Pakistan J. Zool., vol. 57(3), pp 1051-1057, 2025 **DOI**: https://dx.doi.org/10.17582/journal.pjz/20230109070109

Characterization of Alkalophilic Detergent Compatible Amylase from *Bacillus halodurans*, Isolated from a Restaurant's Washing Area

Sana Safdar and Javed Iqbal Qazi*

Institute of Zoology, University of the Punjab, Lahore, Pakistan

Article Information
Received 09 January 2023
Revised 05 November 2023
Accepted 18 November 2023
Available online 19 December 2023 (early access)
Published 18 April 2025

Authors' Contribution
SS performed all experiments. JIQ supervised the work.

Key words
***Bacillus halodurans*, Alkaline amylase, Ionic detergent additive, Detergent compatibility, Stain removal**

ABSTRACT

Bacillus halodurans (NR_025446.1) amylase producing bacterium has been isolated from a restaurant washing influenced soil. The alkaline amylase produced by the *Bacillus halodurans* (NR_025446.1) has been characterized for its compatibility as detergent additive. The yield of alkaline amylase was found optimum after 48 h of incubation (10.97 U/ml) showing pH optima at 10 and temperature optima at 37°C. The amylolytic activity was enhanced by non ionic detergent components; Triton X-100, Tween-80 and moderately decreased in presence of SDS and oxidizing agents such as H_2O_2 and NaClO. The enzyme showed outstanding stability and compatibility with some commercially available laundry detergents. The enzyme maintained more than 85% of its initial activity after being incubated with 7 mg/ml of Sufi, Express and Surf excel locally available commercial detergents at 30◦C for the incubation time of 60 min. The addition of the alkaline amylase to the Sufi brand detergent amended its starch-based stain removal. The detergent compatible bacterium and its amylase appear promising for bio-detergent applications.

INTRODUCTION

Extremophiles are organisms adapted to a variety of niches not suitable for other niches, such as deep-sea hydrothermal vents, hot springs, sulfuric acid fields, soda lakes, hot and cold deserts, salt systems, environments heavily polluted by nuclear waste or heavy metals (Arora and Pansyan, 2019). Extremophiles living in extreme conditions produce extremophiles and aurocholites, which can be valuable resources (Raddadi *et al*., 2015). The industrial use of enzymes has increased significantly over the past decade. This is mainly the result of the discovery of new enzymes from extremophiles (Demirjian *et al*., 2001). Due to their high stability, exozymes are highly resistant to extreme conditions, creating new opportunities for biocatalysis and biotransformation as well as economic development. Despite the advantages of these enzymes, their potential has not been investigated. These enzymes are used in new research and biotechnological applications (Dumorne *et al*., 2017). α-Amylase (E.C.3.2.1.1.) catalyzes the α -1,4 glycosidic linkage of starch (Crabb and Mitchinson, 1997). The starch degrading enzymes are getting great interest of researchers due to their broad-spectrum significance and applications in different industries. Amylases are of great significance with applications ranging from such as detergent, food, textile, and paper industries. Amylases constitute a class of industrial enzymes having approximately 25% of the enzyme market (Das, 2011). Amylases occur widely in nature, but only amylases from microbial sources have attained commercial significance due to their low-cost production, thermostability and high pH stability. Industrial amylase production is largely due to filamentous fungi as well as bacteria belonging to genus Bacillus such as *B. amyloliquefaciens*, *Bacillus licheniformis*, *B. stearothermophilus*, *B. subtilis* are major source of industrial amylase production (Sajedi *et al*., 2005). According to European Commission (EC) 648/2004, Detergent Directive report detergent enzymes constitute 37% of the total production of enzymes across the world-wide enzyme production and the detergents industry is regarded amongst the largest enzyme consuming industry (Hassan *et al*., 2010). Enzymes are used in a very minor concentration in detergent preparations to enhance their cleaning ability (Bajpai and Tyagi, 2007). Detergents that do not contain enzymes may not be able to remove the stains properly and their residue can adhere to surface of fibers after cleaning. The stain removal efficiency of

