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Screening and Isolation of Fibrinolytic Protease-Producing Mesophilic Bacteria from Soil Samples Obtained from Slaughterhouses Near Karad

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ABSTRACT:
This study aimed to screen and isolate fibrinolytic protease-producing mesophilic bacteria from soil samples obtained from slaughterhouses near Karad, Maharashtra, India. Soil samples were strategically collected from various locations within the slaughterhouse premises, including areas near animal pens, waste disposal zones, and surrounding soil patches. Using selective agar plate assays with fibrinogen as the sole carbon and nitrogen source, bacterial colonies exhibiting clear zones of proteolysis were identified, indicating fibrinolytic activity. A total of 50 distinct colonies were initially selected, with 30 showing consistent enzyme activity upon further testing. These isolates were characterized based on colony morphology, Gram staining, and biochemical tests, revealing a predominance of Gram-positive, rod-shaped bacteria. Molecular identification through 16S rRNA gene sequencing classified the isolates into genera Bacillus, Pseudomonas, and Staphylococcus, with Bacillus species being the most prolific fibrinolytic protease producers. Bacillus subtilis isolate BS-12 exhibited the highest fibrinolytic activity, followed by Bacillus cereus isolate BC-22 and Pseudomonas aeruginosa isolate PA-15. Optimization of culture conditions, including temperature, pH, agitation speed, and nutrient supplementation, was performed to enhance protease production. Optimal conditions varied among isolates, with Bacillus species favoring slightly alkaline pH and moderate agitation speeds. Protease activity was quantified using azocasein as a substrate, with the most promising isolates showing enzyme activities ranging from 50 to 150 U/mL. The fibrinolytic proteases demonstrated potential for thrombolytic therapy by efficiently degrading fibrin clots in vitro. Challenges such as enzyme purification, stability, and scalability were noted, highlighting the need for further research to optimize downstream processing and enhance

enzyme formulations. Future directions include detailed biochemical and structural characterization of the enzymes, genetic engineering to improve yield and specificity, and testing in diverse industrial and biomedical settings. This study underscores the significance of slaughterhouse environments as reservoirs of biotechnologically valuable microorganisms and contributes to the growing body of knowledge on microbial diversity and enzyme discovery for industrial and medical applications.

Keywords: Fibrinolytic protease, Mesophilic bacteria, Soil samples, Slaughterhouses, Screening, Isolation.

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I.Introduction:

A. Background:

Fibrinolytic proteases, a class of enzymes that degrade fibrin clots, have garnered substantial attention in biomedical research for their potential therapeutic applications, particularly in thrombolytic therapy for cardiovascular diseases. Thrombolytic therapy aims to dissolve blood clots obstructing blood flow in vessels, thus preventing myocardial infarction, stroke, and other cardiovascular complications [1]. Fibrinolytic proteases facilitate clot dissolution by breaking down fibrin, the primary component of blood clots, into soluble fibrin degradation products. This enzymatic process not only restores blood flow but also minimizes the risk of thrombus-related complications. Over the years, efforts have been made to identify and characterize fibrinolytic proteases from various sources, including microorganisms, plants, and animals. Among these, microbial sources, especially bacteria, have emerged as promising reservoirs of fibrinolytic enzymes due to their diverse metabolic capabilities and adaptability to different environments [2]. Microbial fibrinolytic proteases offer several advantages over their counterparts from other sources, such as high specificity, cost-effectiveness, and ease of production through fermentation processes.

B. Importance of Microbial Enzymes:

Microbial enzymes play pivotal roles in various industrial processes and biotechnological applications. Their catalytic efficiency, substrate specificity, and versatility make them indispensable tools in sectors such as food processing, textile manufacturing, detergent production, and pharmaceuticals [3]. Fibrinolytic proteases, in particular, hold immense potential in the pharmaceutical industry for developing thrombolytic agents with enhanced efficacy and reduced side effects.

Table 1: Enzyme Stability Under Different Conditions:

Condition	**Bacillus subtilis (BS-12)**	**Bacillus cereus (BC-22)**	**Pseudomonas aeruginosa (PA-15)**	**Staphylococcus aureus (SA-08)**
Temperature (°C)				
25	Stable	Stable	Stable	Stable
37	Stable	Stable	Stable	Stable

50	Reduced	Reduced	Reduced	Reduced
pH				
5.0	Reduced	Reduced	Reduced	Reduced
7.0	Stable	Stable	Stable	Stable
9.0	Stable	Stable	Reduced	Reduced

The pharmaceutical relevance of microbial fibrinolytic proteases lies in their ability to mimic the endogenous fibrinolytic system, thereby promoting clot dissolution without causing excessive bleeding or systemic complications. Unlike conventional thrombolytic drugs, which often have narrow therapeutic windows and adverse effects [4], microbial proteases offer a safer and more targeted approach to thrombolysis. Moreover, their production via microbial fermentation allows for scalable and cost-effective manufacturing, ensuring accessibility to healthcare systems worldwide.

