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Original Article

Purification and characterization of alkaline soda-bleach stable protease from *Bacillus* sp. APP-07 isolated from Laundromat soilI.K. Shaikh^{a,b}, P.P. Dixit^{a,*}, T.M. Shaikh^b^a Department of Microbiology, Dr. Babasaheb Ambedkar Marathwada University, Sub Campus, Osmanabad, India^b Department of Antitoxin and Sera, Haffkine Biopharmaceutical Corporation Limited, Pune, India

ARTICLE INFO

Article history:

Received 8 April 2018

Received in revised form 13 July 2018

Accepted 16 July 2018

Available online 20 July 2018

Keywords:

Bacillus sp.

Alkaline protease

Purification and characterization

Laundry detergent

ABSTRACT

The detergent-compatible alkaline protease was produced from the bacterial strain *Bacillus* sp. APP-07 isolated from Laundromat soil of Solapur, Maharashtra, India. The culture was grown in 1000 ml capacity baffled flask with a working volume of 100 ml and incubated at 55 °C for 33 h on a rotary shaker. After incubation, alkaline protease was partially purified by the sequential method of acetone precipitation followed by nominal molecular weight limit (NMWL) cut-off ultrafiltration using 50 K and 10 K filters. Finally, Sephadex G-100 gel filtration chromatographic purification was performed to obtain 3.12 fold purified alkaline protease enzyme with a 66.67% final yield. The purified enzyme showed 31907.269 units (U) of enzyme activity containing 8741.718 U/mg of specific enzyme activity. The molecular weight of the enzyme was confirmed about 33.0 kDa (kDa) by the SDS-PAGE analysis. The purified enzyme was stable at higher pH and temperature range, with an optimum pH 10.5 and temperature 55 °C. The enzyme showed excellent stability and compatibility in various detergents, surfactants, bleach, and oxidizing agents. The enzyme activity enhanced in the presence of Ca²⁺, Cu²⁺, and surfactants, whereas; the phenylmethylsulphonyl fluoride (PMSF) and Diisopropyl fluorophosphate (DFP) completely inhibit the enzymatic activity, which pointed out that the enzyme affiliated to serine-centered metalloproteases family.

In conclusion, the remarkable tolerance and stability of the enzyme explored the promising candidature for the several potential applications in the laundry detergents. The sustainability of the enzyme might serve several possible applications in the laundry detergents, leather industries, and other harsh industrial processes.

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1. Introduction

Several years witnessed the widespread applications and robustness of the enzymes as a biochemical catalyst in numerous industrial and bioprocess engineering. Among all the enzymes, thermostable enzymes are gaining immense potential due to their inherent benefits and sustainability under several harsh industrial processes [3,12,18]. Proteases (EC 3.4.21) represent one of the largest groups of the industrial enzyme, which occupied more than 65% global share market of the enzymes [15,19]. The estimated universal demand of the proteases increased up to 1.2 billion dollars and forecasted to reach about 2.0 billion dollars [15,36]. The alkaline proteases have been a unique demand from the

biotechnological, bioengineering, and industrial perspectives due to their consistency, specificity, wide range pH and temperature tolerance compared to other chemical catalysts [9,20,24,32]. However, thermostable enzymes served an important task in laundry detergents, leather (tannery), protein processing, extraction of silver from X-ray films, diagnostic assays, food, dairy, pharmaceutical, beverages, peptide-synthesizing industries, and many other industrial processes [2,5,16,22,29,37].

Higher thermo-stability with improved enzyme properties will be a primary goal for many enzyme-producing industries. In view of this, many investigators reported the findings of thermostable alkaline proteases from archaea, actinomycetes, bacteria, and fungi survived in the extreme geographical habitats [4,10,31,34]. The *Bacillus* species represent one of the dominating microbial strain used for the production of thermostable alkaline proteases, due to their attractive stability in extreme pH 7–12 and temperatures 10–80 °C [3,9,15]. However, very few studies have been explored the attention towards bleach-stable alkaline proteases, leading to

Peer review under responsibility of National Research Center, Egypt.

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E-mail address: prashantdixit74@gmail.com (P.P. Dixit).<https://doi.org/10.1016/j.jgeb.2018.07.003>1687-157X/© 2018 Production and hosting by Elsevier B.V. on behalf of Academy of Scientific Research & Technology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

the scarcity of detergent-compatible enzymes [17]. The Laundromat soil represents one of the natural habitats for the *Bacillus* sp. where the bacteria can grow up under extreme alkaline condition. The strains derived from such ecological sites are the active producers of extracellular thermostable alkaline proteases, which belong to metallic type or serine centre proteases that exhibited a broad range of pH and temperature stability [9,11,17,35]. The strains with such dual properties had drawn special attention for the production of thermostable alkaline proteases. The enzymes produced from these strains are gaining an immense importance in the leather and detergent industries due to their intrinsic ability to sustain the broad pH and temperature stability with efficient specificity in different detergent additives. These enzymes also proved inherent benefits and cost-effectiveness over the conventional chemical catalysts [6,7,8,13,28,19,21,33].