* Corresponding author: qazi.zool@pu.edu.pk
0030-9923/2025/0003-1051 $ 9.00/00

a detergent not only depends upon the type of stain but depends upon composition of detergent as well. Other factors include washing temperature, hardness of water and procedure of washing (Hasan *et al*., 2010). Amylases that work optimally at higher pH are very important from industrial point of view due to their applications in detergent industry. The criterion for amylases to be used in detergent preparations is not only working optimum at alkaline pH but activity at wide range of temperature, substrate specificity, compatibility, and solidity in the presence of commercial detergents and different components of detergents such as ionic and anionic surfactants, oxidizing and bleaching agents, perfumes are also necessary (Kumar and Takagi, 1999; Adinarayana *et al*., 2003; Choudhary and Jain, 2012). Work of different researchers is available regarding the wash performance analysis of amylases from different microbial sources (Joshi, 2011; Sindhu *et al*., 2011). When used in solid detergents for laundry washing and liquid detergents for dishwashing amylases accelerate the removal of starch-based residues e.g., potato, gravy, pasta, baby food and chocolate stains. Amylases also inhibit swollen starch from penetrating the surface of the fiber and dishes that otherwise may adhere for particulate soiling. Combination of stains or reaction products between starch, protein and/or fat can be found in foods. In such cases combination of enzymes make it possible to remove complex stains even more successfully than using the single enzyme systems (Gormsen *et al*., 1998).

The present study reports optimized starch hydrolyzing potential of detergent compatible alkaline amylase of *Bacillus halodurans* (NR_025446.1) which have been isolated from a restaurant's washing influenced soil.

MATERIALS AND METHODS

Isolation and identification of the bacterium

One gram soil sampled from a depth of 3.5 feet from an area which had been influenced from a restaurant's washing for the last two decades was suspended in 100ml of medium designed by Horikoshi (2004) and then incubated at 37°C and in a shaking incubator at 140rpm for four days. 100 µl was spread on the selective medium agar plate. A well isolated colony was picked and purified using streak plate method on the nutrient agar. The isolate was again streaked on the selective medium. Amylase producing alkalophilic bacterium was selected based on the largest hydrolytic zone on alkaline starch agar medium which contained soluble starch as substrate. Molecular identification of select bacterium was done based on 16S rDNA sequencing; secured commercially. It was accordingly identified as *B. halodurans* (NR_025446.1).

Amylase production

Production of amylase from *Bacillus halodurans* (NR_025446.1) was carried out in a modified mineral medium described by Fritze *et al*. (1990). The medium composed of (g/l): Starch 5; K_2HPO_4 7; KH_2PO_4 2; $MgSO_4.7H_2O$ 0.1; $(NH_4)_2SO_4$ 1; NaCl 5; and Na_2CO_3 10. The medium was autoclaved at 120°C for 20 min. Cultivations were performed on a rotatory shaker (140 rev min^{-1}) for 48 h at 37°C, in 250 ml Erlenmeyer flasks with a working volume of 50 ml. The cultures were then centrifuged, and the supernatants containing crude enzyme were processed for the estimation of amylolytic activity. The bacterial growth was ascertained by measuring absorbance at 600nm.

Amylase activity assay

Amylase activity from *Bacillus halodurans* (NR_025446.1) was determined by measuring the formation of reducing sugars following starch hydrolysis. The reaction mixture contained 0.5 ml of crude enzyme and 0.5 ml of 1% (w/v) potato starch (Sigma) as a substrate in 0.1 M phosphate buffer (pH 10). The reaction was incubated for 20 min at 37 °C. The amount of liberated reducing sugar was determined by the dinitrosalicylic (DNS) acid method (Miller, 1959). One unit of amylase activity was defined as the amount of enzyme that released 1 µmol of reducing end groups per minute. d-glucose was used as standard to prepare the calibration curve. Amylase units of the bacterial culture were then estimated with the help of following formula.

