MICROBIAL ALKALINE PROTEASES AS A GREENER AID TO ECO-SUSTAINABLE DETERGENT: ACTIONS TO ADDITION

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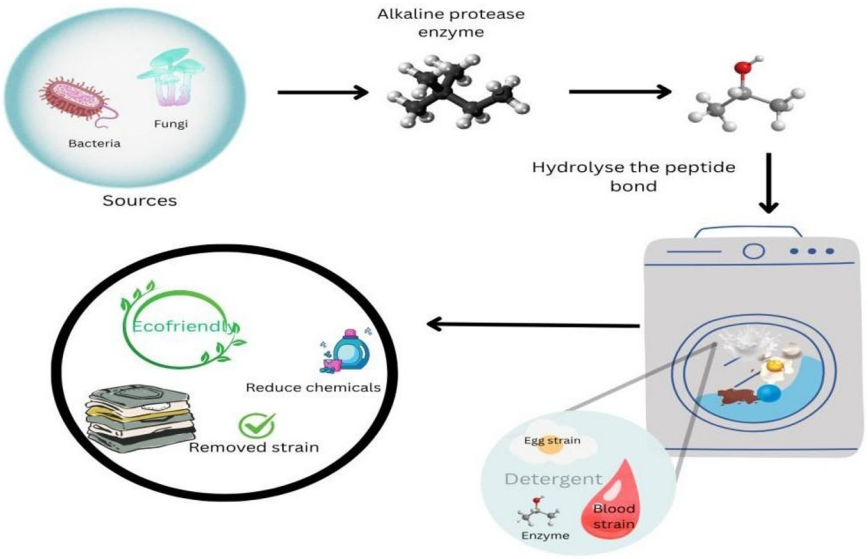
ABSTRACT

Alkaline proteases are among the most important industrial enzymes, widely used in detergent formulations due to their high efficiency, specificity, and eco-friendly nature. These enzymes break down protein-based stains such as blood, milk, eggs, and sweat by converting insoluble proteins into soluble peptides and amino acids under alkaline conditions. This enhances cleaning performance while maintaining fabric quality. Although proteases are produced by plants, animals, and microorganisms, microbial sources are preferred because of their high yield, stability, and ease of genetic modification. Industrially important microorganisms such as *Bacillus*, *Aspergillus*, and *Trichoderma* produce proteases that are more stable and cost-effective compared to plant- and animal-derived enzymes like papain and trypsin. Microbial alkaline proteases play a key role in laundry detergents, dishwashing agents, and industrial cleaners, allowing effective stain removal even at lower temperatures and reducing the need for harsh chemicals. Advances in protein engineering, recombinant DNA technology, and directed evolution are further improving enzyme stability and performance. Additionally, the use of agro-industrial waste for enzyme production offers a sustainable and economical approach. Overall, alkaline proteases provide an efficient and environmentally friendly solution, supporting greener cleaning technologies and sustainable industrial development.

Keywords: Alkaline protease; eco-sustainable detergent; greener aid.

Graphical abstract



INTRODUCTION

Microbial alkaline proteases have emerged as indispensable biocatalysts in modern industry, particularly in the formulation of eco-sustainable detergents. Although a few exceptional enzymes exhibit extreme operational optima—functioning efficiently at pH values approaching 13 and temperatures as high as 80–90°C—the majority of alkaline proteases relevant to detergent applications demonstrate peak catalytic activity within the pH range of 8–12 and at temperatures between 50 and 70°C. Their isoelectric points typically fall between pH 8 and 11, reflecting their inherent alkaliphilic nature. In recent years, several industrially important proteases have been crystallized to elucidate their molecular homology, catalytic architecture, and three-dimensional structural dynamics. Proteases, which constitute one of the most diverse classes of hydrolytic enzymes, are broadly classified into neutral, acidic, and alkaline categories based on their pH optima [20] While plants, animals, and microbes can all synthesize proteolytic enzymes, microorganisms remain the preferred source due to their superior production efficiency, reduced cost, and process scalability[21].