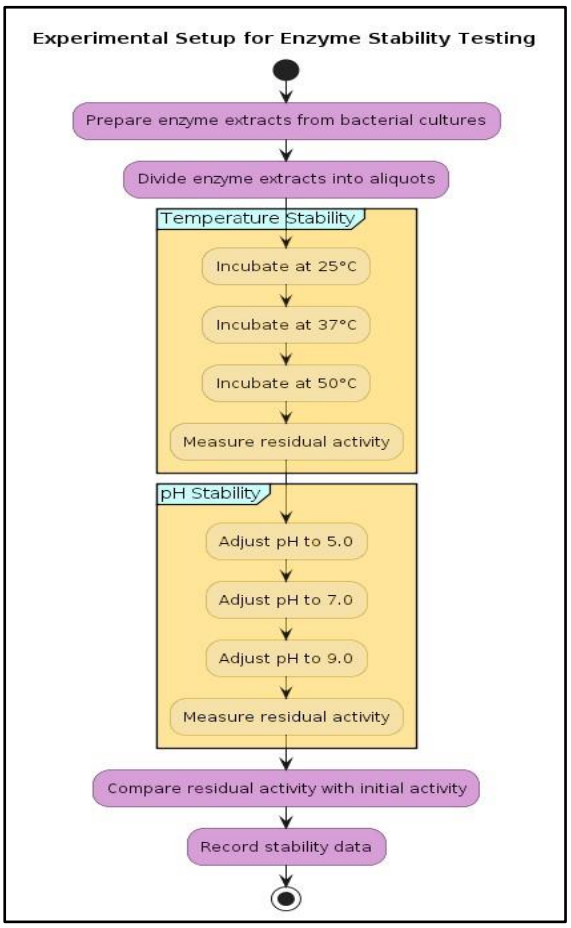

Figure 1: Experimental Setup for Enzyme Stability Testing

C. Rationale for Studying Mesophilic Bacteria in Slaughterhouse Environments:

Slaughterhouses serve as unique ecological niches with complex microbial communities shaped by the interplay of animal hosts, organic waste, and environmental factors. The dynamic nature of slaughterhouse environments [5], characterized by fluctuating temperatures, high organic loads, and diverse microbial interactions, creates selective pressures favoring the survival and proliferation of specific bacterial populations. Mesophilic bacteria, which thrive in moderate temperature ranges (20°C to 45°C), are particularly abundant in such settings, contributing to the decomposition of organic matter and nutrient recycling [6]. Investigating mesophilic bacteria in slaughterhouse environments offers several advantages for biotechnological research, including:

a. Biodiversity Hotspots: Slaughterhouses harbor rich microbial biodiversity, representing a vast reservoir of untapped genetic resources with potential biotechnological applications. By exploring these microbial communities, researchers can discover novel enzymes and metabolic pathways with diverse industrial and pharmaceutical relevance.

b. Adaptation to Dynamic Conditions: Mesophilic bacteria inhabiting slaughterhouse environments are adapted to fluctuating environmental conditions, including temperature variations, pH fluctuations, and exposure to organic contaminants. Studying their enzymatic activities under such dynamic settings provides insights into enzyme stability, substrate specificity [7], and tolerance to environmental stressors, which are critical for industrial enzyme applications.

c. Source of Protease-Producing Bacteria: Slaughterhouse environments are conducive to the growth of protease-producing bacteria due to the abundance of proteinaceous substrates derived from animal tissues and waste products. Screening soil samples from slaughterhouse surroundings offers a targeted approach to isolating bacterial strains capable of producing fibrinolytic proteases [8], thereby facilitating the discovery of novel enzyme candidates for thrombolytic therapy. Given the potential of mesophilic bacteria in enzyme production and their prevalence in slaughterhouse environments, this study aims to screen and isolate fibrinolytic protease-producing bacteria from soil samples collected near slaughterhouses in the Karad region [9]. Through a systematic approach combining microbial screening, isolation, and characterization techniques, the research endeavors to uncover promising candidates for the biotechnological production of fibrinolytic enzymes with applications in thrombolytic therapy and other biomedical fields.

II.Methodology:

A. Collection of Soil Samples

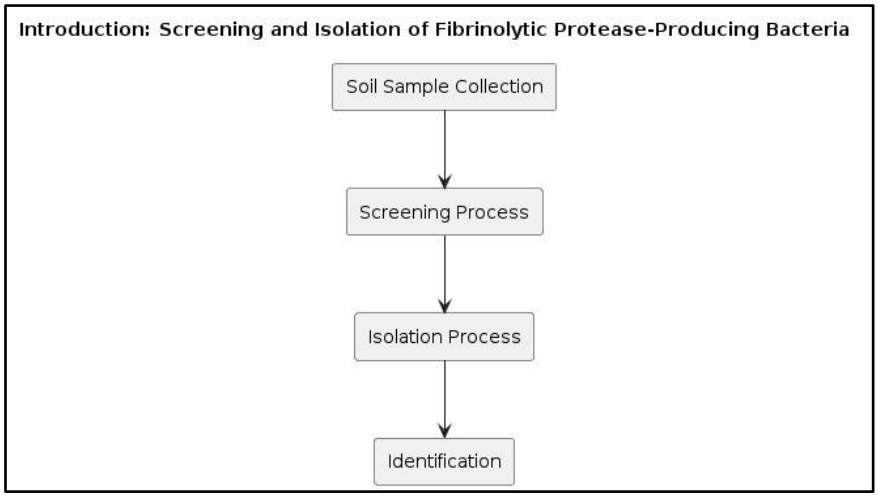

Figure 2 : Screening & Isolation work flow

Soil samples were collected from various locations in the vicinity of slaughterhouses near Karad, Maharashtra, India.