$$\text{Enzyme activity (U/ml/min)} = \frac{\text{Curve value} \times 2 \times \text{O.D of the sample} \times \text{dilution factor}}{\text{Time of incubation} \times \text{molecular weight of the substrate}} \times 1000$$

Optimization of bacterial growth

Bacillus halodurans (NR_025446.1) was exposed to different cultural conditions to identify the optimum conditions for growth. Growth of the bacterium was assessed at (30, 40, 50, and 60°C), at optimized temperature the cultures were initiated with different pH (7.0, 8.5, 10.0 and 11.0) and the pH of different media was adjusted with 0.5, 1, 1.5 and 2% of Na_2CO_3. All the experiments were carried out in 500 ml Erlenmeyer flask containing 100 ml of the starch medium and the growth was assessed by measuring the absorbance at 600nm following 24 h of incubation. Depending on the level of excessive bacterial growth the culture was diluted appropriately from 1 to 5 time to get OD lesser than 1. The final absorbance was then calibrated by multiplying the dilution factor.

Effect of pH on amylase activity

Amylolytic potential of the crude enzyme preparation was studied at different pH using 1% (w/v) soluble starch as substrate. The pH stability of the alkaline amylase was

determined by incubating the crude enzyme in buffers in the range of 5.0–12.0 pH for 20 min, at 37 ◦C. Buffers used were 100mM sodium acetate for pH 5.0, potassium phosphate for pH 6.0–8.0, Tris–HCl for pH 8.0–9.0, glycine– NaOH for pH 9.0–10.0 and carbonate for pH 11.0 to 12.0. Assays were performed for 20 min at 37 ◦C.

Effect of temperature on amylase activity

Thermal stability of the crude enzyme preparation with the substrate was determined by incubating at 40°C, 60°C, 30°C, 37°C, 50°C and 60°C at optimized pH for 20 min and measurement of enzyme activity was done on each temperature.

Effect of incubation time on amylase activity

Optimization of incubation time for crude amylase activity of 48 h old bacterial culture fluid was measured at optimized temperature and pH. The crude amylase extract was incubated at 37° C for 15, 30, 45 and 60 min to asses the optimum time of incubation.

Effect of detergent components on amylase activity

The enzyme was tested for its stability in the presence of detergent components. The surfactants used were SDS, Triton X-100 and Tween-80. Whereas the oxidizing and bleaching agents used were H_2O_2, Sodium perborate and Sodium hypochlorite (Pathak and Deshmukh, 2012). Half (0.5) ml of 0.1%, 0.5% or1% surfactant/oxidizing agent was mixed with 0.5 ml of the crude enzyme solution in 0.1 M glycine–NaOH buffer (pH 10.0) and incubated for 30min at 37°C. The residual activity of amylase was then assessed. The activity of crude enzyme extract without addition of any detergent compound was taken as 100 percent.

Compatibility of the crude alkaline amylase with local commercial detergents

Ariel (Procter and Gamble Pakistan pvt. Ltd), Surf excel (Unilever Pakistan Limited.), Brite (Colgate-Palmolive Pakistan Ltd). Sufi (Hadayat Detergent and chemicals pvt limited., Lahore Pakistan), Express (Colgate-Palmolive Pakistan Ltd.), Bonus (Colgate-Palmolive Pakistan Ltd.), were used to check their compatibility with enzyme. These detergents were diluted to a final concentration of 0.7 % (w/v) in tap water and later heated at 95°C to denature the enzymes present in these detergents (Adinarayana *et al*., 2003; Kalpanadevi *et al*., 2008; Dubey *et al*., 2010). Reaction mixture comprising 0.3 ml of the crude enzyme preparation and 5.7 ml the diluted detergent was pre incubated at room temperature (28±2°C) for 1 h. The procedure was repeated at 60°C as well. Relative enzyme activity was expressed as percentage of the control activity (Ali, 2008).