In light of this, we reported the purification and characterization of the detergent-compatible alkaline protease from *Bacillus* sp. APP-07. The purified enzyme was assayed for the wide range of pH and temperature stability and compatibility using various detergents, surfactants, bleach, and chelating agents.

2. Materials and methods

2.1. Chemicals and materials

Phenylmethylsulphonyl fluoride (PMSF), Diisopropyl fluorophosphate (DFP), Casein, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, USA. Sephadex G-100 was supplied by Pharmacia (Uppsala, Sweden). Amicon ultra-15 centrifugal filter units - Ultracel® membranes with nominal molecular weight limit (NMWL) cut-off 10000 (10 K), 50,000 (50 K) filter units, broad range protein standard marker, and all other analytical grade reagents were procured from Merck, India.

2.2. Screening and selection of microbial strain

Laundromat soil samples were collected, serially diluted, and plated on 10% sterile alkaline milk agar plate-pH 10.5 contained (w/v): 1.0% glucose, 0.5% yeast extract, 0.5% peptone, 0.1% K_2HPO_4 , 0.2% $MgSO_4$, 5.0% NaCl, 1.0% Na_2CO_3 , 10.0% skimmed milk, and 1.5% agar. The skimmed milk, glucose, and Na_2CO_3 were sterilized separately and aseptically added to the media, whereas, Na_2CO_3 used to adjust media pH to 10.5. The inoculated plates were incubated at 55 °C for 24–48 h and colonies observed for the clear zone around the colony. Colonies with a maximum zone of clearance in their vicinity were picked up and subcultured on nutrient agar slants [34].

2.3. Bacterial strain identification

The selected microbial strains were subjected to the various morphological, cultural, and biochemical characteristics for the identification of microbial species. Finally, identification and confirmation of the potent strain APP-07 was performed using the automated VITEK 2 system (Biomérieux, INC., Durham, NC, USA).

2.4. Production of alkaline protease

The maximum alkaline protease was produced according to Shaikh et al. [34]. The medium composed of ingredients (w/v): 1.0% glucose, 0.5% yeast extract, 0.5% peptone, 0.1% K_2HPO_4 , 0.02% $MgSO_4$, 5.0% NaCl, 0.2% $CaCl_2$, 1.0% Na_2CO_3 , 0.5% defatted soya flour, and 1.0% maltodextrin. The glucose, Na_2CO_3 , defatted soya flour, and maltodextrin were sterilized separately. The glucose added immediately to the media and pH adjusted to 10.5

using sterile Na_2CO_3 solution. The culture was grown in 1000 ml baffled flask with a working volume of 100 ml, inoculated with 24 h old 10% (v/v) inoculum and incubated at 55 °C for 33 h on a rotary shaker (100 rpm). After 12 h growth, defatted soya flour and maltodextrin were added aseptically to the media for the enhancement of enzyme production. After completion of 33 h, the cultivated media centrifuged at 10,000g for 10 min and the clear supernatant was assayed for the proteolytic activity [34].

2.5. Estimation of enzymatic activity

The proteolytic activity was measured according to Kembhavi et al. [23] method with some minor modifications. Briefly, 1.0 ml of (1.0%, w/v) casein (substrate) was prepared in 10 mM carbonate-bicarbonate buffer-pH-10.5 and pre-incubated for 5 min at 55 °C. 1.0 ml of suitably diluted enzyme solutions was added to the pre-incubated substrate and incubated for 15 min. Blank test tube incubated without addition of enzyme solution. The enzymatic reaction was terminated by the addition of 2.0 ml trichloroacetic acid (0.4 M) solution. The blank test tube added with 1.0 ml of enzyme solution and reaction mixtures were allowed to stand for 25 min at room temperature. Afterward, the solutions were centrifuged at 10,000g for 10 min to remove the precipitate. The absorbance of the clear supernatant was measured at 660 nm. Tyrosine (0–50 mg/ml) calibration curve was used as a standard calibration plot. One unit of protease activity was defined as the amount of enzyme required to liberate 1 mg of tyrosine/ml/min under the standard experimental conditions.

2.6. Purification of the enzyme

2.6.1. Acetone precipitation

After centrifugation, the clear supernatant from the cultivated media was subjected to the acetone precipitation. Briefly, the cell-free supernatant and chilled acetone (–20 °C) were mixed in 1:2 proportions and centrifuged at 10,000g for 10 min. The precipitate was collected and air-dried at room temperature. The dried pellet was re-suspended in a minimal volume of 10 mM carbonate-bicarbonate buffer-pH-10.5.

2.6.2. Purification of alkaline protease

The NMWL ultracentrifugation filters (50 and 10 K) were used in combination for the partial purification of the enzyme. In brief, 15 ml solution was ultracentrifuged using 50 K centrifugal filters and obtained filtrate solution further passed through 10 K ultracentrifugal filter units. Collect filtrate and retentant solutions in clean glass tubes and assayed for the presence of proteolytic activity.

