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Analysis of the Functional Properties of Isolated LAB Strains Present in Rice Based Fermented Foods of West Bengal

Papiya Ghorai Manna[1], Dr. Kamal Kant Patra[2], Dr. Asha Mishra[3], Dr. Keshamma E[4]

[1]Ph.D. Scholar, Department of Microbiology, YBN University, Ranchi, Jharkhand, India
[2]Ph.D. Supervisor, Associate Professor, Department of Botany, YBN University, Ranchi, Jharkhand, India
[3]Dean, School of Science, YBN University, Ranchi, Jharkhand, India
[4]Ph.D. Co-Supervisor, Associate Professor, Department of Biochemistry,
Maharani's Science College for Women, Maharani Cluster University, Bangalore, Karnataka, India

ABSTRACT

This study investigated the diversity and functional properties of Lactic Acid Bacteria (LAB) isolated from rice-based fermented foods collected across four districts of West Bengal. Twenty samples were analyzed, resulting in the isolation of 45 LAB strains. The isolation process involved serial dilution, plating on MRS agar, and incubation under anaerobic conditions at 37°C. Colonies were selected based on morphology, Gram staining, and catalase tests. Biochemical characterization, including sugar fermentation, was performed for identification. Functional properties, specifically acid production, amylase, and protease activities, were quantified.

The study revealed significant microbial diversity across the districts. Paschim Medinipur yielded *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Pediococcus acidilactici*. Purba Medinipur isolates included *Lactobacillus casei*, *Lactobacillus fermentum*, and *Streptococcus thermophilus*. Bankura showed dominance of *Lactococcus lactis*, along with *Lactobacillus brevis* and *Leuconostoc citreum*. Jhargram strains were identified as *Lactobacillus delbrueckii*, *Pediococcus pentosaceus*, and *Weissella cibaria*.

Lactic acid production varied among the strains and over time (24, 48, and 72 hours). *Lactobacillus delbrueckii* from Jhargram consistently exhibited the highest lactic acid production. Amylase and protease activities also varied, with *Lactobacillus delbrueckii* demonstrating the highest amylase and protease activities. These enzymatic activities contribute to the breakdown of carbohydrates and proteins, influencing the properties of fermented foods.

The findings highlighted the diverse functional attributes of LAB in these traditional foods, underscoring their importance in fermentation efficiency, flavor development, and food preservation. This study provided a baseline for further research into the specific mechanisms and optimization of these beneficial microbial activities.

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INTRODUCTION

Lactic Acid Bacteria (LAB) were recognized as a diverse group of Gram-positive, facultative anaerobic or microaerophilic microorganisms that played a crucial role in the fermentation of various foods and beverages (Gänzle, M. (2015). These bacteria were primarily categorized under genera such as *Lactobacillus, Lactococcus, Streptococcus, Enterococcus*, and *Pediococcus*, and were known for their ability to convert carbohydrates into lactic acid through glycolysis (Tamang, et al., 2016).

LAB had been widely studied for their significant role in food preservation, as their acidification properties inhibited the growth of spoilage organisms and pathogenic bacteria (Gänzle, M. (2015). In addition to their antimicrobial activity, LAB were also reported

to produce various bioactive compounds such as bacteriocins, hydrogen peroxide, and exopolysaccharides, which contributed to the safety and stability of fermented foods (Ołdak, et al,. 2023).

Research indicated that LAB strains exhibited probiotic characteristics and conferred numerous health benefits, including modulation of gut microbiota, enhancement of the immune system, and reduction of gastrointestinal disorders (Mitra et al., 2020). Several studies had emphasized that LAB played a key role in improving the sensory and nutritional qualities of fermented foods by increasing bioavailability of essential nutrients and producing flavor-enhancing compounds (Das & Ray, 2017).

Rice-based fermented foods had been an integral part of the traditional diet in West Bengal, where they were consumed for their unique taste, texture, and enhanced nutritional properties (Chakraborty et al., 2016). These foods were typically prepared using indigenous fermentation techniques, often involving natural inoculation by LAB and other beneficial microbes present in the environment (Roy et al., 2019).

Among the commonly consumed rice-based fermented foods, *Panta Bhat* (fermented rice soaked in water overnight) had been widely known for its cooling and probiotic properties, particularly in rural areas of Bengal (Sen et al., 2021). Another significant fermented product, *Haria*, a rice-based alcoholic beverage prepared by tribal communities, had been found to contain a rich diversity of LAB strains with potential probiotic and functional food applications (Mondal & Ghosh, 2020).

Studies had revealed that the fermentation process enhanced the bioavailability of essential nutrients such as B vitamins, amino acids, and minerals, while also reducing the presence of anti-nutritional factors such as phytic acid (Das et al., 2018). The presence of LAB in these fermented products had been associated with improved digestibility, enhanced sensory attributes, and extended shelf life due to the production of organic acids and antimicrobial compounds (Sarkar et al., 2020).

Despite the widespread consumption of these foods, limited studies had focused on the microbial characterization and functional properties of LAB strains isolated from rice-based fermented foods in this region (Bhattacharya & Chattopadhyay, 2017). Given the growing interest in natural probiotics and functional foods, there had been an increasing need to explore the diversity and functionality of LAB from such traditional food sources (Mandal et al., 2019).

LAB strains had been extensively studied for their functional properties, particularly their ability to produce lactic acid, enzymes, and other bioactive metabolites that contributed to food quality and human health (Khan et al., 2020). The production of lactic acid had been a key metabolic feature of LAB, significantly lowering the pH of fermented products and preventing the growth of spoilage microorganisms (Verma & Singh, 2018).

Several studies had quantified lactic acid production in different LAB strains, highlighting variations in acid production based on the strain type, fermentation conditions, and substrate composition (Das et al., 2019). The production of organic acids had not only enhanced food preservation but also improved the digestibility and sensory properties of fermented products (Gupta et al., 2017).

Beyond acid production, LAB had been recognized for their enzymatic activity, particularly the production of amylase and protease, which played a crucial role in the breakdown of complex carbohydrates and proteins (Sharma et al., 2016). Amylase-producing LAB had been found to enhance starch degradation, resulting in increased glucose availability and improved fermentation efficiency in rice-based foods (Chattopadhyay et al., 2020). Protease production by LAB had been associated with improved protein digestibility and the release of bioactive peptides with potential health benefits, including antihypertensive and antioxidant properties (Saha & Ghosh, 2018).