Among microbial proteases, alkaline proteases hold exceptional economic and industrial significance. Enzymes derived from microbial systems exhibit remarkable stability across a wide spectrum of pH, temperature, and substrate conditions, enabling their deployment in highly variable processing environments [3] Advances in targeted genome editing and protein engineering have further enhanced the catalytic efficiency, stability, and substrate specificity of bacterial proteases. Owing to rapid growth rates, genetic tractability, and minimal space requirements, microbial communities—particularly Bacillus species—have been adopted as the predominant biological platforms for large-scale protease production [12]. Notably, the detergent industry relies heavily on pL4-type alkaline proteases for their robust performance and compatibility with detergent formulations.

Alkaline proteases are inherently eco-friendly, largely because they are produced by non-pathogenic and non-hazardous microbial sources. Although proteolytic enzymes exist across biological kingdoms, microbial-derived proteases are generally favored for sustainable and industrial-scale production [4]. These enzymes work best in an alkaline pH range of 7 to 11, with optimal performance commonly observed around pH 9 [19]. Within the bacterial domain, Bacillus species represent the only commercially dominant producers of alkaline proteases [6].Widely distributed in soil, water, sludge, and diverse environmental niches, Bacillus species secrete alkaline proteases capable of functioning efficiently in pH environments spanning 8–13. Their exceptional operational stability under harsh industrial conditions underpins their extensive use in food processing, cleaning formulations, healthcare products, and environmental management technologies [28].

The advent of molecular biology and recombinant DNA technology has profoundly transformed industrial protease development. Through strategies such as gene overexpression, site-directed mutagenesis, and heterologous expression, it is now possible to engineer proteases with tailored catalytic attributes for specialized applications [9]. Microorganisms produce both intracellular and extracellular proteases, each with distinct physiological and industrial functions. Intracellular proteases regulate metabolic pathways, enzyme turnover, and hormone modulation, while extracellular proteases mediate protein hydrolysis outside the cell, thereby enabling nutrient assimilation and metabolic adaptation [5]. Compared to plant- and animal-derived enzymes, microbial proteases consistently demonstrate broad operational ranges in both pH and temperature, making them highly desirable for industrial use.

Extensive research has characterized alkaline proteases from diverse microbial taxa, including their optimal pH and temperature profiles, responses to solubilizing agents, and susceptibility to inhibitory compounds. Importantly, several of these enzymes exhibit pronounced halo-tolerance and alkaliphilicity, enabling their survival and activity in environments with high salt and elevated pH [29]. Alkaline proteases currently constitute nearly 60% of the global enzyme market, underscoring their immense commercial relevance. Their applications span multiple sectors, including detergent manufacturing, silver recovery from X-ray films, leather dehairing processes, industrial bleaching, and chitin extraction from shrimp waste through protein hydrolysis [26]

Despite their industrial advantages, the cost of enzyme production remains a significant constraint, with growth substrates contributing approximately 30–40% of overall manufacturing expenses. This economic challenge necessitates the development of low-cost and high-yield fermentation media to enhance commercial viability. Given these considerations, the present review aims to provide a comprehensive analysis of microbial alkaline proteases, encompassing their isolation, production strategies, physiological and environmental determinants, and their expanding role as sustainable agents in the detergent industry.

2. Sources of alkaline proteases

Microbial systems including bacteria and fungi are some of the sources of alkaline proteases. In order to lower overall costs and encourage sustainable bioprocessing, agricultural and industrial waste materials have recently been used as efficient substrates for enzyme synthesis. Large-scale alkaline protease production for industrial and environmental applications is made more feasible by the use of such diverse and renewable sources.

In considering this, the goal of the current study was to determine whether it would be possible to use easily available substances in submerged fermentation for the production of alkaline protease by *Bacillus subtilis* by utilizing cheap wastes of bacterial growth conditions and abundant natural resources to increase protease enzyme production, as well as the impact of adding sources of nitrogen and metal salts on enzyme activity.

2.1 Alkaline protease from bacteria

The earliest detailed report on the efficient production of alkaline protease by *Bacillus* species dates back to 1971[8]. Since then, bacterial alkaline proteases have become indispensable across major industries—including leather processing, food technology, detergent formulation, pharmaceuticals, waste management, and silver recovery. Their ability to replace harsh chemicals with biodegradable catalytic alternatives underscores their significance as environmentally benign industrial enzymes.

