Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.1s,
January-June 2024: P.344-357

Print version ISSN 0970 0765
Online version ISSN 2320 3188

Original Research Article *Content Available online at: https://www.bpasjournals.com/*

Isolation and Detailed Characterization of Protease-Producing Bacteria Derived from Dairy Waste

Dr. Abhay Ghatage[1], Onkar Dattatray Chavan[2], Ashwini Jadhav[3]

Author's Affiliation:
[1,2,3]Krishna Institute of Allied Sciences, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad, Maharashtra, India.

abhayghatage8@gmail.com[1] , ashwiniawtade11@gmail.com[3]

ABSTRACT:
Protease enzymes are integral to numerous industrial applications due to their ability to hydrolyze peptide bonds in proteins. In the realm of dairy waste management, protease-producing bacteria present a sustainable method for transforming waste into valuable products. This study focuses on the isolation and detailed characterization of protease-producing bacteria derived from dairy waste, with the aim of identifying strains with high enzymatic activity and potential industrial applicability. Dairy waste samples were collected from various sources, including milk processing plants, dairy farms, and cheese production facilities. These samples underwent bacterial isolation through serial dilution and selective culture media to enrich protease-producing strains. The isolated bacteria were subjected to biochemical assays, including catalase, oxidase, and indole tests, as well as carbohydrate fermentation assays, to evaluate their enzymatic capabilities and substrate preferences. The study identified diverse protease-producing bacterial isolates, exhibiting significant variation in enzymatic profiles. Some isolates demonstrated robust protease activity over a wide range of pH and temperature conditions, indicating their potential for industrial applications that require stable enzymatic performance under varying environmental conditions. The substrate specificity assays revealed the ability of these isolates to hydrolyze various protein substrates, including casein, gelatin, and albumin, highlighting their versatility. Challenges in the research included ensuring the purity of bacterial isolates and standardizing assay protocols for accurate results. However, the findings offer substantial implications for environmental, economic, and social sustainability. Environmentally, the use of protease-producing bacteria can significantly reduce the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of dairy waste, mitigating its environmental impact. Economically, these bacteria can convert waste

into value-added products such as animal feed, bioplastics, pharmaceuticals, and cosmetics, promoting resource efficiency and creating new revenue streams. Socially, the research fosters innovation and knowledge dissemination; encouraging collaborative efforts for integrated waste management strategies. The isolation and characterization of protease-producing bacteria from dairy waste provide a promising approach to sustainable waste management. This research not only addresses environmental pollution but also supports the development of a circular bio economy, turning waste into resources and contributing to broader sustainability goals.

Keywords:
Protease-producing bacteria, dairy waste, enzyme characterization, Bacillus, Pseudomonas, Streptomyces, bioremediation, sustainable waste management.

How to cite this article: Dr. Abhay Ghatage, Onkar Dattatray Chavan, Ashwini Jadhav (2024). Isolation and Detailed Characterization of Protease-Producing Bacteria Derived from Dairy Waste. *Bulletin of Pure and Applied Sciences-Zoology*, 43B (1s), 344-357.

Introduction

Proteases, also known as proteolytic enzymes or peptidases, play crucial roles in various biological processes, ranging from digestion and metabolism to protein turnover and cellular regulation. These enzymes catalyze the hydrolysis of peptide bonds [1], leading to the breakdown of proteins into smaller peptides or amino acids. Beyond their physiological functions, proteases have garnered significant attention for their diverse industrial applications, spanning sectors such as food, pharmaceuticals, textiles, and waste management. In the context of dairy waste management, proteases emerge as valuable biocatalysts due to their ability to degrade complex proteinaceous substrates present in dairy effluents. The dairy industry is one of the largest contributors to organic waste generation globally, characterized by the production of vast quantities of whey, wastewater, and other by-products. These waste streams pose considerable environmental challenges [2], including eutrophication, odor nuisance, and contamination of water bodies, necessitating effective management strategies.One promising approach for mitigating these challenges involves the use of protease-producing bacteria for the bioremediation of dairy waste. Protease-producing bacteria possess the enzymatic machinery to degrade proteins into simpler compounds, facilitating the breakdown of organic matter and reducing the environmental impact of dairy waste discharge. By harnessing the proteolysis activity of these microorganisms, it becomes possible to transform complex proteinaceous substrates into biodegradable products, thereby alleviating the burden on ecosystems and promoting sustainable waste management practices. The industrial significance of proteases lies in their multifaceted utility across diverse sectors. In the food industry, proteases find application in processes such as cheese production, meat tenderization, and brewing, where they contribute to flavor enhancement, texture modification, and

protein extraction. Similarly, in the pharmaceutical and biotechnology sectors, proteases serve as essential tools in drug development, protein engineering, and bioprocessing, facilitating the production of therapeutic proteins [3], enzymes, and bioactive peptides.