2.6.3. Sephadex G-100 gel filtration.

The partially purified enzyme was subjected to gel filtration chromatography on Sephadex G-100 column (2.6 cm × 90 cm) equilibrated with 25 mM carbonate-bicarbonate buffer-pH-10.5 containing 0.5% Triton X-100 (buffer-A). Using buffer-A 30 ml/h flow rate was applied to the column and 5 ml fractions were collected. The collected fractions were assayed for alkaline protease activity. The fraction (25–50) showing alkaline protease activities were pooled and concentrated using 10 K ultracentrifugal filter units.

2.7. Estimation of protein content

The protein content of the samples were estimated according to the Lowry et al. [27]. The absorbance measured at 660 nm and BSA used as a standard protein.

2.8. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

The SDS–PAGE analysis was performed according to Laemmli [25] for the determination of protein profile, molecular weights, and purity of the samples. Briefly, the SDS–PAGE gel slabs were prepared with upper 4% stacking gel and lower 12% resolving gel in gel casting stands. The crude and purified samples were diluted in a native sample buffer solution and applied to the wells, resolved by applying a constant current across the gel. After the run, the resolved bands were visualized by Coomassie brilliant blue R-250 staining method. The molecular weights were estimated by comparing with standard broad range protein marker (Merck, India).

2.9. Zymographic analysis (protease zymogram)

The protease zymogram was performed according to Garcia-Carreno et al. [14] with some modification. Briefly, 12% resolving gel was prepared by incorporating 1.0% milk casein as a substrate. After electrophoresis, the gel was thoroughly washed with distilled water and agitated in 10 mM carbonate-bicarbonate buffer-pH-10.5 containing 2.5% (w/v) Triton X-100 for 60 min. Afterward, the gel was washed with 10 mM carbonate-bicarbonate buffer and incubated at 55 °C for 12 h in collagenase buffer-pH-10.5 composed of (ml/L): 10 mM carbonate-bicarbonate buffer-pH-10.5 (18.32), 0.2 M NaCl (11.68), 5.0 mM CaCl₂ (2.22), and 0.2% Brij-35 (2.0). Finally, the gel was washed with distilled water and stained with Coomassie brilliant blue R-250. The development of clear zones on the blue background indicated the presence of alkaline protease activity.

2.10. Effect of pH and temperature on alkaline protease activity

The influence of pH on the proteolytic activity of the enzyme was assayed by pre-incubating enzyme solution in the pH range 7–12 at 55 °C for 30 min. For the measurement of pH stability, 10 mM buffers (phosphate buffer, pH 7–8; Tris–HCl buffer, pH 8.5–9.5; carbonate-bicarbonate buffer, pH 10–12) were used. The enzyme–substrate mixture solution was pre-incubated in respective buffer solution for 15 min before measuring proteolytic activity at 55 °C. In addition to that, the thermostability of the enzyme also investigated by pre-incubating enzyme at 30–70 °C for 90 min. Then, the proteolytic activity was estimated as per previously described enzymatic assay method. All the assays were performed in triplicates and the mean values were recorded.

2.11. Effect of metal ions on the enzyme activity

The effect of metal ions on the enzyme activity was evaluated by the addition of 5 mM concentration of Mg²⁺, Ca²⁺, Fe²⁺, Cu²⁺, Zn²⁺, Co²⁺, Hg²⁺, Na⁺, and K⁺ metal ions to the enzyme solution. The enzyme solution was pre-incubated with metal ions for 10 min before addition to the substrate and allowed to react for 15 min at 55 °C. The proteolytic activity of the enzyme was estimated as per previously described enzymatic assay method. All the assays were performed in triplicates and the mean values were recorded.

2.12. Effect of enzyme inhibitors, surfactants, bleach, and oxidizing agents on the enzyme activity

The enzyme inhibitors PMSF, DFP, ethylenediaminetetraacetic acid (EDTA), SDS, urea, and β-mercaptoethanol were used to determine the type of alkaline protease. The 5 mM concentration of each inhibitor was added to the enzyme solution and after incubation proteolytic activity was assayed. In addition to that, the influence of surfactants (Triton-X-100, SDS, Tween-80, Tween-20), bleach sodium hypochlorite (NaClO), sodium carbonate (Na₂CO₃), and oxidizing agent (hydrogen peroxide (H₂O₂), sodium perborate) were studied by the addition of 1% concentration of the agent to the enzyme solution and pre-incubated with respective additives for 30 min at 55 °C. The relative enzyme activity was estimated as per previously described method and without any addition of additive enzyme activity considered as positive control activity. All the assays were performed in triplicates and the mean values were recorded.

2.13. Compatibility of the enzyme with detergents

The detergent-compatibility of the purified enzyme was tested using local detergent powders, such as Ariel (Procter & Gamble, India), Nirma (Nirma Limited, India), Surf excel, Rin, and Active wheel (Hindustan Unilever Limited, India). Briefly, detergents (0.7% w/v) were diluted in 10 mM carbonate-bicarbonate buffer-pH-10.5 and heated at 90 °C for 90 min to destroy endogenous protease enzymes present in the detergents. 0.5 ml of the purified enzyme solution mixed with 9.5 ml detergent solutions and incubated for the period of 0, 1, 3, 6, 12, and 24 h at room temperature and 55 °C respectively. The percent residual activity was estimated from the samples at respective temperatures, whereas without any detergent additive was considered as positive control activity. All the assays were performed in triplicates and the mean values were recorded.