Bacteria capable of producing alkaline proteases inhabit a wide spectrum of ecological niches, ranging from soil, water, and air to the gastrointestinal tracts of animals. Marine bacteria, in particular, are ubiquitous across global oceans and inhabit diverse microenvironments such as fish intestines, deep-sea sediments, hydrothermal systems, seamounts, and even the surfaces of macroalgae. These microorganisms have evolved highly specialized adaptation strategies that enable survival in extreme salinity gradients—from hypersaline waters to brackish zones—and temperatures spanning from 33 °C in tropical regions to below 5 °C in deep-sea and polar environments.

Marine organisms, by virtue of their exposure to elevated pressure, salinity, and alkaline conditions, serve as a reservoir of novel bioactive compounds. Their alkaline proteases often display exceptional stability and catalytic efficiency under harsh conditions, making them ideal candidates for detergent formulations. These enzymes effectively degrade protein-based stains such as blood, sweat, food residues, and keratin-rich contaminants. Consequently, bacteria remain one of the most valuable biological sources for the industrial production of alkaline proteases, particularly given their fast growth rates, ease of cultivation, and amenability to genetic improvement.

Table 1 Bacterial species and their applications

Microorganism	Industrial Application	Reference
Bacillus sp. (Bacillus Safensis)	Applied in detergent formulation	[14]
Bacillus pseudofirmus	Employed in detergent manufacturing	[28]
Bacillus sp. (Bacillus atrophaeus)	Enzyme in detergent formulation	[17]
Bacillus Stearothermophilus	Component of detergent formulations	[15]

2.2 Alkaline protease from fungi

Fungal enzymes, including alkaline proteases, are widely utilized in industrial processes owing to their high productivity, rapid secretion, and compatibility with low-cost substrates [16]. Fungi also offer practical advantages over bacteria, such as ease of biomass separation and straightforward recovery of mycelial mass from fermentation broth [1]. These attributes streamline downstream processing and reduce operational costs, thereby contributing to the growing global demand for fungal proteases.

Fungal alkaline proteases are applied extensively in the food and detergent sectors and hold emerging potential for leather treatment, textile processing, bioremediation, and the development of eco-friendly catalytic systems [23]. Despite their industrial importance, only a limited number of fungal species capable of thriving at alkaline pH are well documented. Most fungi exhibit optimal growth in slightly acidic to neutral environments. Consequently, the full biotechnological potential of alkaliphilic and alkali-tolerant fungi remains underexplored.

Fungal proteases are typically categorized based on their pH optima:

- **Acid proteases** operate within pH 2.5–6.0, with maximal activity at pH 4.0–4.5. Their specificity and temperature stability make them ideal for cheese making and dairy fermentations.
- **Neutral proteases** exhibit optimal activity around pH 7.0, are sensitive to chelating agents, and excel in hydrolyzing peptide bonds between hydrophobic amino acids.

The discovery and utilization of fungal alkaline proteases represent a promising frontier for industrial enzyme innovation, particularly given their robustness, broad substrate specificity, and compatibility with green manufacturing processes.

Table 2. Fungi species and their applications

Microorganism	Industrial Application	Reference
Scopulariopsis spp.	Used in detergent preparation and enhancement	[16]
Penicillium verrucosum	Applied in laundry detergent formulation	[11]
Trametes cingulata CTM10101	Utilized for detergent blend improvement	[2]
Aspergillus sp. DHE7	Incorporated into detergent systems for better stain degradation	[24]
Trichoderma longibrachiatum, Aspergillus niger	Effective in eliminating blood stains from fabrics	[25]