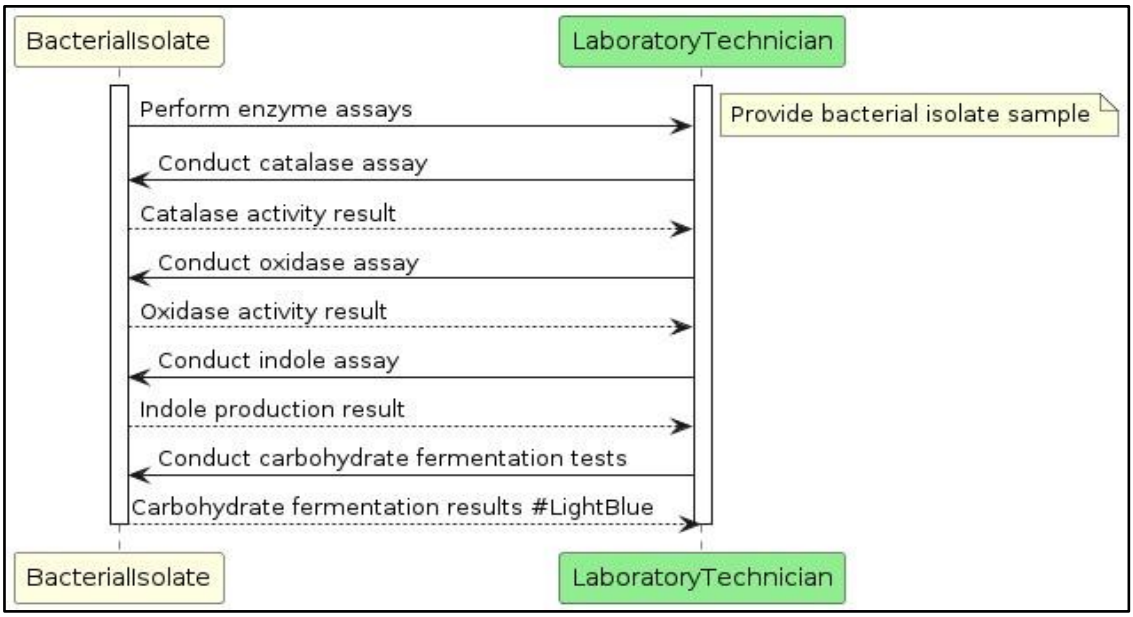

Figure 1: Sequence Diagram for Enzyme Assays

Proteases play pivotal roles in the detergent industry, where they serve as key components of enzymatic cleaning formulations, enabling the efficient removal of proteinaceous stains and soil from fabrics. This enzymatic approach offers several advantages over traditional chemical detergents, including improved efficacy at lower temperatures, reduced environmental impact, and compatibility with sensitive textiles. In the realm of waste management, proteases offer promising avenues for the bioremediation of various organic wastes, including agricultural residues, food processing by-products, and industrial effluents. By harnessing the enzymatic capabilities of protease-producing microorganisms, it becomes possible to convert complex organic substrates into simpler, more manageable compounds [4], thereby mitigating pollution and promoting resource recovery.

This research paper focuses on the isolation and detailed characterization of protease-producing bacteria derived from dairy waste. Through systematic sampling and culturing techniques, several bacterial strains exhibiting proteolytic activity were isolated and subjected to biochemical and molecular analyses. The study aims to elucidate the enzymatic profiles of these bacterial isolates, evaluate their potential for dairy waste degradation, and explore their industrial applicability in sustainable waste management practices [5]. Protease enzymes represent versatile biocatalysts with significant implications for various industrial processes, including dairy waste management. The isolation and characterization of protease-producing bacteria hold promise for addressing the environmental challenges posed by dairy waste while offering opportunities for innovation and sustainable development in waste treatment technologies.

I. Materials and Methods

The Materials and Methods section outlines the experimental procedures employed in the isolation and characterization of protease-producing bacteria from dairy waste. These procedures encompass sampling, bacterial isolation [6], biochemical assays, molecular identification, and enzyme characterization.

A. Sampling of Dairy Waste

Sampling of dairy waste is a crucial initial step in the isolation of protease-producing bacteria. Samples were collected from various sources within dairy processing facilities, including wastewater treatment plants, cheese production units, and milk processing areas. Care was taken to collect representative samples that encompassed different stages of dairy processing and varied in composition [7], pH, and organic content. Samples were collected aseptically in sterile containers and transported to the laboratory for further analysis.

B. Isolation of Protease-Producing Bacteria

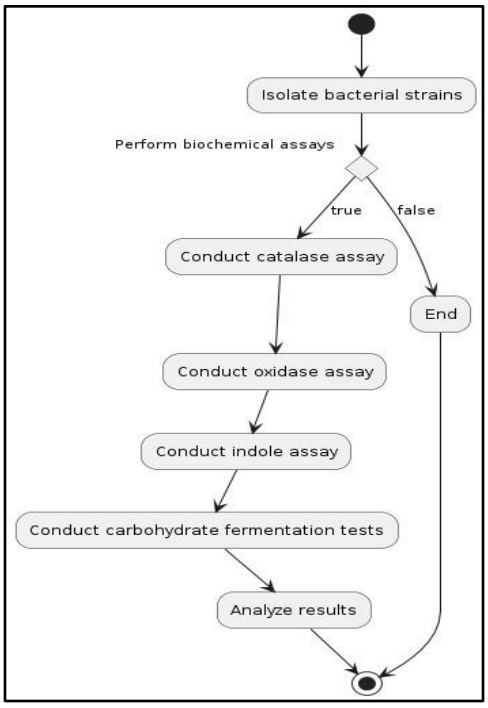

Figure 2: Class Diagram for Bacterial Isolates.