Washing performance of crude amylase extract

Washing performance of amylase from *Bacillus halodurans* (NR_025446.1) in the presence of Sufi detergent was studied on potato curry stains. The selected fiber was cotton (2x2 inches). The stains on the cotton fabric were made by placing 100 µl of potato curry sample on the fabric. The potato curry was prepared by cooking mashed potatoes in oil and spices. The fabrics were then put at room temperature for 30 min and later kept in an electric oven at 60°C for 2 h to fasten the potato curry stains to the surface of cotton fiber. Four different conditions were taken into consideration for determining washing performance of potato curry stains from the fiber surface i.e. 1) distilled water (50ml) 2) distilled water (50 ml) + 1 ml of commercial detergent (7 mg/ml); 3) distilled water (50 ml) + crude amylase solution (4 ml); 5) distilled water (50ml) + 1 ml of detergent (7 mg/ml) + crude amylase solution (5 ml). Washing of potato curry loaded fabrics in the above four different conditions was performed for 1 h in a shaking water bath at 40°C and at 80 rpm. After incubation the cloth pieces were taken out from each set, rinsed with water, dried, and visually examined. Control soiled cloth pieces were without enzyme treatment. Aliquot (2 ml) from experimental as well as control were taken and tests were performed after every 15 min till the completion of 1 h. These aliquots were compared both visually and spectrophotometrically by recording their O.D. at 540nm. Control in performance test was employed as blank.

Statistical analyses

On termination of the experiment, one-way analysis of variance (ANOVA) was performed using SSPSS 2.0. Means were compared by applying Duncan's multiple range test (DMR) at 95% confidence level.

RESULTS AND DISCUSSION

Maximum growth of the *B. halodurans* (NR_025446.1) was observed after 24 h of incubation at 37°C at pH 10.0, whereas maximum enzymatic activity was observed after 48 h of the incubation (Table I). The bacterial amylase was very well active in the pH range of 5.0–12.0 with an optimum at pH of 9.0–10.0 (Fig. 1). This is a very important characteristic for ultimate use in detergent formulations (Kalisz, 1988) because the pH of laundry detergents fell within the range of 9.0–11 (Banik and Prakash, 2004). The amylase was also found very stable over a wide pH range, yielding activity of 12.05U/ml at 09 pH and12.6U/ml at pH 10.0 (Fig. 1). Regarding the thermal profile maximum enzyme activity

Table I. Effect of varying incubation periods on the production of amylase (U/ml) at optimized temperature (37°C) and pH (10).

Incubation period (h.)	Amylase activity (U/ml)
24	7.80±0.75^{c}
48	10.9±1.1^{a}
56	8.90±0.22^{b}
72	5.93±0.20^{d}

Values are meas± S.E.M of triplicates. Values having different superscripts within the column are significantly different from each other. P ≤ 0.05 single factor analysis of variance.

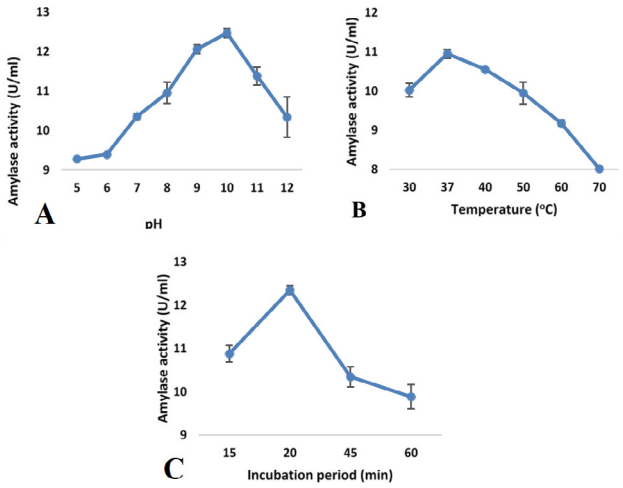

Fig. 1. Effect of pH (A), temperature (B) and incubation period (C) on the amylase activity (U/ml) of the bacterial isolate *Bacillus halodurans* (NR_025446.1).