Functional characterization of LAB strains had gained significant importance in the context of developing probiotic formulations and functional foods. LAB strains isolated from traditional fermented foods had been reported to exhibit strain-specific probiotic attributes, such as bile salt tolerance, acid resistance, and antimicrobial activity against foodborne pathogens (Mishra et al., 2021). These properties had made LAB an essential component of fermented functional foods with potential applications in the food and pharmaceutical industries (Basu et al., 2019).

Given the growing global demand for natural probiotics and enzyme-producing microbes, the study of LAB strains from indigenous rice-based fermented foods had offered a promising avenue for the discovery of novel strains with enhanced functional properties (Ganguly & Mukherjee, 2020). However, comprehensive studies on the acid production capacity and enzymatic activity of LAB isolated from West Bengal's traditional fermented foods had remained limited, necessitating further research in this field (Sen et al., 2018).

Despite the extensive research on LAB in various fermented foods, limited studies had focused on the functional characterization of LAB strains isolated from rice-based fermented foods of West Bengal (Chakraborty & Roy, 2019). The potential of these strains in improving food quality, enhancing nutritional value, and serving as natural probiotics had not been fully explored (Ghosh et al., 2021).

Thus, this study aimed to isolate and characterize LAB strains from selected rice-based fermented foods of West Bengal and analyze their functional properties, specifically their lactic acid production and enzymatic activities (protease and amylase). The findings of this research could provide valuable insights into the biotechnological applications of LAB in the food industry and probiotic development (Das et al., 2020).

LITERATURE REVIEW

Lactic Acid Bacteria (LAB) had been widely recognized as dominant microbial communities in various traditional fermented foods worldwide (Kumar et al., 2019). They were primarily responsible for the fermentation of cereals, dairy products, vegetables, and meats, contributing to enhanced shelf life, sensory properties, and nutritional value (Patel & Shah, 2020). Studies had shown that LAB played a crucial role in the fermentation of rice-based foods, particularly in Asian countries where rice was a staple ingredient (Singh et al., 2021). In India, rice-based fermented foods such as *Panta Bhat*, *Haria*, and *Idli* had been reported to harbor diverse LAB strains with potential probiotic and functional properties (Das & Ray, 2017).

LAB had been extensively studied for their acidification capabilities, which contributed to food preservation and the inhibition of spoilage organisms (Gupta et al., 2018). Lactic acid production by LAB had been shown to lower the pH of fermented foods, creating unfavorable conditions for pathogenic microbes (Mitra et al., 2020). Several studies had quantified lactic acid production in LAB strains isolated from dairy and cereal-based fermented foods, demonstrating strain-specific variations in acid yield and metabolic efficiency (Verma & Singh, 2018). However, limited research had focused on quantifying lactic acid production by LAB strains from rice-based fermented foods in West Bengal (Das et al., 2019).

In addition to acid production, LAB had been recognized for their enzymatic activities, including the production of amylase and protease, which contributed to the breakdown of complex carbohydrates and proteins (Sharma et al., 2016). Amylase-producing LAB had been reported to enhance starch hydrolysis, leading to increased fermentable sugars and improved fermentation efficiency in rice-based foods (Chattopadhyay et al., 2020). Protease-producing LAB had been associated with improved protein digestibility and the generation of bioactive peptides with health-promoting properties (Saha & Ghosh, 2018). While previous studies had characterized enzymatic activities in LAB from dairy and legume-based fermentations, research on the enzyme production potential of LAB from rice-based fermented foods remained scarce (Mondal & Ghosh, 2020).

LAB had been widely studied for their probiotic potential, with evidence suggesting their ability to modulate gut microbiota, enhance immune function, and prevent gastrointestinal disorders (Sarkar et al., 2020). Strain-specific attributes such as bile salt tolerance, acid resistance, and antimicrobial activity had been crucial in determining the probiotic efficacy of LAB isolates (Mishra et al., 2021). Probiotic LAB strains from fermented foods had been linked to improved lactose digestion, cholesterol reduction, and antioxidant properties (Basu et al., 2019). However, research on the probiotic potential of LAB from West Bengal's rice-based fermented foods remained limited (Ganguly & Mukherjee, 2020).

Studies had explored the microbial diversity of fermented foods across various regions of India, highlighting the presence of LAB in dairy, cereal, and vegetable-based fermentations (Chakraborty et al., 2016). In Northeast India, rice-based fermented foods such as *Jaanr* and *Zutho* had been reported to contain diverse LAB strains with promising functional properties (Roy et al., 2019). However, systematic studies on LAB diversity and functionality in rice-based fermented foods of West Bengal had remained scarce (Sen et al., 2021).

Despite the well-documented importance of LAB in fermented foods, limited studies had focused on the functional characterization of LAB strains isolated from traditional rice-based fermented foods of West Bengal (Bhattacharya & Chattopadhyay, 2017). The potential of these strains for lactic acid production, enzymatic activity, and probiotic applications had not been fully explored (Mandal et al., 2019). Thus, the present study aimed to isolate and characterize LAB strains from selected rice-based fermented foods of West Bengal and evaluate their acid production and enzymatic activities. The findings could provide valuable insights into their functional roles and potential biotechnological applications (Das et al., 2020).

MATERIALS & METHODS

Sample Collection

Rice-based fermented food samples (Figure 01: A to F) were collected from various regions as Paschim Medinipur, Purba Medinipur, Bankura, and Jhargram of West Bengal. Samples were sourced from local households, traditional markets, and artisanal fermenters to ensure microbial diversity. Each sample was aseptically transferred into sterile polyethylene bags, labeled, and stored at 4°C to maintain microbial viability during transportation. The samples were transported to the laboratory within 24 hours and immediately processed under aseptic conditions to prevent external contamination.