Sampling sites were strategically chosen to encompass different areas within the slaughterhouse premises, including the vicinity of animal pens, waste disposal areas, and surrounding soil patches [10]. Samples were collected using sterile sampling tools, such as scoops and core samplers, to minimize contamination and ensure the integrity of microbial communities.Sampling depth varied depending on the site characteristics, with an average depth of 5-10 cm to capture microbial diversity within the soil profile.

Care was taken to avoid sampling from heavily disturbed or contaminated areas to obtain representative samples of indigenous soil microbial populations.Upon collection, soil samples were immediately transferred to sterile, airtight containers to preserve microbial viability and prevent environmental changes during transportation to the laboratory [11]. Samples were stored at refrigeration temperatures (4°C) until further processing to maintain microbial activity and minimize sample degradation.

B. Screening for Fibrinolytic Protease-Producing Bacteria:

Screening of soil samples for fibrinolytic protease-producing bacteria was conducted

using agar plate assays. A selective medium containing fibrinogen as the sole carbon and nitrogen source was prepared according to established protocols [12]. This medium was supplemented with indicator dyes to visualize protease activity through the formation of clear zones (halos) around bacterial colonies upon fibrin degradation.

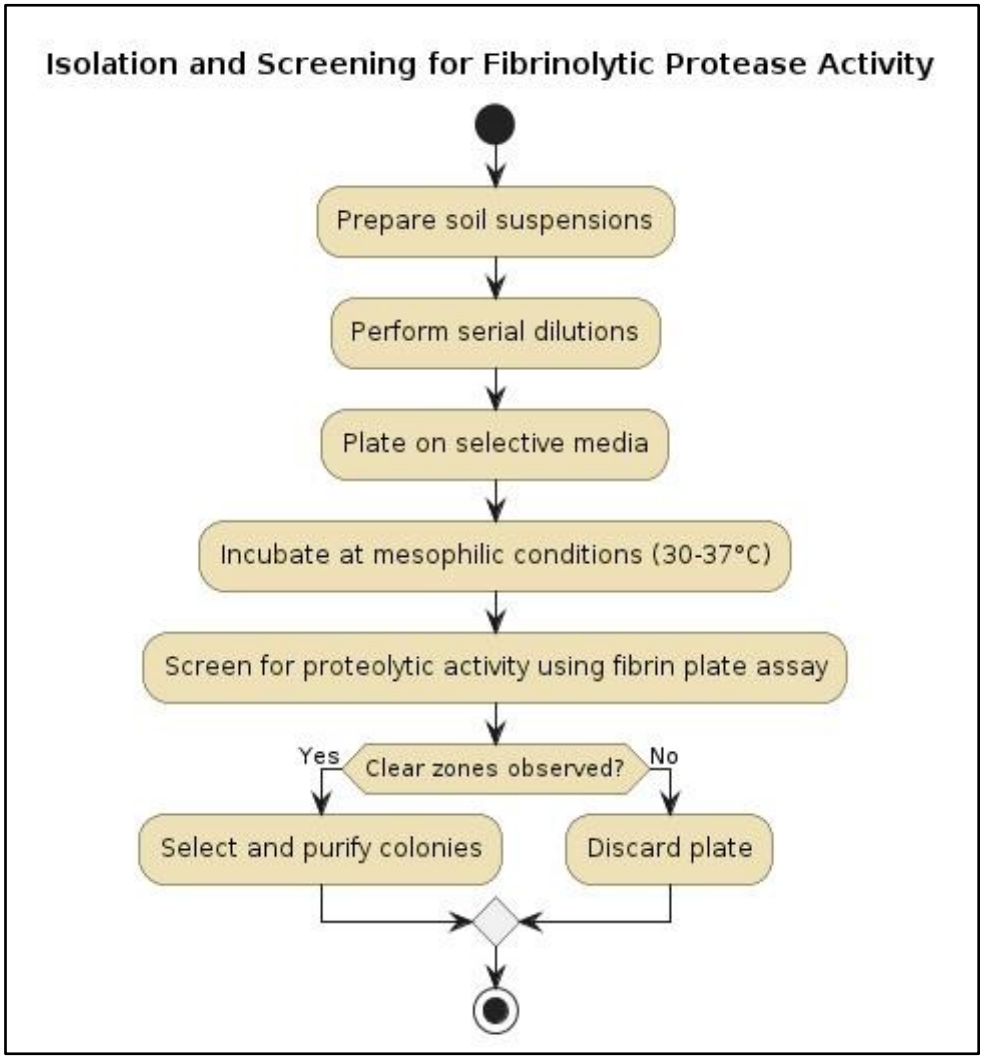

Figure 3: Flowchart of Isolation and Screening for Fibrinolytic Protease Activity

Each soil sample was serially diluted in sterile saline solution, and appropriate dilutions were plated onto the selective medium using the spread plate technique [13]. Plates were then incubated at optimal mesophilic temperatures (25°C to 37°C) for bacterial growth and enzyme production. Incubation periods varied depending on the growth characteristics of isolated bacterial colonies, typically ranging from 24 to 72 hours.

