 ^{0}C with a thermostability of 50-80 ^{0}C [23]. Thermostable amylase from *Anoxybacillus* sp. AH1 also showed increased stability at 60 ^{0}C within 120 mins [24].

Optimum pH of an enzyme increases its rate of reaction. Any alteration in pH above or below the optimum will lead to decline the reaction rate, since the shape of the active sites is not complementary to the shape of their substrate [8]. The alkaline amylase from BCSS retained its activity and stability over a broad range of pH 7 to pH 11 with optimal pH 9 which increases its suitability as an additive of industrial importance. Alkaline amylase from JBWS and RBWS retained its activity and stability over the range of pH 6 to 8 with optimal at pH 8 and pH 7 respectively. Similar results were obtained for alkaline amylase isolated from a *Bacillus* species and found the optimal pH to be 9 [25]. Whereas, alkaline amylase isolated from a thermostable *Bacillus* sp. A3-15 showed an optimal pH of 8.5 [26]. A wide range of pH stability might have a significant advantage of handling the enzyme for the commercial and industrial process [27]. pH stability was checked for a range of pH 6 to 11. BCSS was found to be stable within the pH range for a period of 300 mins but it was found to be much stable at pH 9 with maximum enzyme activity in comparison to RBWS and JBWS. *Bacillus amyloliquefaciens* MTCC 610 was shown to have an optimal pH of 8 with the pH stability of 7 – 9 [28]. The amylase activity of *Bacillus licheniformis* MTCC 1483 was found to be active over a range of pH 6-10 with maximum activity at pH 8 [29]

Metal ions have been known to impact the activity of amylases. They serve many functions in proteins, the most important of which is the modification of protein structures, enhancement of the structural stability of the proteins in the conformation required for biological function, or take part in the catalytic processes of enzymes due to the presence of negatively charged amino acids present on the enzyme structure [30]. Alkaline amylase activity has been known to be enhanced by Zn^{2+}, Ca^{2+} and Na^{+} ions. The enzyme activities of the samples in the current investigation were found to be enhanced by the presence of Na^{+}, Mn^{2+} and Cu^{2+} and decreased due to Mg^{2+} and Hg^{2+}. The metal ions Na^{+}, Ca^{2+}, Co^{2+} & Mg^{2+} enhanced the activity of amylase from *Bacillus sp.* PN5 [31]. $ZnCl_2$ and NaCl inhibited the activity of the enzyme produced by the thermophilic *Bacillus* sp. A3-15 [26]. α-amylase isolated from *Anoxybacillus* sp.AH1 was significantly activated in presence of Mg^{+2}, $Ca^{2+}$and inhibited with $Zn^{2+}$and Cu^{2+}[24]. Metal ions can activate the enzyme activity by stabilizing the structure of ES complex or sensitize the substrate to the attack of enzyme or take part in ion exchange process [32].

Salts and surfactants can improve enzyme activity by stabilizing them against temperature and by improving wetting ability of fabric [30]. Effect of surfactants is tested on alkaline amylases to check whether it is compatible with detergents and can be used at the industrial scale. In this study, the enzymes retained their activity in the presence of SDS. Triton-X-100 was found to have a higher depreciating effect on enzyme activity. Alkaline amylase extracted from BCSS was found to be stable in the presence of Tween-20, SDS, Triton-X. Similar findings were reported from amylase extracted from *B. licheniformis* NH1[20] while a depreciation in amylase activity was observed in the presence of Triton-X-100 by *Bacillus tequilensis* RG-01 [16].

BCSS was identified at the species level by 16S rRNA sequencing and was found to be *Bacillus nanhaiisediminis* NH3. The enzymes to be used in detergents should be stable and perform effectively at wide alkaline pH and at a wide variety of temperatures (from low temperatures for synthetic fibers to high temperatures for cotton) during washing [33]. The wash analysis on stained cotton cloth using the crude amylase obtained from *B. nahaiisediminis* NH3 on tough stains of

blood, chocolate, ketch up and coffee showed better results with amylase as an additive to the detergent indicating its stain removing property. The alkaline amylase from *B. subtilis* strain AS-S01a exhibited a substantial compatibility with the tested commercial laundry detergents and the stain removal of coffee and chocolate-stained cotton cloth was better with the enzyme equated to the washing with only detergent [22]. A similar result was obtained for the wash analysis for all the stains (chocolate, blood and barbecue sauce) washed with the amylase [20]. Therefore, the crude alkaline amylase obtained from marine bacterium identified as *Bacillus nanhaiisediminis* NH3 has been found to be thermostable alkaliphilic as well as halotolerant. This enzyme can find applications in the detergent industry as a detergent additive.

5. Conclusion

The crude enzyme extracted from *Bacillus nanhaiisediminis* NH3 was found to be halotolerant as it grows in a culture medium with 10% NaCl. It is an alkalophile and thermostable strain with its pH and temperature stability for 5 h. It can be used as a laundry detergent for hydrolyzing the starch absorbed on the surface of the fabric thus removing starch from the dirt further allowing the action of the detergent for removal of the stain and in whitening. Thus, it acts as glue for particulate soiling. It can also be used for desizing of fabrics. Desizing involves the removal of starch from the fabric which serves as the strengthening agent to prevent breaking of the warp thread during the weaving process. Further, serving as a promising detergent additive it would find its application in institutional cleaning sectors and many other fields where harsh industrial processes are involved.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest.

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