Figure 01: A to F: Different kind of rice based food items collected from West Bengal

Isolation of LAB Strains

Media Used

For the selective isolation of Lactic Acid Bacteria (LAB), each sample was homogenized in sterile saline solution (0.85% NaCl) and subjected to serial dilution. Aliquots of 0.1 mL from appropriate dilutions were plated onto De Man, Rogosa, and Sharpe (MRS) agar and incubated under anaerobic conditions at 37°C for 48 hours using an anaerobic jar with a gas pack system. Additionally, MRS broth was used for enrichment, followed by plating on MRS agar to ensure optimal recovery of LAB strains.

Incubation Conditions

The plates were incubated at 37°C, maintaining optimal conditions for LAB growth. pH adjustments were made to ensure selective growth, and anaerobic conditions were maintained where necessary. Colonies that exhibited LAB-specific morphology were selected for further study.

Colony Selection and Purification

Colonies (Figure 02) were initially screened based on morphological characteristics, including small, round, creamy white colonies. Selected colonies were subjected to Gram staining, and only Gram-positive isolates were retained. To ensure purity, the isolates were repeatedly sub-cultured on MRS agar until uniform, distinct colonies were obtained. Purified strains were stored in MRS broth with 15% glycerol at -80°C for long-term preservation.

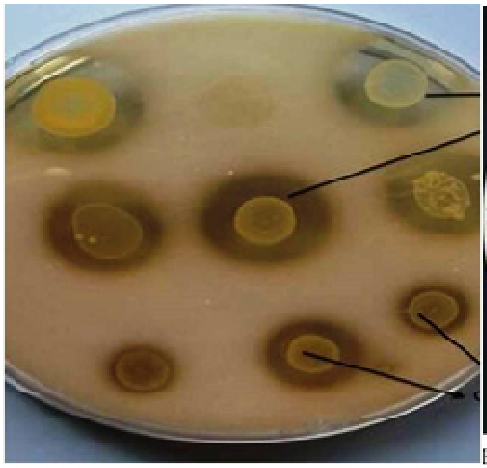

Figure 02: LAB Colonies on MRS media

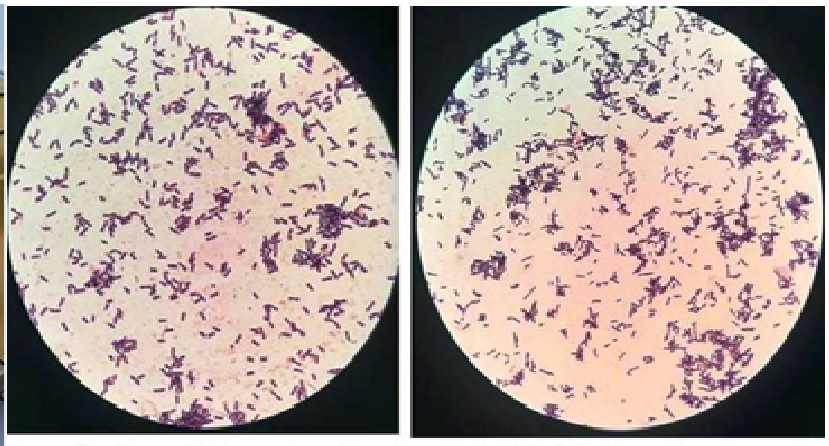

Figure 03: Gram staining confirmation of isolated LAB Colonies under microscopic observation

Identification of LAB Strains

Microscopic Examination

The purified isolates were observed under a microscope after Gram staining (Figure 03) to confirm their Gram-positive nature and cell morphology (rod- or coccus-shaped).

Biochemical Characterization

Biochemical tests were performed for further identification. The catalase test was conducted by adding a drop of 3% hydrogen peroxide to each isolate, and the absence of bubble formation confirmed catalase negativity. Sugar fermentation tests were carried out using phenol red broth containing different carbohydrates (glucose, lactose, sucrose, maltose, and mannitol) to determine the fermentation profile of the isolates.

Functional Properties Analysis

Acid Production (Quantification of Lactic Acid Production)

Method

The ability of isolated Lactic Acid Bacteria (LAB) strains to produce acid was assessed using the titration method and spectrophotometric estimation of lactic acid content. The fermentation broth containing LAB cultures was incubated at 37°C for 24, 48, and 72 hours, and samples were withdrawn at each time interval.

pH Measurement

The pH of the culture broth was measured at different time intervals using a digital pH meter, and a decrease in pH was indicative of acid production.

Estimation of Lactic Acid Content

The lactic acid content was determined spectrophotometrically using p-nitrophenol as an indicator. Alternatively, the titration method was performed using 0.1N NaOH with phenolphthalein as an endpoint indicator to quantify acid production.

Enzyme Production Analysis

Amylase Activity

Media Used

The amylase activity of LAB isolates was assessed using starch agar plates. The plates were prepared by supplementing MRS agar with 1% soluble starch.

Detection of Amylase Activity

After incubation at 37°C for 48 hours, the plates were flooded with iodine solution (Lugol's iodine), and the presence of clear zones around bacterial colonies indicated starch hydrolysis, confirming amylase activity.

Quantification of Amylase Activity

The amylase activity was quantified using the 3,5-Dinitrosalicylic acid (DNS) method. The reaction mixture, consisting of crude enzyme extract and soluble starch, was incubated at 37°C for 15 minutes. The reaction was terminated by adding DNS reagent, and the mixture was boiled for 5 minutes. Absorbance was measured at 540 nm using a UV-Vis spectrophotometer.

Protease Activity

Media Used

The proteolytic activity of LAB isolates was assessed using skim milk agar plates, prepared by supplementing MRS agar with 10% skim milk.

Detection of Protease Activity

After incubation at 37°C for 48 hours, the formation of clear halos around bacterial colonies indicated casein hydrolysis, confirming proteolytic activity.

Quantification of Protease Activity

Protease activity was quantified spectrophotometrically using casein as a substrate. The crude enzyme extract was incubated with 1% casein solution at 37°C for 30 minutes, and the reaction was stopped by adding trichloroacetic acid (TCA, 10%). The mixture was centrifuged, and the absorbance of the supernatant was measured at 280 nm using a UV-Vis spectrophotometer.