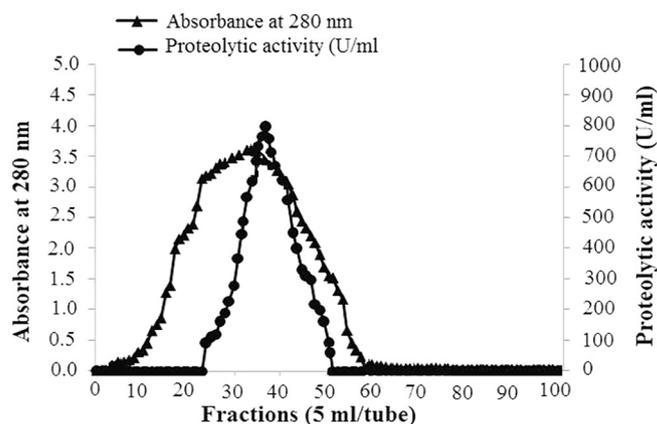


Fig. 1. Purification profile of alkaline protease from *Bacillus* sp. APP-07 by gel filtration chromatography. The partially purified enzyme solution was loaded on Sephadex G-100 (2.6 cm × 90 cm) column. The column operated at 30 ml/h flow rate and 5-ml fractions were collected using elution buffer A.

Table 1
Purification steps for the alkaline protease.

| Purification steps | Total enzyme activity (U) | Total protein content (mg) | Specific activity (U/mg) | Recovery (%) | Fold purification |
|-------------------------------|---------------------------|----------------------------|--------------------------|--------------|-------------------|
| Cell-free supernatant | 119652.26 | 42.71 | 2801.51 | 100 | 1 |
| Acetone precipitation | 77773.97 | 15.63 | 4975.94 | 65.00 | 1.78 |
| NMWL ultrafiltration | 47860.90 | 7.30 | 6556.29 | 61.54 | 2.34 |
| Sephadex G-100 gel filtration | 31907.27 | 3.65 | 8741.72 | 66.67 | 3.12 |

2.14. Blood stain removal ability test

The cleaning capability of the enzyme was evaluated according to Adinarayana et al. [1] with some minor modifications. Briefly, small white colored cloth pieces (5 × 5 cm) were stained with animal blood and air dried at room temperature for 24 h. Three sets were prepared and followed the following procedure to clean the blood stains from the cotton fabric.

- A. Stained cloth + 50 ml tap water
- B. Stained cloth + 1.0 ml Nirma detergent solution (0.7%, w/v) + 50 ml tap water
- C. Stained cloth + 1.0 ml Nirma detergent solution (0.7%, w/v) + 1 ml purified protease solution + 50 ml tap water

The treated sets were incubated for 15 min at 55 °C and rinsed with tap water. The cloths were air dried and visually examined for the removal of blood stain from cloths. Set - A considered as a control blood stained cloth piece.

2.15. Statistical analysis

All the assays were performed in triplicates and average values were interpreted using GraphPad Prism 7 software.

3. Results and discussion

3.1. Screening and microbial strain identification

A total 18 isolates were isolated from Laundromat soil and screened for the maximum alkaline protease producing abilities.