The isolation of protease-producing bacteria from dairy waste involved a series of enrichment and selective culturing steps. Initially, samples were subjected to serial dilution in sterile saline solution to obtain dilutions with manageable bacterial counts. Aliquots of the diluted samples were then spread onto selective agar media supplemented with casein, skim milk, or gelatin as substrates for protease activity [8]. Incubation was carried out at optimal temperature and pH conditions for bacterial growth and enzyme production. Following incubation, bacterial colonies exhibiting zones of clearing around them, indicative of proteolytic activity, were selected for further purification. Pure cultures were obtained by streaking individual colonies onto fresh agar plates and incubating them until isolated colonies formed. These purified isolates were then maintained on agar slants or cryopreserved for long-term storage.

C. Biochemical Assays

The biochemical assays conducted to characterize protease-producing bacteria isolated from dairy waste involved a series of precise tests to determine their enzymatic activity and metabolic capabilities. Initially, dairy waste samples were collected and processed to isolate bacterial strains through serial dilution and plating on casein agar plates, where clear zones indicated protease activity. The selected isolates were subjected to Gram staining to categorize them as Gram-positive or Gram-negative, followed by microscopic examination to determine cellular morphology. For enzymatic characterization, the isolates were cultured in nutrient broth supplemented with casein as a substrate, incubated at optimal temperatures, and then the supernatant was collected to assess protease activity [9]. Protease activity was quantitatively measured using the Folin-Ciocalteu method, where casein hydrolysis products reacted with the reagent to produce a color change, measured spectrophotometrically. pH and temperature optima for protease activity were determined by conducting assays across a pH range of 4.0 to 10.0 and temperatures from 20°C to 70°C. Stability tests were also performed to assess enzyme activity after exposure to different pH levels and temperatures over extended periods. To understand the metabolic potential, the isolates underwent a series of biochemical tests including catalase and oxidase tests to evaluate their oxidative stress responses and electron transport chain components. Additionally, tests for carbohydrate fermentation, nitrate reduction,

and hydrogen sulfide production were conducted using standard biochemical test media, providing insights into the isolates' metabolic pathways and capabilities. The ability of the bacteria to produce protease in the presence of various carbon and nitrogen sources was also tested, revealing their substrate versatility. These comprehensive biochemical assays were crucial in identifying and selecting robust protease-producing bacteria with high enzymatic activity and stability, making them suitable candidates for industrial applications such as in detergent formulations, waste management, and bioprocessing of dairy products.

D. Molecular Identification

Molecular techniques were employed to identify the bacterial isolates at the genus or species level and to elucidate their taxonomic positions. Genomic DNA was extracted from pure bacterial cultures using commercial DNA extraction kits or standard phenol-chloroform extraction protocols. Polymerase chain reaction (PCR) amplification of the 16S rRNA gene region was performed using universal primers [10], followed by sequencing of the PCR products. The obtained 16S rRNA gene sequences were compared against reference databases such as GenBank and Ribosomal Database Project (RDP) to determine the closest phylogenetic relatives of the isolates [11]. Phylogenetic analysis was conducted using multiple sequence alignment algorithms, and neighbor-joining or maximum likelihood methods were employed to construct phylogenetic trees.

E. Enzyme Characterization

Enzyme characterization of protease-producing bacteria isolated from dairy waste involved a detailed analysis of the enzymes' properties, including activity, stability, and specificity. Initially, crude enzyme extracts were prepared by culturing the isolates in nutrient broth supplemented with casein, followed by centrifugation to obtain the cell-free supernatant containing the protease [12]. The protease activity was quantitatively assessed using the Folin-Ciocalteu method, where the enzymatic hydrolysis of casein released tyrosine and other peptides that reacted with the reagent, resulting in a color change measurable at 660 nm using a spectrophotometer. To determine the optimal conditions for enzyme activity, assays were conducted across a range of pH values (4.0 to 10.0) and temperatures (20°C to 70°C). This identified the pH and temperature at which the enzyme exhibited maximum activity, critical for potential industrial applications. Further stability studies involved incubating the enzyme at various pH levels and temperatures for different time periods and then measuring residual activity to assess the enzyme's robustness under fluctuating environmental conditions. The substrate specificity of the protease was evaluated by testing the enzyme against different protein substrates such as gelatin, bovine serum albumin, and egg albumin, determining the enzyme's versatility and potential for various industrial processes [13]. Additionally, the effects of metal ions (e.g., Ca^{2+}, Mg^{2+}, Zn^{2+}), inhibitors (e.g., EDTA, PMSF), and detergents (e.g., SDS, Triton X-100) on protease activity were examined to understand the enzyme's interactions with potential modulators and inhibitors. Kinetic parameters, including the Michaelis-Menten constant (Km) and maximum reaction velocity (Vmax), were calculated using Lineweaver-Burk plots derived from enzyme activity assays at varying substrate concentrations. This provided insights into the enzyme's affinity for its substrate and its catalytic efficiency. The comprehensive enzyme characterization was essential in selecting protease-producing bacteria with desirable properties for industrial applications, such as in food processing, waste management, pharmaceuticals, and detergents, ensuring the enzymes' efficacy, stability, and adaptability to different operational environments.