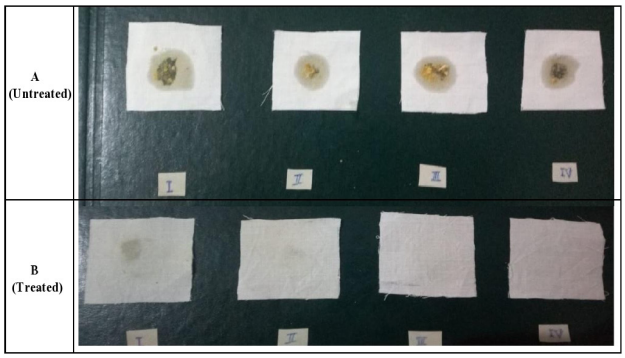

Fig. 2. Wash performance analysis of amylase from *Bacillus halodurans* (NR_025446.1) on potato curry stains using cotton fabrics at 30 °C for 1 h. A, untreated. B, treated; (I) Stain washed with tap water only, (II) Stain washed with detergent diluted in tap water, (III) Stain washed with amylase and tap water, (IV) Stain washed with detergent, tap water and amylase.

was found at 37 ° C (10.948 U/ml, (Fig. 1, Table I). After an incubation period of 30 min, a decline in enzyme activity was observed at higher temperatures. Temperature optima of 37° and 40°C reported for different *Bacillus* sp. (Nusrat and Rahman, 2007). Without any detergent component enzymatic activity of 10.417U/ml/min was considered as 100% (Table II). At 0.1% SDS the amylase activity was recorded 10.034 U/ml/min which slightly increased at1% i.e., 10.489 U/ml/min. An increase of 100.4% in activity of enzyme was observed at 1% SDS. In case of sodium deoxycholate slight declines in enzyme residual activities i.e., 98.7%, 97.6% and 96.5% occurred at 0.1, 0.5 and 1% of the sodium deoxycholate concentrations respectively (Table II). Two anionic detergent components tween and triton up to concentration of 1%, were also

Table II. Stability of alkaline amylase from *B. halodurans* (NR_025446.1) in the presence of various surfactants and detergent components. The enzyme was pre-incubated with surfactants and oxidizing agents for 30 mins at 37°C and at pH10. The value control of amylase activity in absence of any of detergent component was taken as 100(%).

Detergent components	Concentration (%)	Residual activity (%)
Control	None	100
SDS	0.1	96.4
	0.5	96.9
	1	100.6
Sodium deoxycholate	0.1	98.7
	0.5	97.6
	1	96.4
Triton	0.1	101.2
	0.5	105.9
	1	110.15
Tween	0.1	97.36
	0.5	96.78
	1	93.26
Sodium perborate	0.1	101.9
	0.5	106.1
H_2O_2	1	112.13
	0.1	78.28
	0.5	77.76
	1	67.8
NaClO	0.1	81.7
	0.5	77.11
	1	69.63

tested to check their effect on the amylase activity. Slight increase in enzyme activities of 101.2%, 110.1% were observed at 0.1 and 1% of the triton anionic detergent component, respectively. Whereas in the presence of tween residual activity of 93.3% at 1% concentration was recorded. In case of sodium borate an increase in activity of112.5% was observed at its 1%. (Table II). Hydrogen peroxide and sodium hypochlorite caused declines in the enzyme activities. At 0.1, 0.5 and 1% of H_2O_2 decreases of 78.28%, 77.76, 67.8% were observed, respectively whereas at 0.1, 0.5 and 1% of Sodium hypochlorite declines of 81.7, 77.1, 69.63 in enzymatic activities were recorded respectively. Overall, the amylase was found stable with all the different detergent components tested (Table II). In addition to activity and stability in high pH varying temperature ranges (Oberoi *et al*., 2001; Beg *et al*., 2002) a good detergent amylase must be compatible and stable with all commonly used detergent components such as surfactants, bleaches, oxidizing agents and other additives which might be present in the formulation (Gupta *et al*., 1999; Kumar and Takagi, 1999).The crude amylase preparation was incubated for 30 min at 37 °C in the presence of SDS, Tween 20, Triton X-100, sodium perborate and H_2O_2 and the residual activity was then assayed at pH 10.0 and 37°C. The crude enzyme was found highly stable in the presence of the non-ionic surfactants like Tween and Triton X-100 but was found less stable against the bleaching agents.