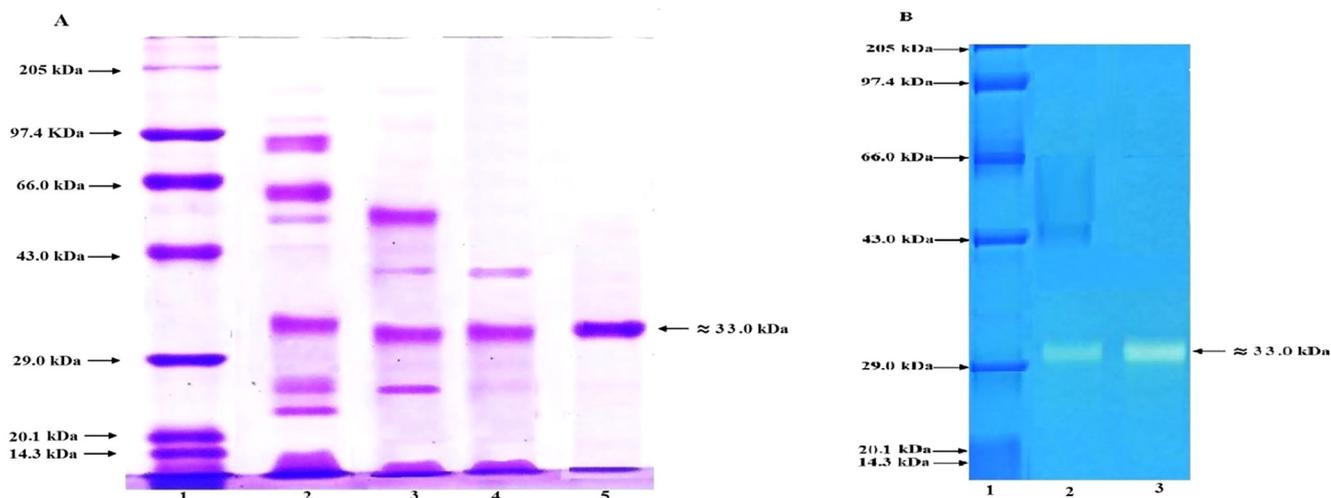


Fig. 2. SDS-PAGE analysis of alkaline protease. (A) 12% gel resolved under the native condition and stained with coomassie brilliant blue G-250. Lane1: standard protein marker, lane 2: cell-free supernatant, lane 3: acetone precipitate sample, lane 4: 10 K ultracentrifugation retentant sample, and lane 5: semi purified enzyme sample. (B) Zymographic analysis of alkaline protease. Lane1: standard protein marker, Lane 2: 10 K ultracentrifugation retentant sample and Lane 3: Sephadex G-100 purified protease sample. The arrow demonstrates molecular weight in kilodalton (kDa).

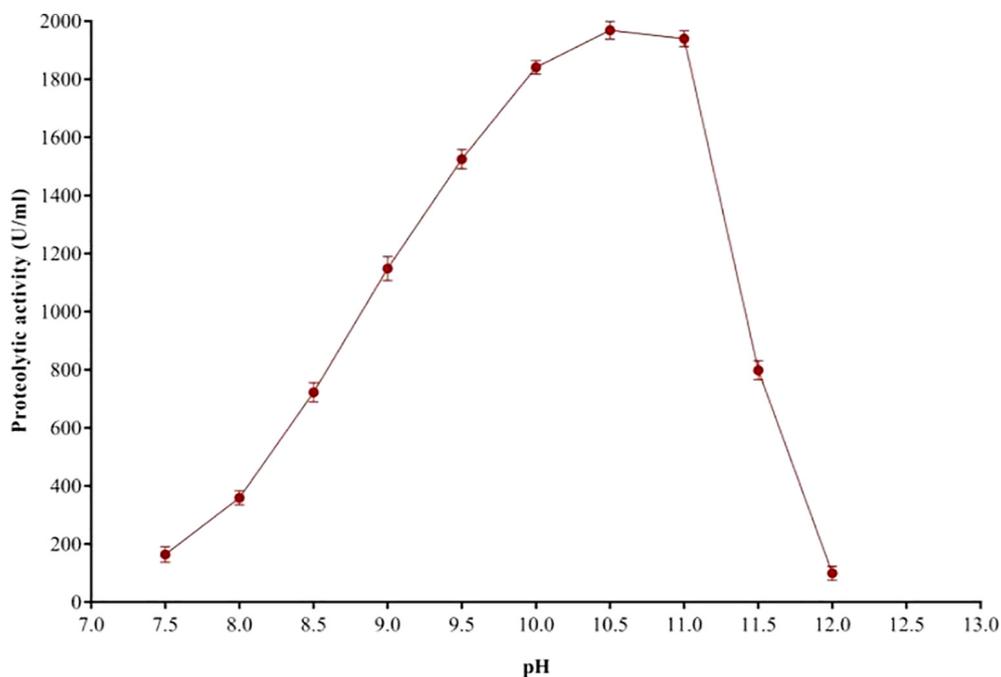


Fig. 3. Effect of pH on the stability of the enzyme. The presented values are the mean values (n = 3) and standard deviation depicted with the error bars.

Isolate no. APP-07 found as a potent alkaline protease producer and identified as *Bacillus* sp. based on their morphological, biochemical, and automated VITEK 2 analysis. Many researchers reported the production of proteases from *Bacillus* species and their applications in tannery and detergent industries [7,9,15,19,33].

3.2. Purification of alkaline protease

After 33 h cultivation, supernatant collected by centrifugation and precipitated using chilled acetone. The obtained pellet showed 4975.942 U/mg of specific enzyme activity, which was further partially purified by the NMWL ultracentrifugation filters. Finally, the partially purified enzyme was subjected to gel filtration chromatography using Sephadex G-100 to yield 3.12 fold-purified alkaline protease having 31907.27 U of total enzyme activity containing 8741.72 U/mg of specific enzyme activity. The purification steps, elution profile, protein content, and proteolytic activity of the enzyme indicated in Table 1 and Fig. 1 respectively.

3.3. Molecular weight determination

The native SDS-PAGE elucidated to determine the relative molecular weight of the alkaline protease. After the run, the resolved gel observed with multiple bands in the crude sample whereas, the single clear distinct band at 33.0 kDa in semi purified sample representing alkaline protease (Fig. 2A). The molecular weight and enzyme activity of the enzyme was confirmed by zymographic analysis using 12% SDS-PAGE incorporated with casein as a substrate. A clear zone of hydrolysis was observed at 33.0 kDa on the blue background of the gel (Fig. 2B). Rahman et al. [31] reported 33.5 kDa alkaline protease from *Bacillus stearothermophilus* F1. However, many researchers reported the relative molecular weights of the bacterial alkaline proteases in the range of 15.0 to 45.0 kDa [10,12,33].