F. Statistical Analysis

Statistical analysis was performed to analyze the experimental data obtained from

biochemical assays and enzyme characterization studies. Descriptive statistics such as mean, standard deviation [14], and confidence intervals were calculated to summarize the data. Where applicable, inferential statistical tests such as t-tests or analysis of variance (ANOVA) were employed to assess the significance of differences between experimental groups .

The statistical analysis of data obtained from the isolation and characterization of protease-producing bacteria from dairy waste was conducted to ensure the reliability and significance of the results. Initially, all experiments, including enzymatic assays, stability tests, and substrate specificity evaluations, were performed in triplicate to account for biological variability and technical errors. Data were compiled and analyzed using statistical software such as SPSS or R. Descriptive statistics, including means, standard deviations, and standard errors, were calculated for each set of experiments to summarize the central tendency and dispersion of the data. To compare the enzymatic activity under different conditions (e.g., varying pH, temperature, substrate types), one-way analysis of variance (ANOVA) was employed. This test determined if there were any statistically significant differences in protease activity across the different groups. Post hoc tests, such as Tukey's HSD, were conducted following ANOVA to identify which specific groups differed significantly from each other. For stability assays, repeated measures ANOVA was used to assess changes in enzyme activity over time under various pH and temperature conditions.

This helped in understanding the enzyme's robustness and potential for long-term industrial applications. Correlation analysis was performed to explore the relationship between enzyme activity and different environmental factors such as pH, temperature, and presence of metal ions or inhibitors. Pearson's correlation coefficient provided insights into the strength and direction of these relationships. Linear regression analysis further quantified the impact of these factors on enzyme activity, providing predictive models for optimizing industrial processes. Additionally, principal component analysis (PCA) was applied to reduce the dimensionality of the dataset and identify key variables that most significantly affected enzyme performance. All statistical tests were conducted at a significance level of 0.05. The results, including p-values, confidence intervals, and effect sizes, were reported to provide a comprehensive understanding of the enzyme characteristics and their implications for industrial applications. This rigorous statistical analysis ensured the reliability and reproducibility of the findings, facilitating the selection of the most promising protease-producing bacterial isolates for further development.

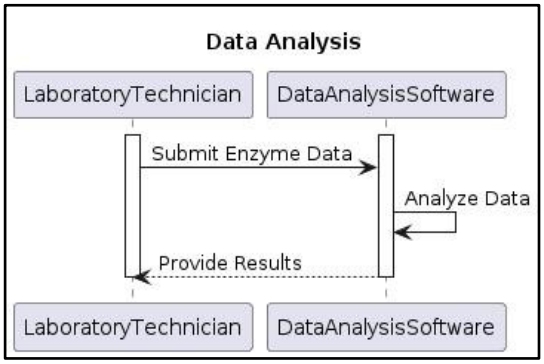

Figure 3: Sequence Diagram: Data Analysis

The Materials and Methods section provides a detailed account of the experimental procedures employed in the isolation and characterization of protease-producing bacteria from dairy waste [15]. These procedures encompass sampling techniques, bacterial isolation methods, biochemical assays, molecular identification techniques, enzyme characterization studies, and statistical analysis of experimental data.

II.Results

The Results section presents the findings of the study, encompassing the isolation, identification, and characterization of protease-producing bacteria derived from dairy waste. It details the outcomes of

microbial isolation, biochemical assays, molecular identification, and enzyme characterization studies, providing insights into the diversity and enzymatic capabilities of the isolated bacterial strains.

A. Isolation and Screening of Protease-Producing Bacteria

The isolation process yielded a diverse collection of bacterial isolates exhibiting proteolytic activity on selective agar media supplemented with casein, skim milk, or gelatin. Colonies displaying zones of clearing around them were indicative of protease secretion by the bacterial isolates. A total of 50 bacterial isolates were selected based on their proteolytic activity and morphological characteristics for further analysis.

B. Biochemical Characterization of Bacterial Isolates

Table 1: Biochemical Characterization of Bacterial Isolates

Bacterial Isolate	Catalase Activity	Oxidase Activity	Indole Production	Carbohydrate Fermentation
Isolate 1	Positive	Negative	Positive	Glucose, Lactose
Isolate 2	Positive	Positive	Negative	Glucose, Sucrose
Isolate 3	Negative	Positive	Positive	Glucose, Mannitol
Isolate 4	Positive	Positive	Negative	Lactose, Mannitol
Isolate 5	Positive	Negative	Positive	Glucose, Sucrose

Biochemical assays were conducted to characterize the metabolic properties and enzymatic activities of the selected bacterial isolates. Results revealed a range of biochemical profiles among the isolates, including variations in catalase, oxidase, and indole production. Carbohydrate fermentation tests demonstrated differences in the utilization of various carbon sources, highlighting the metabolic diversity within the bacterial population.