Stability and activity of amylase in the presence of solid detergents

The bacterial amylase worked optimally at 30°C instead of 50°C. Highest stability was observed in the presence of i.e., Sufi detergent 1.026 U/ml/min (Table III) retaining 98.38% of residual activity at 30°C and 81.4% at 50°C (Table IV). The Enzyme was also found stable in presence of Express, Surf Excel and Ariel detergent at 30°C retaining 89.55, 87.83 and 87.73 % of residual activities, respectively (Table IV).

Table III. Effect of locally available detergents on amylase activity (U/ml/min) at 30^{0}C and 50 °C.

	30 °C	50 °C
Control	1.04±0.11	0.91±0.04
Brite	0.91±0.10	0.69±0.01
S. excel	0.92±0.04	0.81±0.08
Express	0.93±0.05	0.92±0.13
Bonus	0.81±0.20	0.80±0.02
Ariel	0.91±0.04	0.88±0.04
Sufi	1.03±0.03	0.86±0.06

Values are means ± S.E.M. of triplicates.

Table IV. Stability of alkaline amylase of *B. halodurans* (NR_025446.1) in the presence of various laundry detergents at temperature 30 °C and 50 °C.

Detergents	Residual activity (%) at	
	30 °C	50 °C
Brite	86.76	65.68
S. excel	87.83	76.23
Express	89.55	88.12
Bonus	78.34	22.91
Ariel	87.73	83.52
Sufi	98.38	81.4

Adequate number of research studies reporting similar results are presented earlier on compatibility and wash performance for amylases. Joshi (2011) has reported *Bacillus circulans* PN5 amylase producing bacterial strain that show residual enzyme activity of more than 90% after exposure for 30 min to all commercial detergents tested. Similarly, amylase reported from *Bacillus licheniformis* NHI (Hmidet *et al*., 2009) retained excellent stability and compatibility with a variety of solid and liquid detergents tested. Similarly, Correa *et al*. (2011) reported surge in the activity of α-amylase in the presence of different detergents. The amylase from the alkalophilic *Bacillus halodurans* (NR_025446.1) possessed promising compatibility with a wide range of commercial detergents blending of the amylase enzyme of *Bacillus halodurans* (NR_025446.1) to Sufi enhanced its stain removal efficiency. Complete stain removal from cotton clothes was observed after 45 minutes of incubation at 40°C and 80 rpm. It was also observed that the removal of stain by Set- A (only distilled water) was very less (Fig. 2). Similar results for stain removal after 45 min of incubation at 40°C and 80 rpm has been reported by Correa *et al*. (2011).

CONCLUSION AND RECOMMENDATIONS

This work describes the growth optimization of the alkalophilic bacterium *Bacillus halodurans* (NR_025446.1) and characterization and compatibility of its alkaline amylase preparation as a detergent additive. The crude amylase preparation showed a wide range of stability to temperature and pH, ranges 10 and 37°C. Enzyme stability at wide temperature ranges indicates its potential to be used in hot as well as cold wash cycles. The crude alkaline amylase exhibited high stability in the presence different components of detergents as well as with various locally available commercial solid detergents. Maximum stain removal was observed for cotton fabric where the addition of enzyme to the Sufi detergent improved its

cleansing efficiency. Amylase from *Bacillus halodurans* (NR_025446.1) can be considered as a potential candidate for application in the detergent industry. Further work is required to characterize the enzyme produced by *B. halodurans* (NR_025446.1) at molecular level.