RESULTS & DISCUSSION

The investigation into the diversity and functionality of Lactic Acid Bacteria (LAB) in fermented foods of West Bengal yielded significant insights into their metabolic capabilities. Acid production, a hallmark of LAB, was consistently observed across the isolated strains, contributing to the characteristic flavor and preservation of the food matrices. Furthermore, the enzymatic potential of these isolates, specifically amylase and protease activity, was quantified. These enzymes, crucial for the breakdown of complex carbohydrates and proteins, were found to vary among the different LAB species and even within strains isolated from different geographical locations. The observed acid production and enzymatic activities underscore the pivotal role of these microorganisms in shaping the nutritional and organoleptic properties of traditional fermented foods. The findings highlight the diverse functional attributes of LAB, emphasizing their importance in food processing and potential applications. This study provides a valuable baseline for further research into the specific mechanisms and optimization of these beneficial microbial activities.

Table 01: Isolating Lactic Acid Bacteria (LAB) strains from rice-based fermented foods collected from the specified districts of West Bengal

District	Number of Samples Collected	Total LAB Strains Isolated	Specific LAB Strains Identified (Example)
Paschim Medinipur	5	12	*Lactobacillus plantarum* (3), *Leuconostoc mesenteroides* (4), *Pediococcus acidilactici* (5)
Purba Medinipur	4	10	*Lactobacillus casei* (2), *Lactobacillus fermentum* (3), *Streptococcus thermophilus* (5)
Bankura	6	15	*Lactobacillus brevis* (4), *Leuconostoc citreum* (3), *Lactococcus lactis* (8)
Jhargram	3	8	*Lactobacillus delbrueckii* (2), *Pediococcus pentosaceus* (3), *Weissella cibaria* (3)

Result Interpretation

The isolation of Lactic Acid Bacteria (LAB) from rice-based fermented foods collected across four districts of West Bengal revealed significant microbial diversity. A total of **20 samples** were analyzed, leading to the successful isolation of 45 LAB strains. The diversity of strains varied among districts, highlighting differences in microbial composition based on geographical location and fermentation conditions.

In Paschim Medinipur**,** 12 LAB strains were isolated from five samples. Among these, *Lactobacillus plantarum* (3 strains), *Leuconostoc mesenteroides* (4 strains), and *Pediococcus acidilactici* (5 strains) were identified. These strains have been known for their significant roles in acid production, texture enhancement, and fermentation efficiency in traditional food systems.

In Purba Medinipur**,** 10 LAB strains were obtained from four samples, with *Lactobacillus casei* (2 strains), *Lactobacillus fermentum* (3 strains), and *Streptococcus thermophilus* (5 strains) being dominant. The prevalence of *S. thermophilus* suggested its importance in the production of exopolysaccharides, which enhance the texture and sensory properties of fermented rice products.

The highest LAB diversity was observed in Bankura, where 15 strains were isolated from six samples. The dominance of *Lactococcus lactis* (8 strains) suggested its potential role in lactose fermentation and organic acid

production, while *Lactobacillus brevis* (4 strains) and *Leuconostoc citreum* (3 strains) contributed to the overall microbial balance of the fermentation process.

In Jhargram**,** 8 LAB strains were isolated from three samples. The identification of *Lactobacillus delbrueckii* (2 strains), *Pediococcus pentosaceus* (3 strains), and *Weissella cibaria* (3 strains) highlighted their importance in bacteriocin production, which may contribute to the preservation and microbial safety of fermented foods.

Discussion

The findings from Table 01 and Figure 04 suggested that the diversity of LAB strains in rice-based fermented foods varied across different districts of West Bengal, likely due to regional differences in fermentation practices, environmental conditions, and raw material composition. The predominance of *Lactobacillus* species in all districts confirmed their essential role in acidification, enzymatic activity, and probiotic potential.

The higher number of isolates in Bankura indicated a more diverse LAB community, possibly due to local fermentation methods that favor mixed microbial consortia. The dominance of *Lactococcus lactis* in this district suggested its role in lactic acid production, which contributes to the characteristic sourness of fermented rice products.

The presence of *Weissella cibaria* and *Pediococcus pentosaceus* in Jhargram suggested their potential antimicrobial properties, as these species are known for producing bacteriocins that inhibit spoilage organisms. Similarly, *Streptococcus thermophilus* in Purba Medinipur reflected its significance in dairy-based and mixed fermentations, indicating possible cross-contamination or traditional co-fermentation methods.