3.4. Effect of pH and temperature on enzyme stability

The influence of pH on the proteolytic activity of the enzyme was examined at different pH range (7.0–12) at 55 °C and optimal

activity of the purified enzyme was observed at pH 10.5. However, the enzyme retained about 80–90% activity at pH 9–10 and pH 11.0, after that, the enzyme activity rapidly decreased (Fig. 3). The effect of temperature range (30–70 °C) also investigated and the results showed that the purified enzyme has the optimum activity at 55 °C and remained stable up to 60 °C, but its stability decreases above 60 °C (Fig. 4). The results revealed that the enzyme exhibit a wide range of pH and temperature stability, which eventually suitable for the detergent industries. The similar results were reported by Raheman et al. [31] during the studies of heat-stable protease obtained from *Bacillus stearothermophilus* F, which showed optimal stability at pH 9.0 and 70 °C [31]. Genckal and Tari in 2006 reported a novel alkaline protease from alkalophilic *Bacillus* sp L21 showed optimal stability at pH 11.0 and temperature 60 °C [15]. However, many investigators reported a wide range of thermo-stability and pH tolerance of the alkaline proteases from various microbial sources [11,20,28]

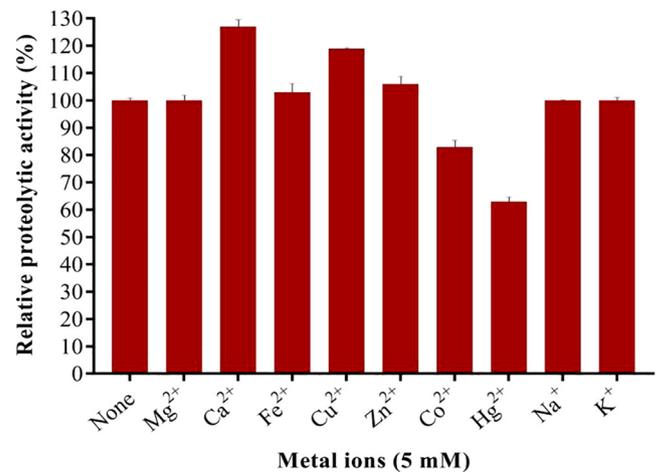


Fig. 5. Effect of various metal ions on the proteolytic activity of the enzyme. The presented values are the mean values (n = 3) and standard deviation depicted with the error bars.

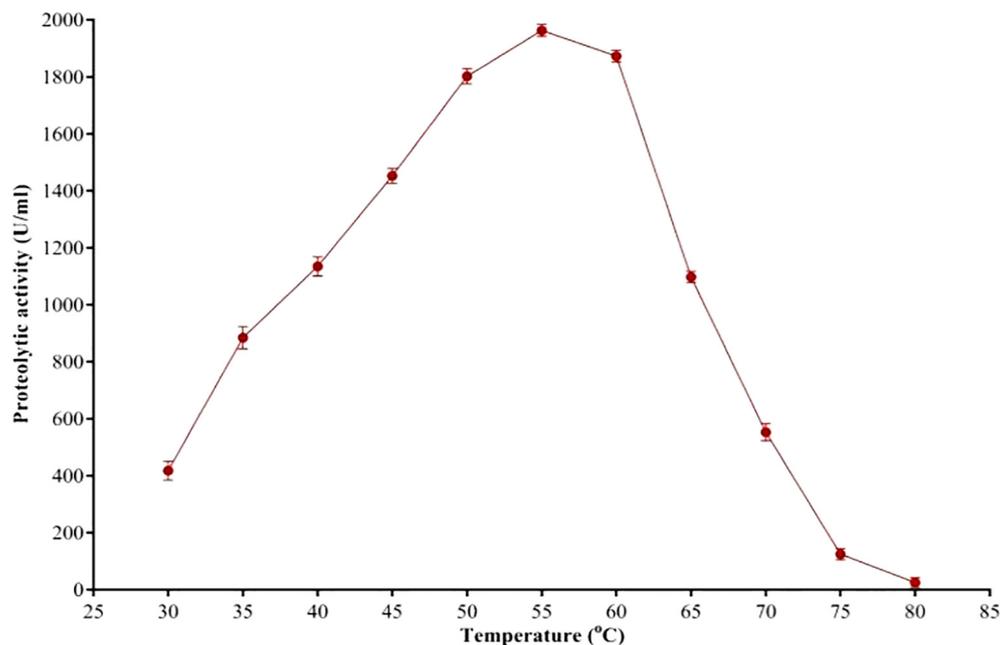


Fig. 4. Effect of temperature on the stability of the enzyme. The presented values are the mean values (n = 3) and standard deviation depicted with the error bars.