ACKNOWLEGEMENT

We are grateful to members of our laboratory for constructive comments on this report and for fruitful discussions.

Funding

No any funding was received for the current research work.

Statement of conflict of interest

The authors declare that no conflicts of interest, and financial or other, exists. The work described has not been published elsewhere and is not under consideration by another journal. Both of the authors have approved the manuscript and agree with its submission to this journal.

REFERENCES

Adinarayana, K., Ellaiah, P. and Prasad, D.S., 2003. Purification and partial characterization of thermostable serine alkaline protease from a newly isolated *Bacillus subtilis* PE-11. *AAPS Pharm. Sci. Tech*., **4**: 1–9. https://doi.org/10.1208/pt040456

Ali, U.F., 2008. Utilization of whey amended with some agro-industrial by products for the improvement of protease production by *Aspergillus terreus* and its compatibility with commercial detergents. *Res. J. Agric. Biol. Sci*., **4**: 886–891.

Arora, N.K. and Hovik, P., 2019. Extremophiles: Applications and roles in environmental sustainability. *Environ. Sustain*., **2**: 217218. https://doi.org/10.1007/s42398-019-00082-0

Bajpai, D. and Tyagi, V.K., 2007. Laundry detergents: An overview. J. Oleo Sci., **56**: 327-340. https://doi.org/10.5650/jos.56.327

Banik, R.M. and Prakash, M., 2004. Laundry detergent compatibility of the alkaline protease from *Bacillus cereus*. *Microbiol. Res*., **159**: 135–140. https://doi.org/10.1016/j.micres.2004.01.002

Beg, O.K., Saxena, R.K. and Gupta, R., 2002. Kinetic constants determination for an alkaline protease from *Bacillus mojavensis* using response surface methodology. *Biotechnol. Bioeng*., **78**: 289–295. https://doi.org/10.1002/bit.10203

Choudhary, V. and Jain, P.C., 2012. Screening of alkaline protease production by fungal isolates from different habitats of Sagar and Jabalpur district (MP). *J. Acad. Ind. Res*., **1**: 215–220.

Correa, T., Moutinho, S.S., Martins, M. and Martins, M., 2011. Simultaneous α-amylase and protease production by the soil bacterium *Bacillus* sp. SMIA2 under submerged culture using whey protein concentrate and corn steep liquor: compatibility of enzymes with commercial detergents. *Fd. Sci. Technol*., **31**. https://doi.org/10.1590/S0101-20612011000400003

Crabb, W.D. and Mitchinson, C., 1997. Enzymes involved in the processing of starch to sugars. *Trends Biotechnol*., **15**: 349-352. https://doi.org/10.1016/S0167-7799(97)01082-2

Das, S., 2011. Biotechnological applications of industrially important amylase enzyme. *Int. J. Pharm. Biol. Sci*., **2**: 486-496.

Demirjian, D.C., Morís-Varas, F. and Cassidy, C.S., 2001. Enzymes from extremophiles. *Curr. Opin. Chem. Biol*., **5**: 144-151. https://doi.org/10.1016/S1367-5931(00)00183-6

Dubey, R., Adhikary, S., Kumar, J. and Sinha, N., 2010. Isolation, production, purification, assay and characterization of alkaline protease enzyme from *Aspergillus niger* and its compatibility with commercial detergents. *Dev. Microbiol. Mol. Biol*. **1**: 75–94.

Dumorne, K., Córdova, D.C., Astorga-Eló, M. and Renganathan, P., 2017. Extremozymes: A potential source for industrial applications. *J. Microbiol. Biotechnol.*, **27**: 649-659. https://doi.org/10.4014/jmb.1611.11006

Fritze, D., Flossdorf, J. and Claus, D., 1990. Taxonomy of alkaliphilic *Bacillus* strains. *Int. J. Syst. Evol. Microbiol*., **40**: 92-97. https://doi.org/10.1099/00207713-40-1-92

Gormsen, E. and Marcussen, E.T., 1998. *Damhus, in powdered detergents*. (Edited by M.S. Showell and E.J. Baas), Marcel Dekker Inc., New York. Surfactant Sci. Ser., **71**: 137–163.