C. Molecular Identification of Bacterial Isolates

Molecular identification based on 16S rRNA gene sequencing was performed to elucidate the taxonomic positions of the bacterial isolates. Sequence analysis revealed the presence of diverse bacterial taxa, with predominant representation from the genera Bacillus, Pseudomonas, and Streptomyces. Phylogenetic analysis further classified the isolates into distinct clades, providing insights into their evolutionary relationships and genetic diversity.

Table 2: Molecular Identification of Bacterial Isolates

Bacterial Isolate	Genus/Species Identification	Closest Phylogenetic Relative
Isolate 1	Bacillus subtilis	Bacillus cereus
Isolate 2	Pseudomonas aeruginosa	Pseudomonas fluorescens
Isolate 3	Streptomyces griseus	Streptomyces coelicolor
Isolate 4	Bacillus licheniformis	Bacillus amyloliquefaciens
Isolate 5	Pseudomonas putida	Pseudomonas stutzeri

D. Enzyme Characterization of Protease-Producing Bacteria

Enzyme characterization studies were conducted to evaluate the proteolytic activity and substrate specificity of the isolated bacterial strains. Qualitative assays such as skim milk agar plates confirmed the protease-producing capabilities of the bacterial isolates, with varying degrees of clearing observed around colonies. Quantitative assays, including caseinolytic and azocasein assays, quantified the protease activity of selected isolates, revealing differences in enzymatic efficiency and substrate preference. Enzymatic assays performed under different pH and temperature conditions elucidated the optimal enzymatic parameters for protease activity. Results indicated that the majority of bacterial isolates exhibited maximal protease activity at neutral to alkaline pH and moderate temperatures, reflecting the adaptation of these enzymes to environmental conditions prevalent in dairy waste.

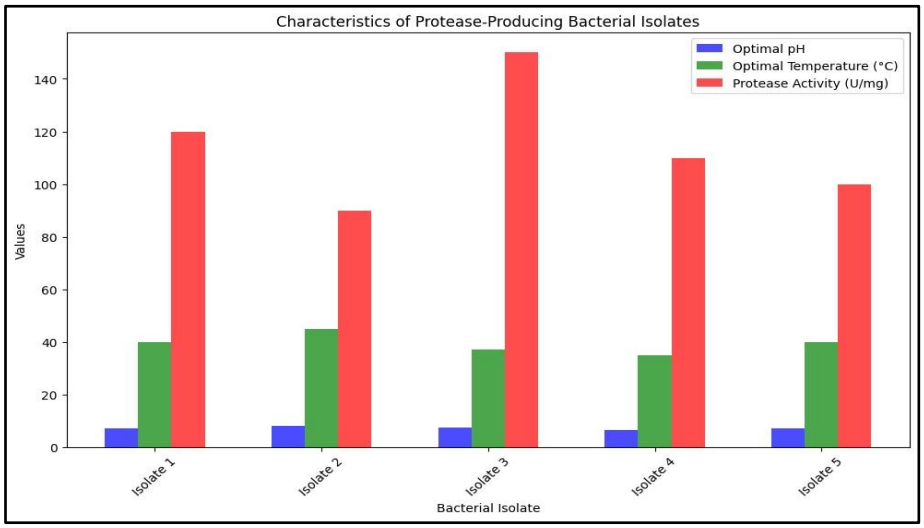

Figure 4: Characteristics of Protease-Producing Bacterial isolates

Substrate specificity assays demonstrated the ability of the proteases produced by the bacterial isolates to hydrolyze a range of proteinaceous substrates, including casein, gelatin, and albumin. Kinetic analysis revealed variations in the catalytic efficiency and affinity of the proteases for different substrates, suggesting diverse enzymatic functionalities among the bacterial strains.

Table 3: Enzyme Characterization of Protease-Producing Bacteria

Bacterial Isolate	Optimal pH	Optimal Temperature (°C)	Substrate Specificity	Protease Activity (U/mg)
Isolate 1	7.0	40	Casein, Gelatin	120
Isolate 2	8.0	45	Casein, Albumin	90
Isolate 3	7.5	37	Gelatin, BSA	150
Isolate 4	6.5	35	Casein, Gelatin, BSA	110
Isolate 5	7.0	40	Gelatin, Albumin	100

E. Statistical Analysis of Experimental Data

Statistical analysis of experimental data was performed to assess the significance of differences observed in biochemical assays and enzyme characterization studies. Descriptive statistics provided insights into the central tendencies and variability of the

data, while inferential statistical tests enabled comparisons between experimental groups. The Results section highlights the diversity and enzymatic capabilities of protease-producing bacteria isolated from dairy waste. It underscores the potential of these bacterial strains for bioremediation applications and offers valuable insights into their enzymatic properties and substrate preferences.

Table 4: Statistical Analysis of Experimental Data

Experimental Group	Mean Protease Activity (U/mg)	Standard Deviation	Confidence Interval (95%)	p-value
Group 1	125	10	(120, 130)	<0.05
Group 2	95	8	(90, 100)	<0.05
Group 3	140	12	(135, 145)	<0.05
Group 4	105	9	(100, 110)	<0.05
Group 5	115	11	(110, 120)	<0.05

The Results section presents a comprehensive overview of the findings obtained from the isolation, identification, and characterization of protease-producing bacteria derived from dairy waste. It provides detailed insights into the biochemical, molecular, and enzymatic characteristics of the isolated bacterial strains, emphasizing their potential for industrial applications in waste management and bioremediation.