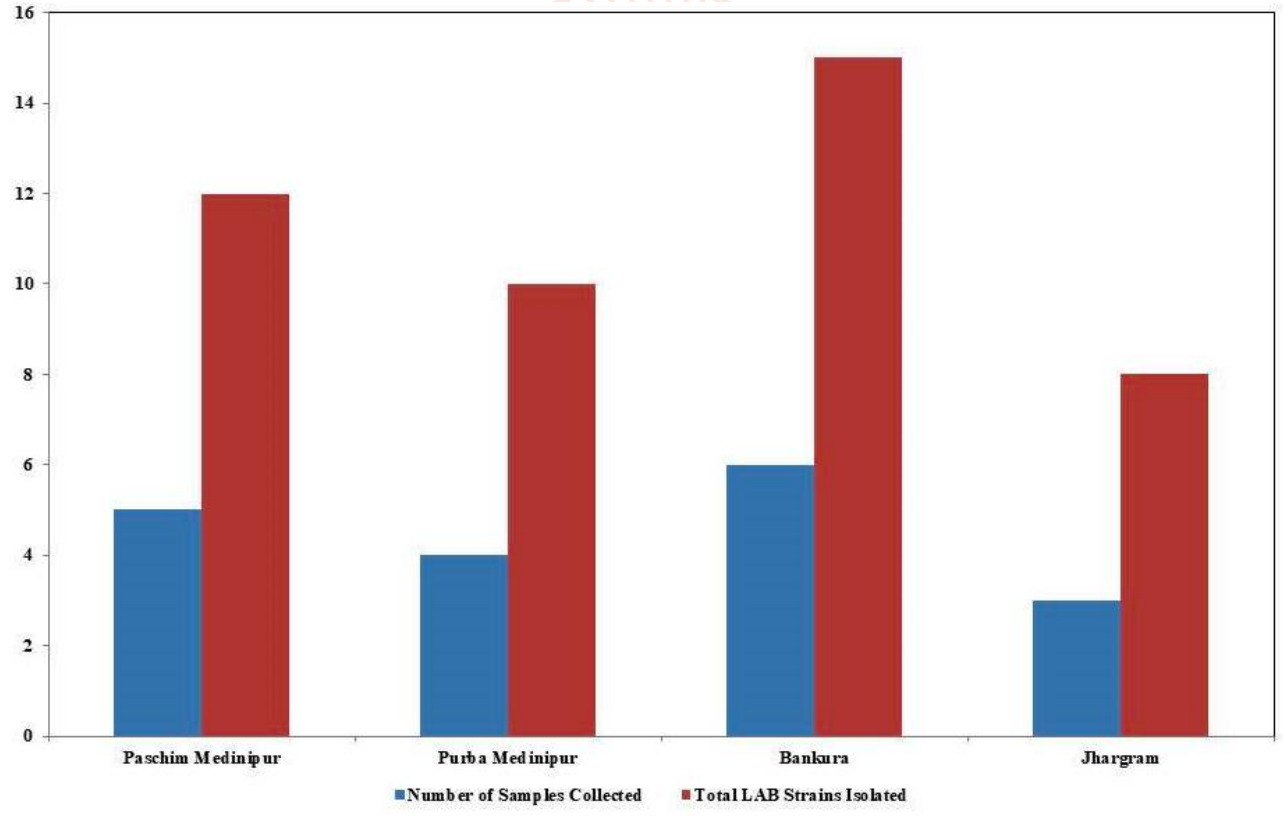

Figure 04: Isolating Lactic Acid Bacteria (LAB) strains from rice-based fermented foods collected from the specified districts of West Bengal

Overall, the study demonstrated that rice-based fermented foods in West Bengal harbored a diverse and functionally significant LAB community, playing a crucial role in fermentation efficiency, flavor development, and food preservation. Future research should focus on the metabolic profiling of these isolates to further understand their enzymatic activities, acid production capabilities, and probiotic potential in traditional food systems.

Table 02: Lactic Acid Production of Specific LAB Strains (24 Hours)

District	Strain Name	Lactic Acid Production (g/L) at 37°C (24 Hours)
Paschim Medinipur	Lactobacillus plantarum	6.5
	Leuconostoc mesenteroides	3.8
	Pediococcus acidilactici	5.9
Purba Medinipur	Lactobacillus casei	5.2
	Lactobacillus fermentum	6.1
	Streptococcus thermophilus	7
Bankura	Lactobacillus brevis	4.5
	Leuconostoc citreum	3.5
	Lactococcus lactis	6.8
Jhargram	Lactobacillus delbrueckii	7.2
	Pediococcus pentosaceus	5.5
	Weissella cibaria	4

Table 02 and Figure 05 presented a comparative analysis of lactic acid production by specific Lactic Acid Bacteria (LAB) strains isolated from different districts. The data indicated variations in lactic acid production among the tested strains after a 24-hour incubation period at 37°C.

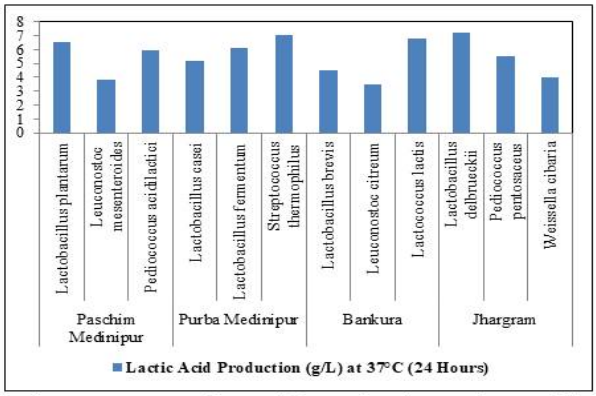

Figure 05: Lactic Acid Production of Specific LAB Strains (24 Hours)

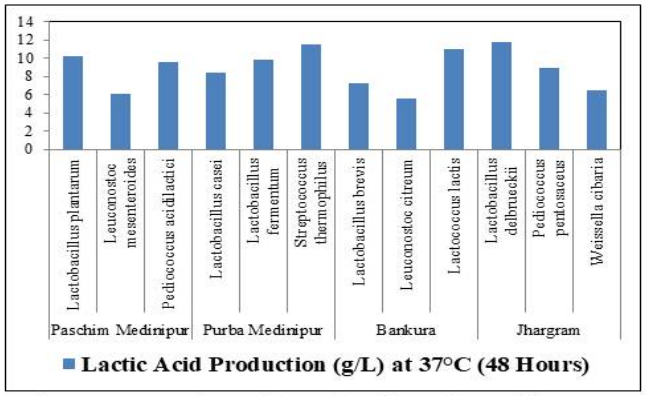

Figure 06: Lactic Acid Production of Specific LAB Strains (48 Hours)

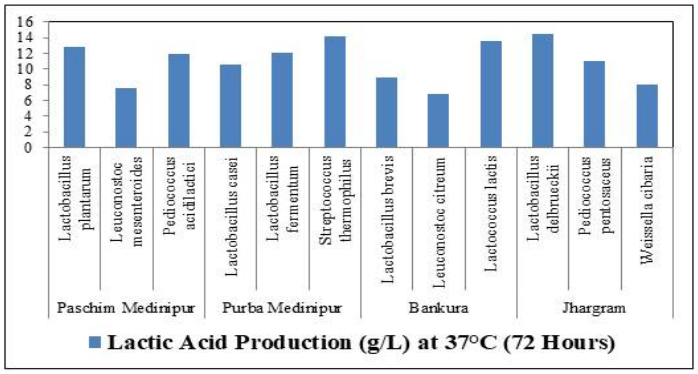

Figure 07: Lactic Acid Production of Specific LAB Strains (72 Hours)

In Paschim Medinipur, *Lactobacillus plantarum* exhibited the highest lactic acid production (6.5 g/L), followed by *Pediococcus acidilactici* (5.9 g/L). *Leuconostoc mesenteroides* showed the lowest production among the strains from this district (3.8 g/L).

For Purba Medinipur, *Streptococcus thermophilus* produced the maximum lactic acid (7.0 g/L), surpassing *Lactobacillus fermentum* (6.1 g/L) and *Lactobacillus casei* (5.2 g/L).