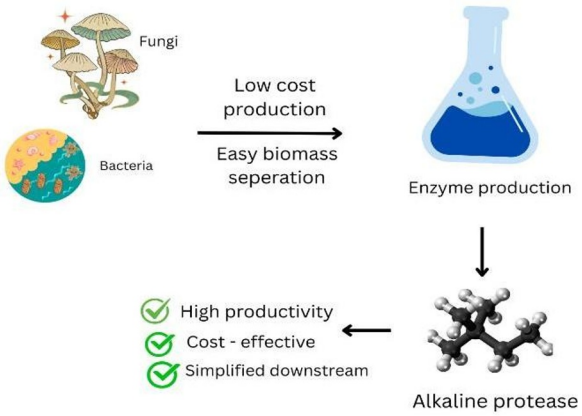


Fig. 1 Microbial sources of alkaline protease

3. Mechanism of detergent action of alkaline protease

Detergents function by lowering the energy required for soil removal, whether the energy originates from mechanical washing or manual scrubbing. The incorporation of alkaline proteases significantly enhances detergent performance by accelerating stain degradation, reducing washing time, and decreasing overall energy consumption.

When detergent formulations are supplemented with proteolytic enzymes, the breakdown of complex proteinaceous stains becomes more efficient, resulting in superior cleaning performance. The efficacy of protease-enhanced detergents is typically evaluated using analytical techniques such as visual scoring, turbidity measurements, gravimetric assessment, reflectance spectroscopy, densitometric scanning, and colorimetry.

Key mechanisms involved in protease-assisted detergent action include:

- **Hydrolysis of peptide bonds:** Proteases cleave peptide linkages in protein-based stains, converting them into soluble peptides and amino acids.
- **Breakdown of stain matrices:** Enzymatic action disrupts the structural integrity of proteinaceous soils, facilitating their release from fabric fibers.
- **Synergistic interaction with detergent components:** Surfactants, builders, and proteases act cooperatively to optimize soil dispersion and stain removal.
- **Removal of blood stains:** Since blood contains plasma proteins, alkaline proteases rapidly hydrolyze these components into smaller peptides, enhancing the removal of stubborn stains.

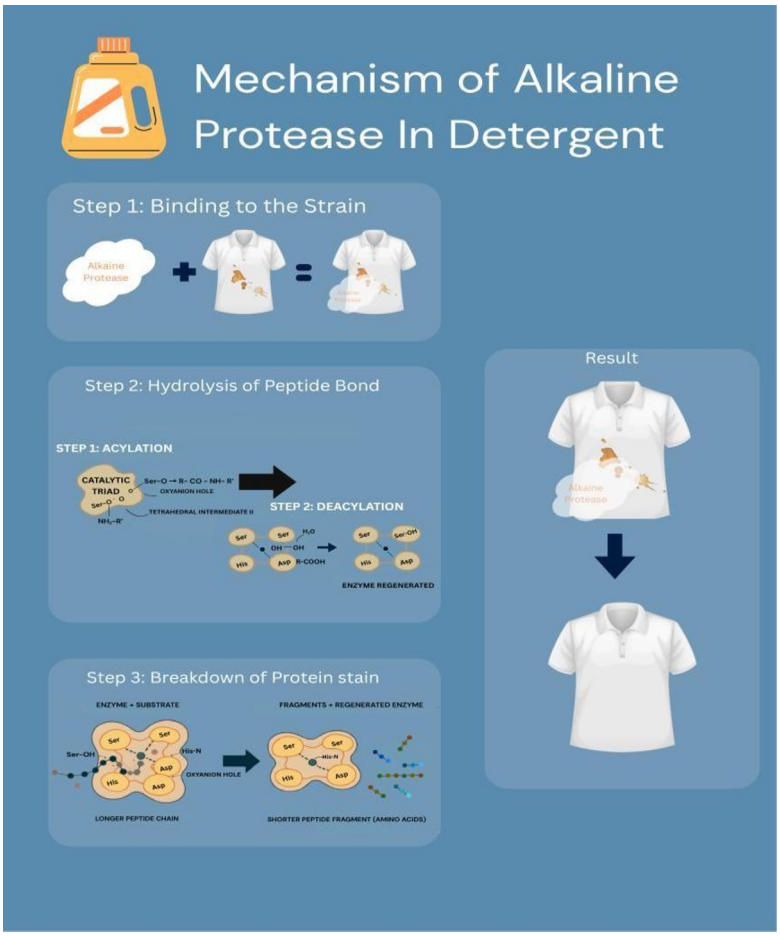


Fig. 2 Mechanism of alkaline protease in detergent

4. Detergent applications of alkaline protease

Alkaline proteases have a long history of use in the detergent industry, where they account for the largest portion of the global enzyme market [19]. The most common usage of alkaline protease is as an ingredient in detergents. 60–65% of the industrial enzyme market worldwide is made up of alkaline proteases. Subtilisins are being developed as detergent proteases. Use began in 1913, made with *Bacillus* species. Shorter agitation times and lower wash temperatures are made possible by increased washing efficiency, high stability and activity throughout a wide pH and temperature range. Low levels of activity. The detergent industry likewise places a high value on serine proteases produced by *Bacillus* strains.