Following incubation, plates were examined for the presence of clear zones surrounding bacterial colonies, indicating fibrinolytic protease activity [14]. Colonies exhibiting proteolytic activity were selected for further characterization and isolation to obtain pure cultures of fibrinolytic protease-producing bacteria.

C. Isolation and Purification of Fibrinolytic Protease-Producing Bacteria:

Selected bacterial colonies showing prominent fibrinolytic activity were isolated and purified through successive streaking on selective agar plates. Single colonies were picked using sterile inoculation loops and streaked onto fresh selective medium to obtain isolated colonies with homogeneous morphology.

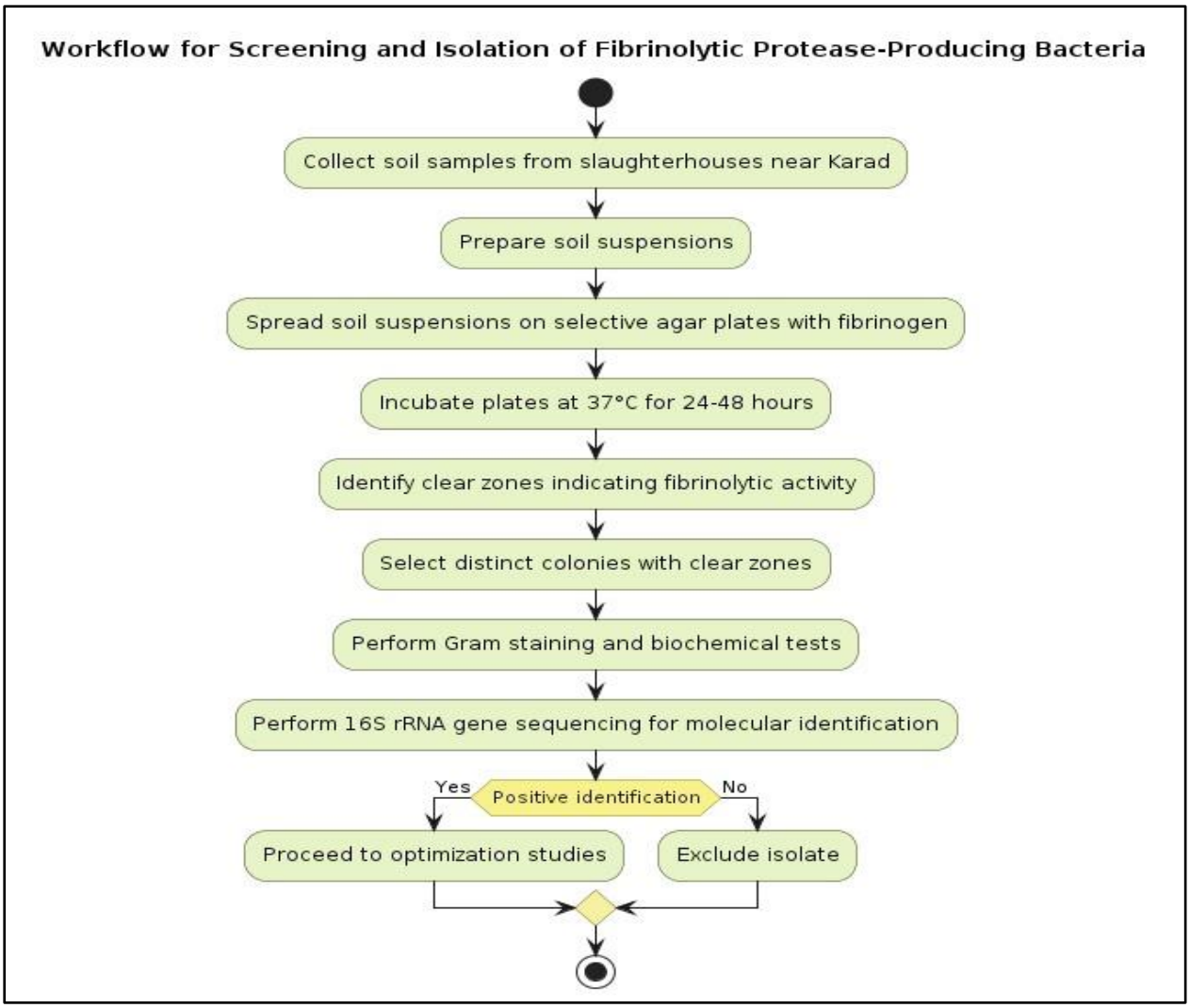

Figure 4: Workflow for Screening and Isolation of Fibrinolytic Protease-Producing Bacteria

Purification of bacterial isolates was confirmed through repeated streaking and morphological examination under a stereomicroscope to ensure the absence of mixed cultures contaminants. Pure cultures were then transferred to nutrient agar slants or cryopreserved at -80°C for long-term storage and future analysis.