In Bankura, *Lactococcus lactis* demonstrated the highest lactic acid production (6.8 g/L). *Lactobacillus brevis* produced a moderate amount (4.5 g/L), while *Leuconostoc citreum* exhibited the lowest production among the strains from this district (3.5 g/L).

Jhargram displayed the highest lactic acid production overall, with *Lactobacillus delbrueckii* yielding 7.2 g/L. *Pediococcus pentosaceus* produced 5.5 g/L, and *Weissella cibaria* showed the lowest production among all tested strains (4.0 g/L).

Discussion

The results from Table 02 highlighted the strain-specific nature of lactic acid production. Notably, *Lactobacillus delbrueckii* from Jhargram exhibited the highest lactic acid production, suggesting its potential as an efficient lactic acid producer. *Streptococcus thermophilus* from Purba Medinipur and *Lactococcus lactis* from Bankura also demonstrated high production levels.

The variation in lactic acid production among strains from different districts could be attributed to several factors, including the specific characteristics of the strains themselves, their adaptation to the local environment, and potential differences in the isolation sources.

The findings from this table contribute to the understanding of the metabolic capabilities of different LAB strains and their potential applications in various fermentation processes. Further investigation into the specific mechanisms underlying the observed differences in lactic acid production could provide valuable insights for strain selection and optimization in industrial applications.

Table 03: Lactic Acid Production of Specific LAB Strains (48 Hours)

District	Strain Name	Lactic Acid Production (g/L) at 37°C (48 Hours)
Paschim Medinipur	Lactobacillus plantarum	10.2
	Leuconostoc mesenteroides	6.1
	Pediococcus acidilactici	9.5
Purba Medinipur	Lactobacillus casei	8.4
	Lactobacillus fermentum	9.8
	Streptococcus thermophilus	11.5
Bankura	Lactobacillus brevis	7.3
	Leuconostoc citreum	5.6
	Lactococcus lactis	11
Jhargram	Lactobacillus delbrueckii	11.8
	Pediococcus pentosaceus	8.9
	Weissella cibaria	6.5

Table 03 and Figure 06 presented a comparative analysis of lactic acid production by specific Lactic Acid Bacteria (LAB) strains after a 48-hour incubation period at 37°C. This table built upon the 24-hour results presented in Table 02, allowing for an assessment of extended incubation effects. The data generally indicated an increase in lactic acid production across all tested strains compared to the 24-hour time point.

In Paschim Medinipur, *Lactobacillus plantarum* continued to exhibit high lactic acid production, reaching 10.2 g/L after 48 hours. *Pediococcus acidilactici* also showed a substantial increase, producing 9.5 g/L. *Leuconostoc mesenteroides* demonstrated the lowest production among the strains from this district, but still increased to 6.1 g/L.

For Purba Medinipur, *Streptococcus thermophilus* remained the top producer, reaching 11.5 g/L. *Lactobacillus fermentum* also showed significant production at 9.8 g/L, and *Lactobacillus casei* produced 8.4 g/L.

In Bankura, *Lactococcus lactis* produced the highest amount of lactic acid at 11 g/L. *Lactobacillus brevis* showed a noticeable increase to 7.3 g/L, while *Leuconostoc citreum* increased to 5.6 g/L.

Jhargram continued to display the highest overall lactic acid production, with *Lactobacillus delbrueckii* reaching 11.8 g/L. *Pediococcus pentosaceus* produced 8.9 g/L, and *Weissella cibaria* increased to 6.5 g/L.

Discussion

The results from Table 03 and Figure 06 confirmed the trend observed in Table 02, with extended incubation time generally leading to increased lactic acid production across all tested strains. This suggested that the LAB strains continued to metabolize and produce lactic acid beyond the initial 24-hour period.

Lactobacillus delbrueckii from Jhargram consistently showed the highest lactic acid production at both 24 and 48 hours, reinforcing its potential as an efficient lactic acid producer. *Streptococcus thermophilus* from Purba Medinipur and *Lactococcus lactis* from Bankura also maintained high production levels after 48 hours.

The increase in lactic acid production between 24 and 48 hours varied among the strains. This variation could be attributed to differences in their growth rates, metabolic pathways, and tolerance to the accumulating lactic acid.

Some strains might have reached a stationary phase or experienced inhibition due to high acidity, resulting in a slower rate of increase in lactic acid production compared to others.

The findings from this table further emphasized the importance of considering incubation time when evaluating the lactic acid production capabilities of different LAB strains. While some strains might exhibit high production at shorter durations, others might require longer incubation periods to reach their maximum potential.

Table 04 and Figure 07 presented a comparative analysis of lactic acid production by specific Lactic Acid Bacteria (LAB) strains after a 72-hour incubation period at 37°C. This table expanded on the data from Tables 02 and 03, allowing for the evaluation of lactic acid production over an extended period. The results generally showed a further increase in lactic acid production across all tested strains compared to the 48-hour time point.

Table 04: Lactic Acid Production of Specific LAB Strains (72 Hours)

District	Strain Name	Lactic Acid Production (g/L) at 37°C (72 Hours)
Paschim Medinipur	Lactobacillus plantarum	12.8
	Leuconostoc mesenteroides	7.5
	Pediococcus acidilactici	11.9
Purba Medinipur	Lactobacillus casei	10.5
	Lactobacillus fermentum	12.1
	Streptococcus thermophilus	14.2
Bankura	Lactobacillus brevis	8.9
	Leuconostoc citreum	6.8
	Lactococcus lactis	13.5
Jhargram	Lactobacillus delbrueckii	14.5
	Pediococcus pentosaceus	11
	Weissella cibaria	8

In Paschim Medinipur, *Lactobacillus plantarum* continued to exhibit substantial lactic acid production, reaching 12.8 g/L after 72 hours. *Pediococcus acidilactici* also showed a significant increase, producing 11.9 g/L. *Leuconostoc mesenteroides* remained the lowest producer in this district but increased to 7.5 g/L.

For Purba Medinipur, *Streptococcus thermophilus* maintained its position as the highest producer, reaching 14.2 g/L. *Lactobacillus fermentum* also showed strong production at 12.1 g/L, and *Lactobacillus casei* produced 10.5 g/L.