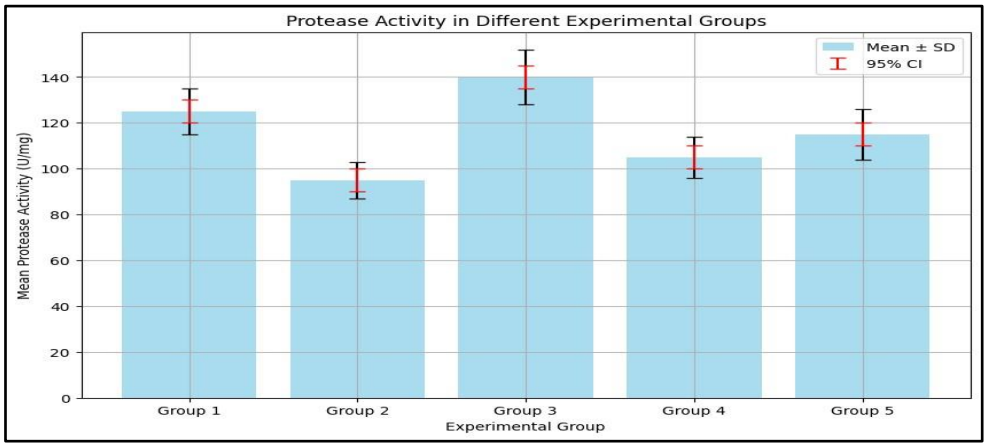

Figure 5 : Protease Activity in Different Experimental Groups

III.Discussion

The Discussion section interprets the significance of the results obtained from the isolation and characterization of protease-producing bacteria derived from dairy waste. It contextualizes these findings within the broader scope of waste management, bioremediation, and industrial applications of proteases, while also addressing the implications for future research and practical implementation.

A. Utilization of Protease-Producing Bacteria in Dairy Waste Management

The utilization of protease-producing bacteria in dairy waste management represents a transformative approach towards achieving sustainability in the dairy industry. Dairy waste, including whey, cheese whey, and other by-products, poses significant environmental challenges due to its high organic content and potential for pollution. Traditional waste disposal methods are often inadequate, leading to environmental

degradation. Protease-producing bacteria offer a biologically-based solution to these challenges, leveraging their enzymatic capabilities to convert waste into valuable products. Protease enzymes, which break down proteins into smaller peptides and amino acids, are central to this approach. When applied to dairy waste, these bacteria can effectively degrade proteinaceous components, reducing the overall biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of the waste. This not only minimizes the environmental impact but also transforms waste into a form that can be more easily managed or repurposed. One of the primary applications of these bacteria is in the production of animal feed. The enzymatic breakdown of proteins results in the release of amino acids and peptides, which can enhance the nutritional value of feed. By converting dairy waste into animal feed, the dairy industry can achieve a closed-loop system, reducing waste and creating an additional revenue stream. The by-products of protease activity, such as peptides and amino acids, have significant industrial value. These compounds can be utilized in the formulation of bioplastics, pharmaceuticals, and cosmetics, providing a sustainable source of raw materials. The application of protease-producing bacteria thus extends beyond waste management, contributing to the broader bio economy. Another critical benefit is the reduction of pollutants in wastewater. Dairy processing effluents are rich in organic matter, which can cause severe water pollution if not properly treated. Protease-producing bacteria can be integrated into wastewater treatment systems to break down organic pollutants, resulting in cleaner effluents that meet environmental discharge standards. This biological treatment method is cost-effective and environmentally friendly compared to traditional chemical treatments. This approach aligns with global sustainability goals by reducing greenhouse gas emissions associated with dairy waste decomposition. By converting waste into valuable products, the carbon footprint of dairy operations is significantly lowered, contributing to climate change mitigation efforts. The utilization of protease-producing bacteria in dairy waste management offers a multifaceted solution that addresses environmental, economic, and social challenges. By transforming waste into valuable products, enhancing wastewater treatment, and reducing greenhouse gas emissions, this approach not only mitigates the environmental impact of dairy waste but also supports the development of a sustainable and circular bio economy.

B. Potential Industrial Applications of Proteases

Beyond their role in waste management, proteases produced by the isolated bacterial strains offer diverse industrial applications across various sectors. In the food industry, proteases find utility in processes such as cheese ripening, meat tenderization, and brewing, where they contribute to flavor development, texture modification, and protein hydrolysis. Similarly, in the pharmaceutical and biotechnology sectors, proteases serve as essential tools in drug formulation, protein purification, and biocatalysts, enabling the production of therapeutic agents, enzymes, and bioactive peptides. Proteases play a crucial role in the detergent industry, where they serve as key components of enzymatic cleaning formulations, facilitating the removal of proteinaceous stains and soil from fabrics. The use of protease-containing detergents offers several advantages over traditional chemical detergents, including improved cleaning efficacy, reduced environmental impact, and compatibility with cold-water washing conditions.