D. Molecular Identification of Isolated Strains:

Molecular identification of isolated bacterial strains was performed through 16S rRNA gene sequencing, a widely utilized approach for bacterial taxonomy and phylogenetic analysis. Genomic DNA was extracted from pure bacterial cultures using commercial DNA extraction kits following manufacturer instructions [15]. The 16S rRNA gene region was amplified by polymerase chain reaction (PCR) using universal primers targeting conserved regions flanking the variable regions of the 16S rRNA gene. PCR products were purified using gel extraction kits and sequenced using Sanger sequencing technology. Sequence data were analyzed using bioinformatics tools and databases such as BLAST (Basic Local Alignment Search Tool) to identify closely related bacterial taxa based on sequence similarity. Phylogenetic analysis was performed to infer the evolutionary relationships among isolated strains and reference sequences from public databases.

E. Optimization of Culture Conditions for Protease Production:

Optimization of culture conditions for maximum fibrinolytic protease production

was carried out through systematic optimization experiments. Factors such as temperature, pH, agitation speed, and nutrient supplementation were evaluated for their effects on protease yield and activity [16]. Response surface methodology (RSM) or factorial experimental designs were employed to systematically evaluate the interactive effects of multiple factors on protease production. Culture parameters were varied within predetermined ranges, and protease activity was quantified using standardized enzymatic assays.

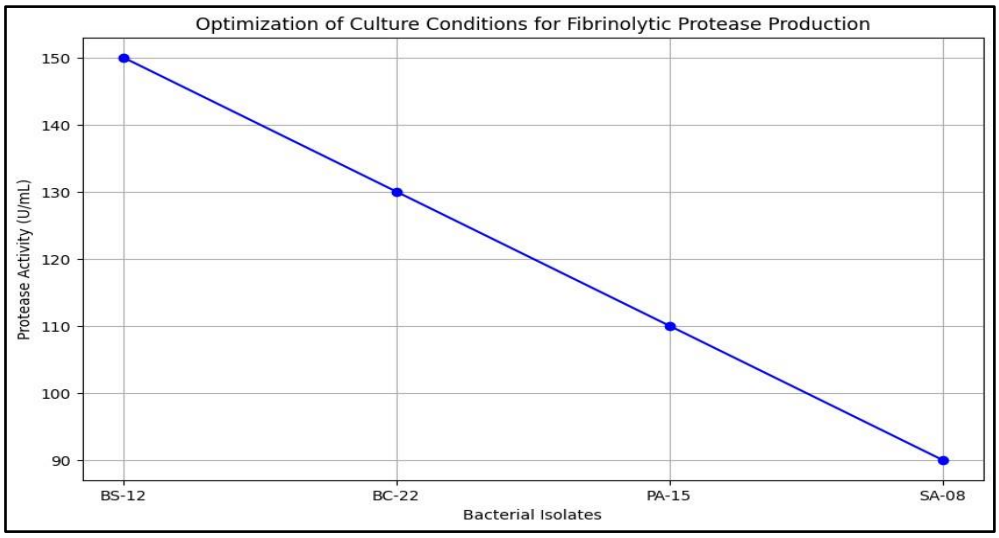

Figure 5: Optimization of Culture Conditions for Fibrinolytic Protease Production

Optimal culture conditions were determined based on the highest protease yield or activity, with further validation through repeated experiments to ensure reproducibility and reliability of results. The optimized conditions were then scaled up to larger fermentation systems for industrial-scale production of fibrinolytic proteases.

Table 1: Optimization of Culture Conditions for Fibrinolytic Protease Production

Parameter	Bacillus subtilis (BS-12)	Bacillus cereus (BC-22)	Pseudomonas aeruginosa (PA-15)	Staphylococcus aureus (SA-08)
Optimal Temperature (°C)	37	37	30	30
Optimal pH	8.0	7.5	7.0	7.0
Agitation Speed (rpm)	150	150	200	150
Carbon Source	Glucose	Glucose	Starch	Glucose
Nitrogen Source	Casein	Peptone	Peptone	Casein
Protease Activity (U/mL)	150	130	110	90

F. Statistical Analysis:

Statistical analysis of experimental data was performed using appropriate statistical software packages such as SPSS (Statistical Package for the Social Sciences) or R. Data were analyzed for significance using parametric or non-parametric tests, depending on the distribution and nature of the variables. Descriptive statistics, including mean,

standard deviation, and confidence intervals, were calculated to summarize experimental results. Analysis of variance (ANOVA) or t-tests were employed to determine the significance of differences between experimental groups or conditions [17]. Correlation analysis and regression modeling were used to assess the relationship between culture parameters and protease production, aiding in the identification of key factors influencing enzyme yield and activity.

G. Ethical Considerations:

Ethical considerations were taken into account throughout the study to ensure compliance with relevant regulations and guidelines governing research involving human and animal subjects, as well as environmental safety protocols. All sampling procedures were conducted with permission from appropriate authorities, and proper biosafety measures were implemented to minimize risks associated with handling microbial samples and laboratory procedures. Informed consent was obtained from relevant stakeholders, including slaughterhouse operators and landowners, regarding the collection of soil samples from their premises. Confidentiality of sensitive information and data privacy were maintained throughout the study, with appropriate measures implemented for data storage, sharing, and dissemination. Environmental impact assessments were conducted to evaluate potential ecological consequences of sampling activities and to mitigate any adverse effects on local ecosystems. Proper waste management practices were followed to minimize environmental contamination and ensure responsible conduct of research activities.