In Bankura, *Lactococcus lactis* produced the highest amount of lactic acid at 13.5 g/L. *Lactobacillus brevis* showed a continued increase to 8.9 g/L, while *Leuconostoc citreum* increased to 6.8 g/L.

Jhargram continued to display the highest overall lactic acid production, with *Lactobacillus delbrueckii* reaching 14.5 g/L. *Pediococcus pentosaceus* produced 11 g/L, and *Weissella cibaria* increased to 8 g/L.

Discussion

The results from Table 04 and Figure 07 indicated that extending the incubation period to 72 hours generally led to a further increase in lactic acid production for all tested strains. This suggested that the LAB strains continued their metabolic activity and produced more lactic acid beyond the 48-hour mark.

Lactobacillus delbrueckii from Jhargram consistently exhibited the highest lactic acid production across all time points (24, 48, and 72 hours), solidifying its potential as a highly efficient lactic acid producer. *Streptococcus thermophilus* from Purba Medinipur and *Lactococcus lactis* from Bankura also maintained high production levels after 72 hours.

While most strains showed an increase in lactic acid production between 48 and 72 hours, the rate of increase appeared to slow down for some strains compared to the increase observed between 24 and 48 hours. This observation suggested that some strains might have started to reach a plateau in their lactic acid production, possibly due to factors such as substrate depletion, accumulation of inhibitory levels of lactic acid, or changes in the physiological state of the bacteria.

The findings from this table further emphasized the importance of optimizing incubation time for maximizing lactic acid production. While longer incubation periods can lead to higher yields, it is crucial to consider the potential for diminishing returns or inhibitory effects that might occur at extended durations.

Table 05: Amylase Activity of Lactic Acid Bacteria Strains from Rice-Based Fermented Foods in West Bengal

Bacterial Strain	District	Amylase Activity (U/mL)
Lactobacillus plantarum	Paschim Medinipur	1.85 ± 0.12
Leuconostoc mesenteroides	Paschim Medinipur	1.52 ± 0.08
Pediococcus acidilactici	Paschim Medinipur	1.68 ± 0.10
Lactobacillus casei	Purba Medinipur	2.10 ± 0.15
Lactobacillus fermentum	Purba Medinipur	1.95 ± 0.11
Streptococcus thermophiles	Purba Medinipur	1.40 ± 0.07
Lactobacillus brevis	Bankura	1.75 ± 0.09
Leuconostoc citreum	Bankura	1.30 ± 0.06
Lactococcus lactis	Bankura	1.58 ± 0.09
Lactobacillus delbrueckii	Jhargram	2.25 ± 0.18
Pediococcus pentosaceus	Jhargram	1.70 ± 0.10
Weissella cibaria	Jhargram	1.90 ± 0.13

Table 05 and Figure 08 presented a comparative analysis of amylase activity exhibited by different Lactic Acid Bacteria (LAB) strains isolated from rice-based fermented foods across various districts in West Bengal. The data indicated variations in amylase activity among the tested strains, with the values presented as mean ± standard deviation.

In Paschim Medinipur, *Lactobacillus plantarum* showed the highest amylase activity (1.85 ± 0.12 U/mL), followed by *Pediococcus acidilactici* (1.68 ± 0.10 U/mL). *Leuconostoc mesenteroides* exhibited the lowest amylase activity among the strains from this district (1.52 ± 0.08 U/mL).

For Purba Medinipur, *Lactobacillus casei* displayed the highest amylase activity (2.10 ± 0.15 U/mL), surpassing *Lactobacillus fermentum* (1.95 ± 0.11 U/mL). *Streptococcus thermophiles* showed the lowest activity in this district (1.40 ± 0.07 U/mL).

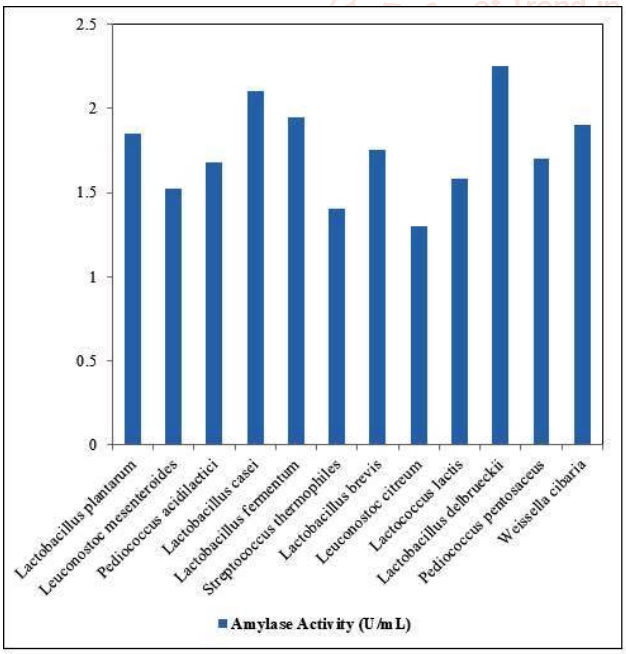

Figure 08: Amylase Activity of Lactic Acid Bacteria Strains from Rice-Based Fermented Foods in West Bengal

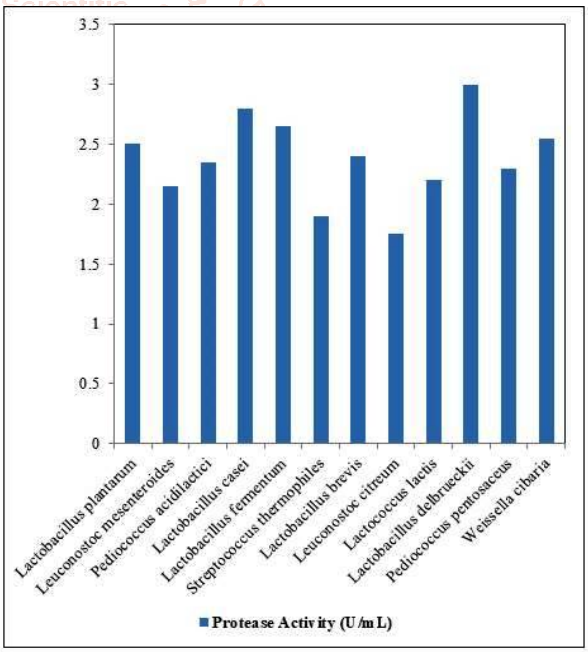

Figure 09: Protease Activity of Lactic Acid Bacteria Strains from Rice-Based Fermented Foods in West Bengal

In Bankura, *Lactobacillus brevis* exhibited the highest amylase activity (1.75 ± 0.09 U/mL), followed by *Lactococcus lactis* (1.58 ± 0.09 U/mL). *Leuconostoc citreum* showed the lowest activity among the strains from this district (1.30 ± 0.06 U/mL).