The detergent industry has long been interested in alkaline protease enzymes because of their effectiveness in removing protein-based stains, such as blood, proteins released by our bodies, and foods like dairy products, eggs, meat, and fish. They also offer unique cleaning benefits that are challenging to obtain with conventional detergent formulations. Detergent manufacturers are becoming more and more dependent on enzyme-based technologies due to a number of factors, including consumer recognition of cleaning efficiency, the introduction of completely new cleaning functionalities, fabric renewal effects, and improved cost-to-performance ratios made possible by the availability of highly efficient enzymes and continuous product price reductions.

The evolution of enzymes employed in detergent formulations is being shaped by current market dynamics and customer needs, with an emphasis on those with increased catalytic efficiency, higher stability, and greater compatibility with other detergent components. Additionally, producers of enzymes and detergent firms are always investigating new classes of enzymes to satisfy consumer demands for better cleaning results, fabric protection, and antibacterial qualities. These enzymes are frequently used not just in laundry formulations but also in industrial and institutional cleaning agents and automated dishwashing detergents [22].

Detergents are commonly used in home laundry and as contact lens cleansers. Proteases are now an essential part of every detergent. These enzymes produce detergents that are effective for washing [30].Because of their ionic strength, these proteases are ideal for use in detergents. It is believed that proteases work best when their ionic strength is equal to the pH of the detergent solution, increasing the detergent's efficacy. Blood stains are frequently removed using Spilosoma oblique protease [13]. Because alkaline proteases are more effective at removing stains from textiles and more stable across a pH and temperature range, they are frequently used in conjunction with commercial detergents.

Because they can eliminate any type of proteinaceous debris, proteases are a necessary and common addition in detergents [10]. In 1913, the first enzymatic solution known as "Brunus" was created using sodium carbonate and crude pancreatic extract [18]. In 1956, a detergent called "BIO-40," which contained a bacterial enzyme, was first put on the market. Because proteases work on a variety of substrates, it is beneficial to remove food, blood, and bodily secretion stains. Alkaline proteases are an essential option for their use in the detergent industry due to features including stability at alkaline pH and high temperatures, as well as the capacity to tolerate surfactants, oxidizing agents, and chelating agents [19].

Aspergillus clavatus ES1's alkaline serine-protease showed remarkable stability (100%) in non-ionic surfactants (5% (v/v) Tween 80 and Triton X-100); yet, the enzyme maintained 90% of its normal activity when examined with 0.1% SDS, a powerful anionic surfactant. Additionally, the enzyme retained 71 and 53% activity, respectively, when exposed to 1% (w/v) and 2% (w/v) sodium perborate, demonstrating considerable stability. Similar findings were found for the protease generated by *Scopulariopsis* spp. in another study, which showed that Tween-80, SDS, and Triton X-100 increased protease activity while maintaining 50% enzyme activity in industrial detergents (enzymatic and nonenzymatic) [27]. In the presence of several commercial detergents, including Tide, Hattic, Savo, Surf, Henko, Persil, Wheel, and Aerial, Anoth A. terreus maintained 50–80% of its initial activity.

The compatibility of the enzymes produced by two fungal isolates—*Graphiumputredinis,* also known as *Trichoderma harzianum*, and an interlinked derived fusant—was further investigated by a group of researchers. All three enzymes retained 58.25–73.82% and 61.58–70.24% of residual activity when exposed to SDS and sodium perborate, respectively. However, when tested for compatibility with the commercial detergent Rin Advanced, the enzyme from fusant was more stable than its parents,

exhibiting 76.74% of activity. The compatibility and incorporation of alkaline protease generated by *Aspergillus niger* and *Aspergillus sp.* DHE7 as an addition in detergent formulation was the subject of similar investigations [7].