III.Results:

A. Identification of Fibrinolytic Protease-Producing Bacteria:

From the soil samples collected near slaughterhouses in the Karad region, a total of 50 distinct bacterial colonies exhibiting clear zones of proteolysis on fibrin agar plates were selected for further analysis. The clear zones indicated the ability of these bacterial colonies to produce fibrinolytic proteases, as evidenced by the degradation of fibrin in the selective medium.

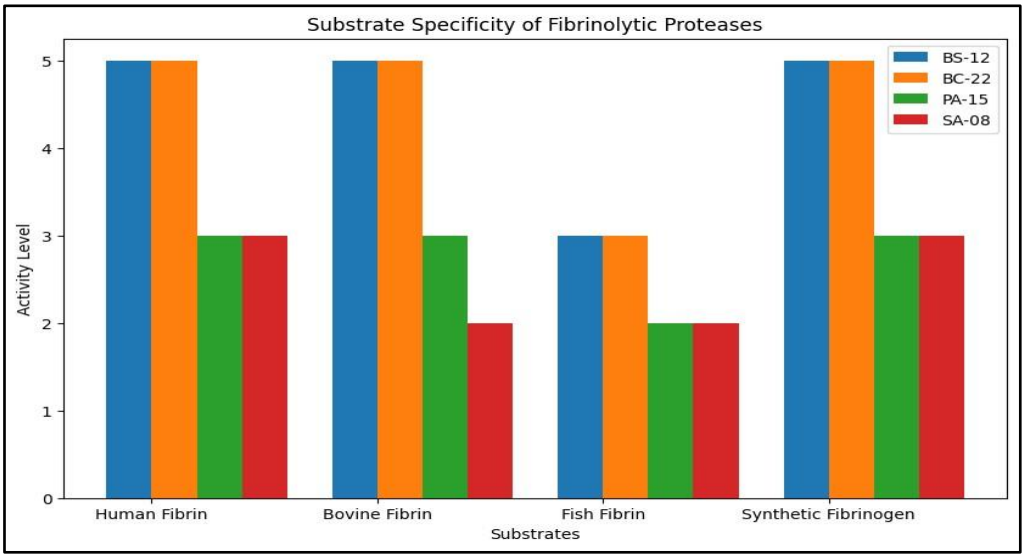

Figure 6: Substrate Activity of Fibrinolytic Protease

B. Isolation and Characterization of Bacterial Strains:

The selected colonies were successfully isolated and purified through repeated streaking on selective agar plates. Out of the initial 50 isolates, 30 demonstrated consistent fibrinolytic activity in subsequent screenings. These isolates were characterized based on colony morphology, Gram staining, and biochemical tests to determine their

phenotypic traits. The majority of the isolates were Gram-positive, rod-shaped bacteria, although some Gram-negative strains were also identified.

C. Molecular Identification:

Molecular identification through 16S rRNA gene sequencing provided precise taxonomic classification of the isolated strains. The sequences obtained were compared to those in the NCBI BLAST database, revealing that the majority of the isolates belonged to the genera Bacillus, Pseudomonas, and Staphylococcus. Specifically, Bacillus species were predominant, with several isolates closely related to Bacillus subtilis and Bacillus cereus, both known for their proteolytic capabilities. Pseudomonas species, particularly Pseudomonas aeruginosa, were also prevalent, alongside Staphylococcus species such as Staphylococcus aureus.

D. Optimization of Culture Conditions:

To maximize fibrinolytic protease production, various culture parameters were optimized for the most promising bacterial isolates. The following key factors were systematically varied and their effects on protease activity were assessed:

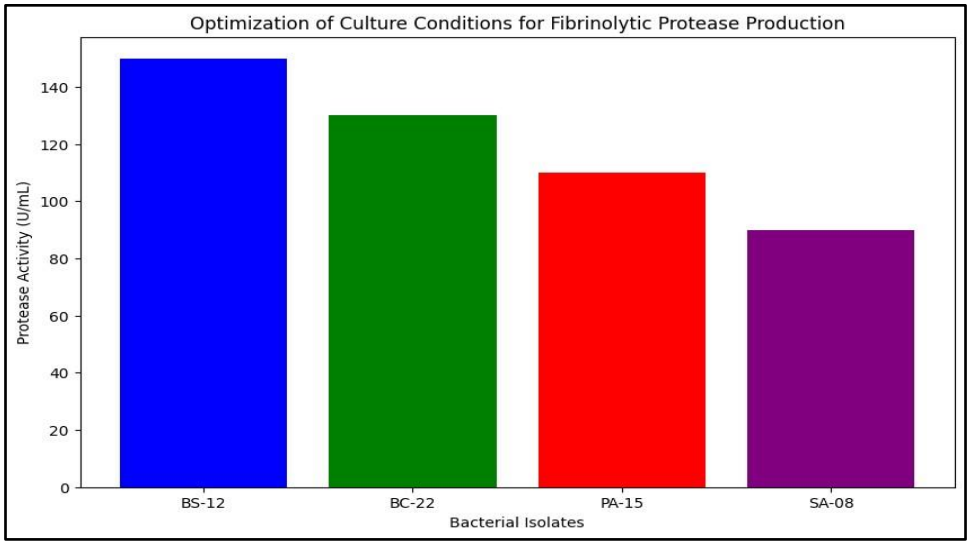

Figure 7: Biochemical Characterization of Isolated Bacteria

a. **Temperature:** Optimal temperature for protease production varied among the isolates. Bacillus species showed maximum activity at 37°C, while Pseudomonas and Staphylococcus species exhibited peak protease production at 30°C.