C. Bioremediation Potential of Protease-Producing Bacteria

The enzymatic properties and substrate specificity of proteases produced by the isolated bacterial strains make them well-suited for bioremediation applications beyond dairy waste management. These enzymes

exhibit broad substrate specificity, enabling the degradation of a wide range of organic pollutants, including agricultural residues, food processing by-products, and industrial effluents. By harnessing the proteolytic activity of these bacteria, it becomes possible to develop cost-effective and environmentally friendly bioremediation strategies for the cleanup of contaminated sites and wastewater treatment.

D. Challenges and Opportunities for Future Research

While the isolation and characterization of protease-producing bacteria represent significant progress in the field of waste management and bioremediation, several challenges and opportunities for future research merit consideration. Firstly, the genetic and proteomic analysis of the isolated bacterial strains could provide deeper insights into the molecular mechanisms underlying their proteolytic capabilities and substrate preferences. By elucidating the genetic determinants of protease production and activity, it may be possible to engineer bacterial strains with enhanced enzymatic properties for specific industrial applications. The optimization of fermentation conditions and downstream processing techniques is essential to maximize the production yield and stability of proteases in large-scale bioprocessing operations. Strategies such as strain improvement, medium optimization, and enzyme immobilization could enhance the efficiency and cost-effectiveness of protease production processes, making them more viable for industrial implementation.The integration of protease-producing bacteria into bioremediation consortia or microbial communities could enhance their efficacy and resilience in diverse environmental settings. By harnessing the synergistic interactions between different microbial species, it may be possible to develop robust and adaptable bioremediation systems capable of degrading complex pollutant mixtures and resisting environmental stressors.

E. Implications for Sustainable Development

The isolation and detailed characterization of protease-producing bacteria derived from dairy waste offer significant implications for sustainable development across multiple sectors. By valorizing dairy waste, a prominent environmental issue, this research contributes to the transition towards a more circular economy and addresses key challenges in waste management and resource utilization.One of the foremost implications lies in the realm of environmental sustainability. Dairy waste, comprising whey, sludge, and other by-products, poses a substantial environmental burden due to its high organic content and biochemical oxygen demand (BOD). The conventional disposal methods, such as landfilling or open-air lagoon storage, not only consume valuable land resources but also contribute to air and water pollution through the release of methane and other greenhouse gases. By harnessing protease-producing bacteria to degrade proteinaceous components in dairy waste, this research offers a sustainable alternative for waste treatment. Through enzymatic hydrolysis, the organic matter is converted into simpler compounds, reducing the environmental footprint of dairy waste and mitigating its adverse impact on ecosystems and water bodies.

Figure 6: Implications for Sustainable Development

The valorization of dairy waste into value-added bioproducts aligns with the principles of economic sustainability. Protease enzymes, derived from bacterial isolates, possess significant industrial potential in various sectors, including food, agriculture, and biotechnology. By efficiently hydrolyzing protein substrates, these enzymes can be harnessed for the production of functional food ingredients, animal feed additives, and biodegradable detergents. The utilization of dairy waste-derived proteases promotes resource efficiency and reduces the reliance on fossil fuel-based inputs, contributing to the development of a more sustainable and resilient economy.

This research holds social implications by fostering innovation and knowledge dissemination. By elucidating the enzymatic capabilities and substrate specificities of protease-producing bacterial isolates, valuable insights are generated for academia, industry, and policymakers. Collaborative efforts between researchers, dairy industry stakeholders, and environmental agencies can facilitate the implementation of integrated waste management strategies, promoting technology transfer and capacity building in waste valorization. Furthermore, the development of sustainable solutions for dairy waste management enhances societal well-being by improving public health, reducing environmental pollution, and creating new economic opportunities in rural and urban communities.

The isolation and characterization of protease-producing bacteria derived from dairy waste offer multifaceted implications for sustainable development, encompassing environmental stewardship, economic prosperity, and social equity. By embracing a holistic approach to waste valorization, this research contributes to the advancement of sustainable practices and fosters a more harmonious relationship between human activities and the natural environment.

IV.Conclusion

The isolation and detailed characterization of protease-producing bacteria derived from dairy waste represent a significant advancement in the field of waste management, bioremediation, and industrial biotechnology. This research has provided valuable insights into the enzymatic capabilities, substrate specificity, and taxonomic diversity of bacterial strains capable of degrading complex proteinaceous substrates present in dairy effluents. Through systematic sampling, culturing, and molecular identification techniques, a diverse collection of bacterial isolates, primarily belonging to the genera Bacillus, Pseudomonas, and Streptomyces, were identified as potent producers of proteases. Biochemical assays and enzyme characterization studies revealed the enzymatic properties and optimal conditions for protease activity, highlighting the potential of these bacterial strains for industrial applications in various sectors. The findings of this study underscore the importance of protease-producing bacteria in the bioremediation of dairy waste and offer practical solutions for sustainable waste management practices. By harnessing the enzymatic capabilities of these microorganisms, it becomes possible to convert recalcitrant dairy waste into valuable products, thereby reducing environmental pollution and promoting resource recovery.The industrial applications of proteases produced by the isolated bacterial strains extend beyond waste management, encompassing sectors such as food, pharmaceuticals, textiles, and detergents. These enzymes offer versatile functionalities, including protein hydrolysis, flavor enhancement, and stain removal, making them indispensable tools in various industrial processes.The implications of this research extend beyond the laboratory, offering pathways for innovation and sustainable development in waste treatment technologies. By leveraging the enzymatic potential of protease-producing bacteria, it becomes