The introduction of third-generation cold-adapted alkaline proteases to the detergent industry was highlighted in recent literature. These proteases have been identified to have excellent activity and stability in surfactants and bleaches [24].

5. Future prospects

Research on alkaline proteases continues to advance rapidly, propelled by their extensive utility across industrial, environmental, and biomedical sectors. Ongoing investigations address not only the microbiological aspects of enzyme production but also the regulatory and pharmacological dimensions that govern their performance and safety. As microbial systems remain the dominant platforms for industrial protease synthesis, microbial alkaline proteases are expected to play increasingly central role in future biotechnological innovations.

The integration of protein engineering, directed evolution, and gene cloning has transformed enzyme development by enabling the creation of tailor-made proteases with enhanced stability, broader substrate specificities, and improved catalytic efficiency. These tools allow researchers to manipulate molecular structures in vitro, thereby generating enzymes optimized for highly specific industrial processes such as detergent formulation, leather processing, and bioremediation.

Moreover, extremophilic microorganisms—organisms thriving in extreme pH, temperature, salinity, and pressure—represent a promising resource for discovering intrinsically robust proteases suited for challenging industrial environments. Enzymes derived from alkaliphiles, thermophiles, halophiles, and psychrophiles display exceptional resistance to denaturation, making them ideal candidates for next-generation detergent applications.

Future research is expected to emphasize:

a. High-throughput screening of microbial diversity for novel alkaline proteases.
b. Rational and semi-rational enzyme design to enhance catalytic performance.
c. Metagenomic exploration of uncharacterized ecological niches.
d. Development of eco-efficient, low-cost substrate systems for sustainable enzyme production.
e. Integration of synthetic biology approaches to construct optimized microbial cell factories.

Collectively, these advancements will pave the way for more stable, application-specific, and environmentally compatible alkaline proteases suitable for large-scale deployment.

6. Conclusion

Proteases, particularly alkaline proteases, continue to be indispensable in the detergent industry due to their exceptional ability to hydrolyze proteinaceous stains under a wide range of washing conditions. Their incorporation into detergent formulations not only enhances cleaning efficiency but also reduces reliance on harsh chemical agents, supporting the development of eco-friendly and sustainable cleaning technologies. Owing to their inherent stability at elevated pH and temperature, alkaline proteases remain the most widely employed class of detergent-compatible enzymes.

Recent biotechnological advancements—including protein engineering, recombinant DNA technology, and the isolation of enzymes from extremophilic microorganisms—are opening new avenues to produce proteases that are more

thermostable, alkaline-resistant, and cost-efficient. These engineered enzymes are tailored to meet evolving consumer demands while simultaneously addressing global priorities for environmental sustainability.

Beyond detergents, alkaline proteases serve as vital biocatalysts across industries such as leather processing, food technology, pharmaceuticals, and bio-waste management. Microbial sources—especially bacteria and fungi—have revolutionized enzyme manufacturing by offering high yields, reduced production costs, and adaptability to industrial fermentation systems. Their stability under harsh processing conditions, such as high pH, elevated temperatures, or saline environments, further underscores their industrial relevance.

Emerging studies highlight the promising potential of diverse microbial taxa—including marine, estuarine, and cold-adapted organisms—in generating proteases with unique catalytic characteristics. For example, *Aspergillus ustus*, *Pseudoalteromonas atlantica*, and *Serratia marcescens* have been reported to produce alkaline or cold-tolerant proteases, expanding the scope of enzyme applications in challenging operational environments. Developments in genetic engineering and bioprocess optimization have significantly increased the yields, specificity, and functional diversity of these enzymes.

In conclusion, alkaline proteases represent a cornerstone of modern industrial biotechnology. Their continued development and refinement will be instrumental in advancing sustainable manufacturing, improving detergent performance, and supporting global efforts toward environmentally conscious industrial solutions.

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Author contributions

R.V wrote the manuscript text. All authors reviewed and revised the manuscript.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests

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