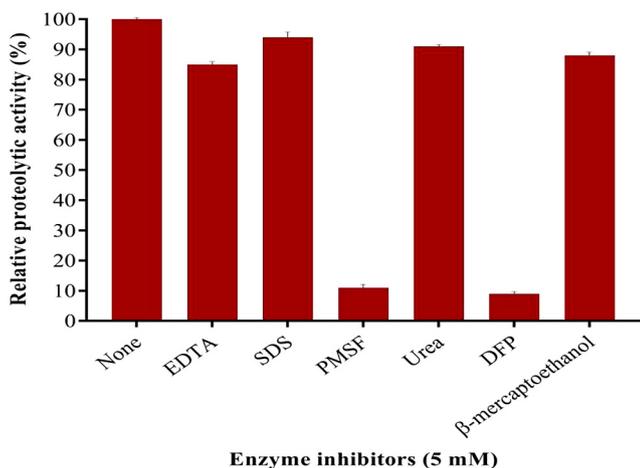


Fig. 6. Effect of various enzyme inhibitors on proteolytic activity of the enzyme. The presented values are the mean values ($n = 3$) and standard deviation depicted with the error bars.

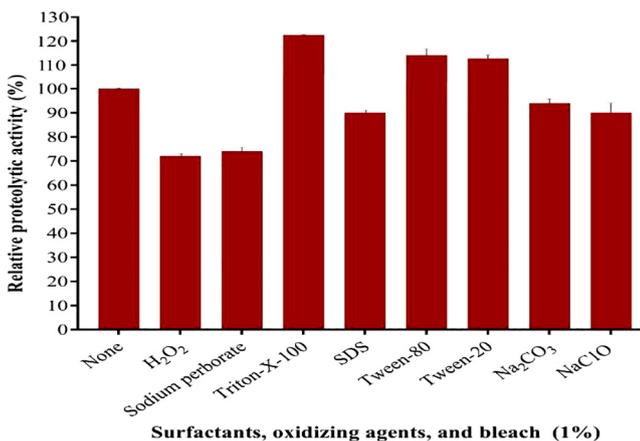


Fig. 7. Effect of various detergent additives on the proteolytic activity of the enzyme. The presented values are the mean values ($n = 3$) and standard deviation depicted with the error bars.

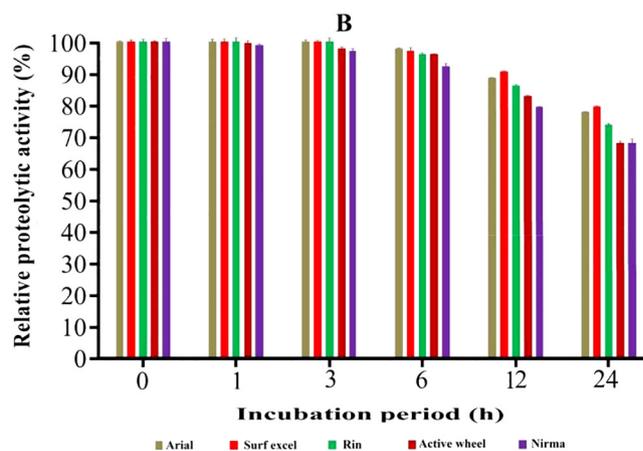
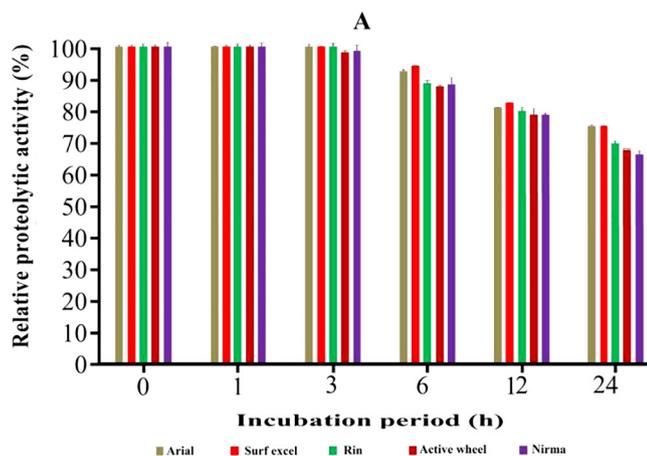


Fig. 8. Detergent compatibility of the alkaline protease with local detergents. (A) Represent the effect of detergents on enzyme stability at 55 °C, whereas; (B) represent the effect of detergents on enzyme stability at room temperature (34 ± 2 °C). The presented values are the mean values ($n = 3$) and standard deviation depicted with the error bars.

3.5. Effect of metal ions on the enzymatic activity

The influence of various metal ions on the proteolytic activity of the enzyme was determined at pH 10.5 and 55 °C. The result showed that the protease activity was significantly improved in the presence of Ca²⁺ and Cu²⁺. The Zn²⁺ and Fe²⁺ had a little influence in the increment of the enzyme activity, whereas; Co²⁺, Hg²⁺ slowdown the enzyme activity (Fig. 5). Many researchers have been reported divalent metal ions like Ca²⁺, Zn²⁺, Fe²⁺ etc. are the more common requirement for the enhancement of enzyme activity as well as protection from the thermal inactivation and retention of enzymatic activity at high temperatures [3,9,19].