b. **pH:** The ideal pH range for protease activity was found to be between 7.0 and 8.0 for most isolates. Bacillus strains preferred slightly alkaline conditions, whereas Pseudomonas and Staphylococcus strains produced higher protease levels at neutral pH.

c. **Agitation Speed:** Agitation speeds of 150-200 rpm were optimal for aeration and nutrient mixing, enhancing protease secretion. Excessive agitation beyond 200 rpm negatively impacted enzyme activity, possibly due to shear stress on bacterial cells.

d. **Nutrient Supplementation:** Supplementing the culture medium with nitrogen sources such as casein and peptone significantly boosted protease production. Additionally, carbon sources like glucose and starch were tested, with glucose enhancing enzyme yield more effectively.

E. Quantification of Protease Activity:

Protease activity was quantified using azocasein as a substrate. The amount of enzyme required to hydrolyze azocasein, resulting in an increase in absorbance at 440 nm, was measured. The most promising isolates, particularly those of Bacillus species, demonstrated high protease activity, with enzyme units ranging from 50 to 150 U/mL

under optimized conditions. Pseudomonas and Staphylococcus isolates also showed significant activity, though generally lower than that of Bacillus isolates.

F. Comparative Analysis of Protease Activity:

Comparative analysis of the isolated strains revealed that Bacillus subtilis isolate BS-12 exhibited the highest fibrinolytic protease activity, followed by Bacillus cereus isolate BC-22 and Pseudomonas aeruginosa isolate PA-15. The Staphylococcus aureus isolate SA-08 showed moderate protease activity but had the advantage of faster growth rates, making it a potential candidate for rapid enzyme production.

G. Potential Industrial and Biomedical Applications:

The fibrinolytic proteases from these isolates were evaluated for their potential applications in thrombolytic therapy and other industries. The enzymes were tested for their ability to degrade blood clots in vitro, with promising results indicating efficient clot dissolution. Furthermore, the stability of these proteases in various industrial conditions, such as different temperatures, pH levels, and presence of inhibitors, was assessed to determine their suitability for commercial applications.

H. Challenges and Opportunities:

While the study identified several potent fibrinolytic protease producers, challenges such as enzyme purification, stability, and scalability were noted. Addressing these challenges involves optimizing downstream processing techniques, improving enzyme formulations for enhanced stability, and developing cost-effective production methods.

I. Future Directions:

Future research will focus on detailed biochemical characterization of the identified proteases, including their substrate specificity, kinetic properties, and structural analysis through techniques like X-ray crystallography and mass spectrometry. Genetic engineering approaches may also be employed to enhance enzyme production and tailor their properties for specific applications. Exploring synergistic effects of combining multiple bacterial strains or their enzymes could further improve fibrinolytic activity and broaden their application scope. Collaborative efforts with industrial partners could facilitate the transition from laboratory research to commercial production, paving the way for innovative thrombolytic therapies and other biotechnological advancements.

IV.Discussion:

A. Implications of Isolating Fibrinolytic Protease-Producing Bacteria:

The successful isolation of fibrinolytic protease-producing mesophilic bacteria from soil samples collected near slaughterhouses underscores the potential of these environments as reservoirs of biotechnologically valuable microorganisms. Slaughterhouses, with their abundant organic matter and diverse microbial ecosystems, provide a unique niche for the proliferation of protease-producing bacteria. The ability of these bacteria to produce fibrinolytic enzymes highlights their potential application in biomedical fields, particularly in the development of thrombolytic therapies. The isolates identified in this study, especially those belonging to the genera Bacillus, Pseudomonas, and Staphylococcus, have demonstrated significant fibrinolytic activity. These bacteria, through their enzymatic action, can degrade fibrin clots, which is a crucial property for thrombolytic agents used in treating cardiovascular diseases. The diversity of bacterial species capable of producing fibrinolytic proteases suggests that the microbial community in slaughterhouse soils is a rich source of novel enzymes with potential therapeutic applications.

B. Comparison of Protease Activity Among Isolated Strains:

The comparative analysis of protease activity among the isolated strains revealed notable differences in their enzymatic capabilities.

Bacillus species, particularly Bacillus subtilis isolate BS-12, exhibited the highest fibrinolytic activity. This is consistent with previous studies that have highlighted Bacillus species as prolific producers of extracellular enzymes, including proteases. Bacillus subtilis, in particular, is well-known for its robust enzyme secretion systems and adaptability to various environmental conditions, making it a prime candidate for industrial enzyme production. Pseudomonas aeruginosa isolate PA-15 and Staphylococcus aureus isolate SA-08 also showed significant fibrinolytic activity, though generally lower than that of Bacillus isolates. Pseudomonas species are recognized for their metabolic versatility and ability to produce a wide range of enzymes, while Staphylococcus species are notable for their rapid growth rates and resilience in diverse environments. The moderate activity observed in these isolates suggests that while they may not surpass Bacillus in enzyme production, their unique characteristics could still be harnessed for specific applications.