Gupta, R., Gupta, K., Saxena, R.K. and Khan, S., 1999. Bleach-stable, alkaline protease from *Bacillus* sp. *Biotechnol. Lett*., **21**: 135–138. https://doi.org/10.1023/A:1005478117918

Hasan, F., Shah, A., Javed, S. and Hameed, A., 2010. Enzymes used in detergents: Lipases. *Afr. J. Biotechnol*., **9**: 4836-4844.

Hmidet, N., El-Hadj, Ali., Haddar, A., Kanoun, S., Alya, S.K. and Nasri, M., 2009. Alkaline proteases and thermostable alpha-amylase coproduced by *Bacillus licheniformis* NHI- characterization and

potential application as detergent additive. *Biochem. Eng. J.,* **47**: 71-79. https://doi.org/10.1016/j.bej.2009.07.005

Horikoshi, K., 2004. Alkaliphiles. *Proc. Jpn. Acad. B Phys. Biol. Sci. Ser. B*, **80**: 166-178. https://doi.org/10.2183/pjab.80.166

Joshi, B.H., 2011. A novel thermostable alkaline α-amylase from *Bacillus circulans* PN5: Biochemical characterization and production. *Asian J. Biotechnol*., **3**: 58-67. https://doi.org/10.3923/ajbkr.2011.58.67

Kalisz, H.M., 1988. Microbial proteinases. In: (ed. A. Fietcher). *Adv. Biochem. Eng. Biotech*., **36**: 1–65. https://doi.org/10.1007/BFb0047944

Kalpanadevi, M., Banu, A.R., Gnanaprabhal, G.R., Pradeep, B.V. and Palaniswamy, M., 2008. Purification, characterization of alkaline protease enzyme from native isolate *Aspergillus niger* and its compatibility with commercial detergents. *Ind. J. Sci. Technol*., **1**: 1–6. https://doi.org/10.17485/ijst/2008/v1i7.8

Kumar, C.G. and Takagi, H., 1999. Microbial alkaline protease from bioindustrial viewpoint. *Biotechnol. Adv*., **17**: 561–594. https://doi.org/10.1016/S0734-9750(99)00027-0

Miller, G.L., 1959. Use of Dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*., **31**: 426–429. https://doi.org/10.1021/ac60147a030

Nusrat, A. and Rahman, S.R., 2007. Comparative studies on the production of extracellular amylase by three mesophilic Bacillus isolates. *Bangladesh J. Microbiol*., **24**: 129-132. https://doi.org/10.3329/bjm.v24i2.1257

Oberoi, R., Beg, K., Puri, S., Saxena, R.K. and Gupta, R., 2001. Characterization and wash performance analysis of an SDS-stable alkaline protease from a Bacillus sp. *J. Microbiol. Biotechnol*., **17**: 493–497. https://doi.org/10.1023/A:1011918806201

Raddadi, N., Cherif, A., Daffonchio, D., Neifar, M. and Fava, F., 2015. Biotechnological applications of extremophiles, extremozymes and extremolytes. *Appl. Microbiol. Biotechnol*., **99**: 7907-7913. https://doi.org/10.1007/s00253-015-6874-9

Sajedi, R.H., Naderi-Manesh, H., Khajeh, K., Ahmadvand, R., Ranjbar, B. and Asoodeh, M.F., 2005. A Ca- independent alpha-amylase that is active and stable at low pH from the *Bacillus* sp. KR-8104. *Enzyme Microb. Technol*., **36**: 666-671. https://doi.org/10.1016/j.enzmictec.2004.11.003

Sindhu, R., Suprabha, G.N. and Shashidhar, S., 2011. Purification and characterization of alpha –amylase from *Penicillium janthinellum* (NCIM 4960) and its application in detergent industry. *Biotechnol. Bioinform. Bioeng*., **1**: 25-33.