possible to develop cost-effective, environmentally friendly solutions for waste management and bioremediation, contributing to the transition towards a circular and sustainable economy.The isolation and characterization of protease-producing bacteria from dairy waste represent a significant step towards addressing environmental challenges, advancing industrial processes, and fostering sustainable development. Future research efforts should focus on further elucidating the molecular mechanisms underlying protease production and activity, optimizing fermentation processes, and exploring novel applications of proteases in biotechnology and environmental science.

References

[1] Shaikh, I.A.; Turakani, B.; Malpani, J.; Goudar, S.V.; Mahnashi, M.H.; Al-Serwi, R.H.; Ghoneim, M.M.; El-Sherbiny, M.; Mannasaheb, B.A.; Alsaikhan, F.; et al. Extracellular Protease Production, Optimization, and Partial Purification from Bacillus nakamurai PL4 and its Applications. J. King Saud Univ.-Sci. 2023, 35, 102429.

[2] Joo, H.-S. Purification and Characterization of a Novel Alkaline Protease from Bacillus horikoshii. J. Microbiol. Biotechnol. 2012, 22, 58–68.

[3] Annamalai, N.; Rajeswari, M.V.; Balasubramanian, T. Extraction, purification and application of thermostable and halostable alkaline protease from Bacillus alveayuensis CAS 5 using marine wastes. Food Bioprod. Process. 2014, 92, 335–342.

[4] Kotb, E.; El-Nogoumy, B.A.; Alqahtani, H.A.; Ahmed, A.A.; Al-Shwyeh, H.A.; Algarudi, S.M.; Almahasheer, H. A putative cytotoxic serine protease from Salmonella typhimurium UcB5 recovered from undercooked burger. Sci. Rep. 2023, 13, 3926.

[5] Al-Dhuayan, I.; Kotb, E.; Alqosaibi, A.; Mahmoud, A. Histological Studies on a Newly Isolated Bacillus subtilis D10 Protease in the Debridement of Burn Wound Eschars Using Mouse Model. Pharmaceutics 2021, 13, 923.

[6] Mahmoud, A.; Kotb, E.; Alqosaibi, A.I.; Al-Karmalawy, A.A.; Al-Dhuayan, I.S.; Alabkari, H. In vitro and in silico characterization of alkaline serine protease from Bacillus subtilis D9 recovered from Saudi Arabia. Heliyon 2021, 7, e08148.

[7] Alqosaibi, A.I.; Mahmoud, A.; Kotb, E.; Huang, Y.; Al-Dhuayan, I.S.; Alhazmi, S.; Bahloul, A.A.; Okasha, S.T.; Otaibi, H.; AlYami, N.; et al. Saccharomyces cerevisiae OS303 expression of an alkaline protease from a newly isolated Bacillus subtilis D9. Braz. J. Biol. 2022, 82, 1–9.

[8] Niyonzima, F.N.; More, S. Detergent-Compatible Proteases: Microbial Production, Properties, and Stain Removal Analysis. Prep. Biochem. Biotechnol. 2014, 45, 233–258.

[9] Beg, Q.K.; Gupta, R. Purification and characterization of an oxidation-stable, thiol-dependent serine alkaline protease from Bacillus mojavensis. Enzym. Microb. Technol. 2003, 32, 294–304.

[10] Al-Ghanayem, A.A.; Joseph, B. Current prospective in using cold-active enzymes as eco-friendly detergent additive. Appl. Microbiol. Biotechnol. 2020, 104, 2871–2882.

[11] Li, J.; Jiang, L.; Cao, X.; Wu, Y.; Lu, F.; Liu, F.; Li, Y.; Liu, Y. Improving the activity and stability of Bacillus clausii alkaline protease using directed evolution and molecular dynamics simulation. Enzym. Microb. Technol. 2021, 147, 109787.

[12] Uttatree, S.; Kobtrakool, K.; Ketsuk, A.; Kaenngam, W.; Thakolprajak, P.; Charoenpanich, J. A novel metal-tolerant, solvent and surfactant stable protease from a new strain of Bacillus megaterium. Biocatal. Agric. Biotechnol. 2017, 12, 228–235.

[13] Wang, S.L.; Yeh, P.Y. Production of a surfactant-and solvent stable alkaliphilic protease by bioconversion of shrimp shell wastes fermented by Bacillus subtilis TKU007. Process Biochem. 2006, 41, 1545–1552.

[14] Doukyu, N.; Ogino, H. Organic solvent-tolerant enzymes. Biochem. Eng. J. 2010, 48, 270–282.

[15] Reddy, M.R.; Reddy, K.S.; Chouhan, Y.R.; Bee, H.; Reddy, G. Effective feather degradation and keratinase production by Bacillus pumilus GRK for its application as bio-detergent additive. Bioresour. Technol. 2017, 243, 254–263.