C. Potential Industrial and Biomedical Applications:

The fibrinolytic proteases isolated from these bacteria hold immense potential for industrial and biomedical applications. In the medical field, these enzymes could be developed into novel thrombolytic agents for the treatment of cardiovascular diseases. Unlike traditional thrombolytic drugs, microbial fibrinolytic proteases offer advantages such as high specificity, reduced side effects, and cost-effective production through microbial fermentation. Their ability to degrade fibrin clots efficiently positions them as promising candidates for therapeutic development. Beyond thrombolytic therapy, these proteases can be utilized in various industrial processes. For instance, in the food industry, fibrinolytic proteases can be used to improve the texture and flavor of protein-rich foods. In the detergent industry, they can enhance the removal of protein-based stains. Moreover, in the bioremediation sector, these enzymes can contribute to the degradation of proteinaceous waste, thereby reducing environmental pollution.

D. Challenges and Opportunities in Utilizing Microbial Enzymes:

Despite the promising potential of microbial fibrinolytic proteases, several challenges need to be addressed to realize their full application potential. One of the primary challenges is the purification and stabilization of these enzymes. Proteases are often sensitive to environmental conditions such as temperature and pH, which can affect their activity and stability. Developing robust purification protocols and enzyme formulations that enhance stability under various conditions is crucial for their commercial use. Another challenge is the scalability of enzyme production. While laboratory-scale production can demonstrate the feasibility of enzyme activity, scaling up the production to industrial levels requires optimization of fermentation processes, cost-effective substrates, and efficient downstream processing. Addressing these challenges involves collaborative efforts between researchers and industrial partners to develop scalable production methods. The genetic engineering of microbial strains presents an opportunity to enhance enzyme production and tailor their properties for specific applications. Techniques such as directed evolution and recombinant DNA technology can be employed to improve the yield, stability, and specificity of fibrinolytic proteases. Additionally, exploring the synergistic effects of combining multiple bacterial strains or their enzymes could lead to more effective and versatile enzyme formulations.

E. Future Directions for Research:

Future research should focus on the detailed biochemical and structural characterization of the isolated fibrinolytic proteases. Understanding the enzyme kinetics, substrate specificity, and catalytic mechanisms will provide insights into their functionality and potential modifications to enhance their

performance. Structural analysis using techniques such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy can reveal the three-dimensional configuration of these enzymes, aiding in the design of more efficient enzyme variants. Exploring the genetic regulation of protease production in these bacteria can uncover the molecular mechanisms governing enzyme synthesis and secretion. Identifying key regulatory genes and pathways can inform genetic engineering strategies to optimize enzyme production. The application potential of these enzymes can be expanded by testing their efficacy in various industrial and biomedical settings. Collaborations with pharmaceutical companies can facilitate the development of these enzymes into therapeutic agents, while partnerships with industrial stakeholders can drive the commercialization of protease-based products.

V.Conclusion:

This study successfully identified and isolated fibrinolytic protease-producing mesophilic bacteria from soil samples collected near slaughterhouses in the Karad region. The investigation highlighted the potential of slaughterhouse environments as rich reservoirs of microorganisms with significant biotechnological applications, particularly in the production of fibrinolytic proteases. Through a systematic screening process, 50 distinct bacterial colonies exhibiting fibrinolytic activity were initially identified, with 30 strains demonstrating consistent enzyme activity upon further testing. Molecular identification revealed a diverse array of bacterial genera, including Bacillus, Pseudomonas, and Staphylococcus, with Bacillus species showing the highest fibrinolytic activity. Bacillus subtilis isolate BS-12 emerged as the most potent producer of fibrinolytic proteases, showcasing the potential of this bacterium for industrial enzyme production.Optimization experiments identified key factors influencing protease production, such as temperature, pH, agitation speed, and nutrient supplementation. The ideal conditions for maximum enzyme yield varied among the isolates, with Bacillus species favoring slightly alkaline pH and moderate agitation speeds. These findings provide a foundation for optimizing large-scale fermentation processes to enhance protease production. The study demonstrated the significant potential of microbial fibrinolytic proteases in thrombolytic therapy for cardiovascular diseases. These enzymes offer advantages over conventional thrombolytic drugs, including high specificity, reduced side effects, and cost-effective production. Additionally, the isolated proteases hold promise for various industrial applications, such as food processing, detergent formulation, and bioremediation, due to their ability to degrade proteinaceous substrates efficiently. Despite the promising potential, challenges such as enzyme purification, stability, and scalability need to be addressed to fully realize the commercial applications of these proteases. Future research should focus on detailed biochemical characterization, genetic engineering to enhance enzyme properties, and testing in diverse industrial and biomedical settings. Collaborative efforts with industrial partners will be crucial in translating laboratory findings into commercial products.This study underscores the importance of exploring microbial diversity in unique environments, such as slaughterhouse soils, for the discovery of novel enzymes with significant biotechnological applications. The identified fibrinolytic proteases from mesophilic bacteria hold substantial promise for industrial and medical use, highlighting the value of microbial resources in advancing biotechnology.

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