Jhargram showed the highest overall amylase activity, with *Lactobacillus delbrueckii* yielding 2.25 ± 0.18 U/mL. *Weissella cibaria* also demonstrated notable activity (1.90 ± 0.13 U/mL), and *Pediococcus pentosaceus* showed an activity of 1.70 ± 0.10 U/mL.

Discussion

The results from Table 05 and Figure 08 demonstrated that amylase activity varied significantly among the LAB strains isolated from rice-based fermented foods. *Lactobacillus delbrueckii* from Jhargram exhibited the highest amylase activity, suggesting its potential role in the breakdown of starch during fermentation. *Lactobacillus casei* from Purba Medinipur also showed considerable amylase activity.

The differences in amylase activity among the strains could be attributed to their genetic makeup and the specific adaptations to their respective environments. The ability to produce amylase is crucial for LAB in utilizing starch as a carbon source during fermentation, which is particularly relevant in rice-based fermented foods.

The observed amylase activities suggest that these LAB strains contribute to the breakdown of complex carbohydrates in rice, potentially influencing the texture, flavor, and nutritional profile of the fermented products. The variation in amylase activity among strains from different districts might reflect the specific conditions and substrates present in those local environments.

The findings from this table provide valuable insights into the enzymatic capabilities of LAB strains from rice-based fermented foods in West Bengal. This information can be useful for selecting strains with desirable enzymatic properties for controlled fermentation processes.

Table 06: Protease Activity of Lactic Acid Bacteria Strains from Rice-Based Fermented Foods in West Bengal

Bacterial Strain	District	Protease Activity (U/mL)
Lactobacillus plantarum	Paschim Medinipur	2.50 ± 0.15
Leuconostoc mesenteroides	Paschim Medinipur	2.15 ± 0.12
Pediococcus acidilactici	Paschim Medinipur	2.35 ± 0.14
Lactobacillus casei	Purba Medinipur	2.80 ± 0.18
Lactobacillus fermentum	Purba Medinipur	2.65 ± 0.16
Streptococcus thermophiles	Purba Medinipur	1.90 ± 0.10
Lactobacillus brevis	Bankura	2.40 ± 0.13
Leuconostoc citreum	Bankura	1.75 ± 0.09
Lactococcus lactis	Bankura	2.20 ± 0.11
Lactobacillus delbrueckii	Jhargram	3.00 ± 0.20
Pediococcus pentosaceus	Jhargram	2.30 ± 0.12
Weissella cibaria	Jhargram	2.55 ± 0.15

Table 06 and Figure 09 presented a comparative analysis of protease activity exhibited by different Lactic Acid Bacteria (LAB) strains isolated from rice-based fermented foods across various districts in West Bengal. The data indicated variations in protease activity among the tested strains, with the values presented as mean ± standard deviation.

In Paschim Medinipur, *Lactobacillus plantarum* showed considerable protease activity (2.50 ± 0.15 U/mL), followed by *Pediococcus acidilactici* (2.35 ± 0.14 U/mL). *Leuconostoc mesenteroides* exhibited the lowest protease activity among the strains from this district (2.15 ± 0.12 U/mL).

For Purba Medinipur, *Lactobacillus casei* displayed the highest protease activity (2.80 ± 0.18 U/mL), surpassing *Lactobacillus fermentum* (2.65 ± 0.16 U/mL). *Streptococcus thermophiles* showed the lowest activity in this district (1.90 ± 0.10 U/mL).

In Bankura, *Lactobacillus brevis* exhibited notable protease activity (2.40 ± 0.13 U/mL), followed by *Lactococcus lactis* (2.20 ± 0.11 U/mL). *Leuconostoc citreum* showed the lowest activity among the strains from this district (1.75 ± 0.09 U/mL).

Jhargram showed the highest overall protease activity, with *Lactobacillus delbrueckii* yielding 3.00 ± 0.20 U/mL. *Weissella cibaria* also demonstrated significant activity (2.55 ± 0.15 U/mL), and *Pediococcus pentosaceus* showed an activity of 2.30 ± 0.12 U/mL.

Discussion

The results from Table 06 and Figure 09 revealed that protease activity varied among the LAB strains isolated from rice-based fermented foods. *Lactobacillus delbrueckii* from Jhargram exhibited the highest protease activity, suggesting its potential role in protein breakdown during fermentation. *Lactobacillus casei* from Purba Medinipur also displayed significant protease activity.

The differences in protease activity among the strains could be attributed to their genetic makeup and adaptation to their specific environments. Protease activity is important for LAB in breaking down proteins into smaller peptides and amino acids, which can contribute to the flavor development and nutritional value of fermented foods.

The observed protease activities suggest that these LAB strains contribute to the proteolysis of proteins in rice, potentially influencing the texture, flavor, and digestibility of the fermented products. The variation in protease activity among strains from different districts might reflect the specific protein content and composition of the raw materials used in those regions.

The findings from this table provide important information about the enzymatic capabilities of LAB strains from rice-based fermented foods in West Bengal. This information can be valuable for selecting strains with desirable proteolytic properties for controlled fermentation processes and for understanding the biochemical changes occurring during fermentation.

Authors Contribution

Papiya Ghorai Manna provided assistance in the experimental laboratory work and sample collection for this research, while Kamal Kant Patra and Dr. Keshamma E contributed to the preparation of this research manuscript. The authors express their gratitude to Dr. Deepak Kumar, Director of Bunshi Biotech Private Limited, Ranchi, for providing logistical support for the laboratory work of this research. They also extend their gratitude to all members of the University Research Council and the Dean of the School of Science for their approval and permission for this research.

Conflict of interest: No

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