3.6. Effect of enzyme inhibitors, surfactants, bleach, and oxidizing agents

The effects of enzyme inhibitors were assayed to determine the nature of protease. The result represented in Fig. 6 revealed that PMSF and DFP completely inhibited the proteolytic activity compared to other inhibitors indicating that enzyme belongs to the serine protease family. The PMSF, DFP, 1-10-phenanthroline enzyme inhibitors strongly inhibited the active site of the proteases by sulfonating essential serine residues. However, EDTA, Ethylene glycol-bis (β -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and Tosyl phenylalanyl chloromethyl ketone (TPCK), are also used as preferred enzyme inhibitors. [3,12,20].

The influence of surfactant, bleach, and oxidizing agents on the activity and stability of the enzyme were studied by adding additives at pH 10.5 and 55 °C. The results revealed that the surfactants stimulated the proteolytic activity of the enzyme, whereas, other additives have a modest influence on the enzyme activity (Fig. 7). The ideal enzyme should sustain a broad range of pH and temperature, along with compatibility and stability with various detergent ingredients [17,24,33,35].

3.7. Detergent compatibility

The stability and suitability of the alkaline protease were tested with 5 locally available detergent powders at 55 °C and room temperature (34 ± 2 °C). The result shown in Fig. 8 represented that purified protease enzyme retained 100% stability up to 3.0 h in all detergents except Active wheel and Nirma. About 30–35%

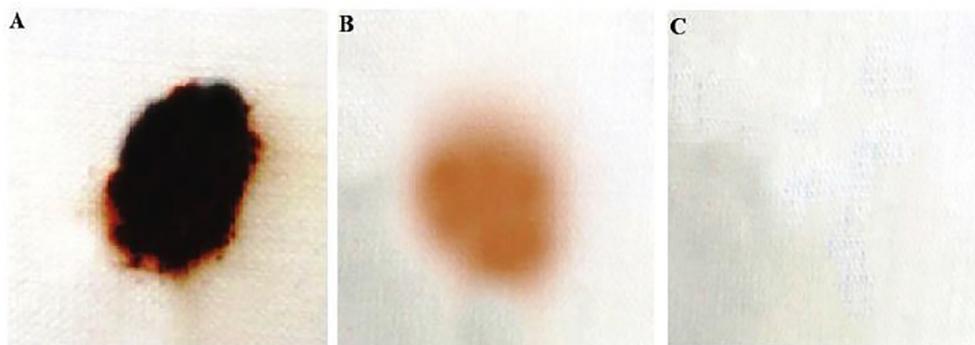


Fig. 9. The cleaning performance of purified enzyme in presence of local (Nirma) detergent. (A) A blood-spotted cotton fabric; (B) The blood-spotted fabric cleaned with local (Nirma) detergent solution only; and (C) blood-spotted cotton fabric cleaned with a mixture of detergent and enzyme solution.

proteolytic activity of the enzyme was decreased at the end of 24 h. The enzyme demonstrated remarkable sustainability in tested local detergents in a broad range of pH and temperatures. However, Adinarayana et al. [1] reported compatibility of the alkaline protease obtained from *Bacillus subtilis* PE-11 with commercial and local detergents. Niyonzima and More [30] reported almost similar results for the protease obtained from *Aspergillus terreus* gr. with 100% stability and compatibility at 60 °C for 2 h. The study of Haddar et al. [17] also reported detergent-compatible protease enzyme from *Bacillus mojavensis* A21, which exhibited excellent stability in the presence of solid and liquid detergents.

3.8. Cleaning performance of the enzyme

The alkaline protease expressed an excellent stain removal ability in the presence of 0.7% (w/v) detergent solution. The blood stain was completely removed from the cotton fabric when applied with Nirma detergent and 1 ml purified enzyme solution (Fig. 9). Many studies demonstrated the applicability and suitability of alkaline protease in commercial detergents, which served an excellent performance to accelerate the cleaning ability of detergents [16,21,30,33]. Similarly, Li et al. [26] represented the promising stability and compatibility of the alkaline protease obtained from *Cel-lulomonas bogoriensis* with locally available detergent in China. The competent ability of the enzyme revealed an ideal suitability for the laundry detergents to exhilarate the cleansing capacity of the detergents.

4. Conclusion

In the current study, we isolated *Bacillus* sp. APP-07 from Laundromat soil, which exhibited potent thermostable alkaline protease producing ability. After purification a low molecular weight (33.0 kDa), alkaline protease was obtained, which belongs to serine centred protease family. The enzyme showed excellent stability in the wide range of temperature and pH, also it has a good range of compatibility with various detergents, surfactants, and oxidizing agents. The novel parameter and characteristics of the enzyme may find potential applications in the laundry detergents, leather industries, and many other industrial aspects. Further, a complete characterization work is required to improve quality, stability, and superior performance of the thermostable alkaline protease.

5. Conflict of interest

The authors declare that they have no conflict of interest.

6. Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

The authors are grateful to all the staff members of the department of Microbiology, Dr. BAMU, sub-campus, Osmanabad and Haffkine Biopharmaceutical Corporation Ltd, Pune for providing constant support, guidance, and suggestion during research work.

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