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RESEARCH ARTICLE

Extracellular Enzyme-Producing Bacteria from Soil and Poultry Waste: Industrial and Environmental Potential

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ABSTRACT

Microbial enzymes play a crucial role in industrial and environmental biotechnology due to their biodegradability, specificity, and ability to function under mild conditions. This study aimed to isolate and characterize extracellular enzyme-producing bacteria from soil and poultry waste collected in Mirer Bazar, Tongi, Bangladesh. A total of 40 bacterial isolates were screened for amylase, cellulase, pectinase, lipase, and protease activities through primary plate assays (Table 1) and subsequent shake-flask fermentation experiments (Tables 2–6). Significant variation in enzyme production was observed among isolates. Strong amylase and cellulase activity were detected in PLW 5EH, PLW 5FH, GRDF 2, PEDF 10^{-3} 1, and PLW 5CH, with halo diameters reaching 40–46 mm, indicating their potential for starch and cellulose hydrolysis in industrial processes such as food processing, biomass conversion, and biofuel production. Moderate lipase activity was observed in PLW 1AH and PLW 2CH, suggesting their suitability for lipid degradation and biocatalysis applications. Pectinase and protease activities were generally low, implying that optimized culture conditions or substrate induction may be required for enhanced production. The study highlights the diversity of enzyme-producing bacteria from environmental sources and identifies promising strains for industrial and environmental biotechnological applications. Future research should focus on optimizing fermentation conditions, enzyme purification, biochemical characterization, and molecular identification of top-performing isolates.

Keywords: Extracellular enzymes; amylase, cellulase; lipase; soil and poultry waste; industrial biotechnology

1. Introduction

Enzyme-based industrial processes offer several advantages, including cost-effectiveness, environmental sustainability, and energy efficiency. Unlike chemical catalysts, enzymes are biodegradable, function under mild conditions, shorten reaction times, conserve energy, and do not degrade industrial materials. Among the most widely used enzymes are proteases, cellulases, lipases, and amylases[1]. Microbial

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enzymes, due to their stability and scalability, are often preferred over those derived from plants and animals in industrial applications. Bacteria are ideal candidates for enzyme production, as they can grow on diverse substrates, and the process of generating enzymes on a large scale is relatively straightforward in biotechnology industries. The discovery of novel microbial strains and enzymes is an ongoing research focus, given the vast diversity of organisms in nature[2].

Amylases, which constitute approximately 25% of all enzymes produced globally, are crucial in the hydrolysis of starch into smaller sugar units. These enzymes are predominantly sourced from bacteria, yeast, and fungi, with bacterial amylases being the preferred choice for industrial applications[3]. This preference is due to the advantages bacterial enzymes offer, such as shorter growth periods, cost-effective production, eco-friendly production methods, and ease of manipulation of bacterial genes. The food industry is the largest consumer of amylase enzymes, primarily for starch conversion, but amylases also find applications in automated dishwashing detergents, textile desizing, and the pulp and paper industries. Moreover, amylases with enhanced thermostability, low pH tolerance, and raw starch digestibility are particularly valuable in various industrial processes. In the pharmaceutical sector, amylases are utilized as digestive aids[4,5].

Although amylases are derived from multiple sources, bacterial amylases are the most suitable for industrial production due to their shorter growth periods, higher productivity, and ease of handling. Bacillus species, such as *B. amyloliquefaciens*, *B. subtilis*, *B. licheniformis*, and *B. stearothermophilus*, are commonly used in the industrial production of amylase. Additionally, microbial cellulases, which are secreted as enzyme complexes, play a significant role in the breakdown of cellulose into fermentable sugars[6,7]. These enzymes are used in industries such as food processing, animal feed, bioethanol production, and paper manufacturing.

Proteases, categorized by their pH preference and substrate action mode, are another major group of industrial enzymes, predominantly produced by Bacillus species. Proteases are widely utilized in detergents, food processing, leather treatments, and bioremediation processes. Lipases, which hydrolyze fatty acid esters, are essential in industries like detergents, food processing, and wastewater treatment, and they are also crucial for biodiesel production[8].

The industrial demand for microbial enzymes is growing due to their specificity, the mild reaction conditions they require, and their lower energy consumption compared to traditional chemical processes. Enzymes from microbial sources can be purified using techniques such as ultrafiltration, dialysis, and column chromatography. Characterizing microbial enzymes in terms of optimal temperature, pH stability, and thermostability is critical for determining their industrial applications[9,10].

Despite extensive research on enzyme-producing bacteria, no studies have been conducted on bacteria isolated from soil or poultry waste in Mirer Bazar, Tongi, Bangladesh. The aim of this study is to isolate and identify amylase-producing bacteria from soil and poultry waste in Mirer Bazar.

2. Materials and methods

2.1. Sample collection

Soil samples were randomly collected from various agricultural fields, including a jungle, paddy field, spinach field, and gourd field, from a depth of 15 cm below the soil surface. Poultry waste samples were also collected from polystyrene containers in Mirer Bazar, Tongi, Gazipur, Bangladesh. All samples were transported to the laboratory for further analysis.

2.2. Isolation of amylase-producing bacteria

For the isolation of amylase-producing bacteria, 10 g of each sample (soil or poultry waste) was dissolved in 90 mL of sterile 0.85% NaCl solution to prepare a suspension. The suspension was serially diluted, and 100 µL aliquots from each dilution were spread onto nutrient agar plates (g/L) and incubated at 37°C for 24 hours. After incubation, colonies with distinct morphological characteristics were selected and streaked onto fresh nutrient agar plates to obtain pure isolates. The purity of the bacterial isolates was confirmed by Gram staining, followed by microscopic examination at 1000X magnification.

2.3. Bacterial identification

The morphological characteristics of the bacterial colonies, including shape, size, texture, elevation, and color, were recorded. Additional phenotypic identification was carried out using the IMViC test, Gram staining, motility tests, and biochemical assays, including catalase, oxidase, urease, and Triple Sugar Iron (TSI) test[11]. The results were used to classify the bacteria at the genus and species level.

2.4. Enzyme activity determination

Amylase Activity

Amylase-producing bacteria were first identified by culturing the pure isolates on starch agar plates. After 24 hours of incubation at 37°C, the plates were flooded with Lugol's iodine solution. Clear zones around the colonies indicated amylase activity, as iodine does not stain areas where starch has been hydrolyzed. The diameters of the clear zones were measured using a ruler to calculate the Halo: Colony ratio.

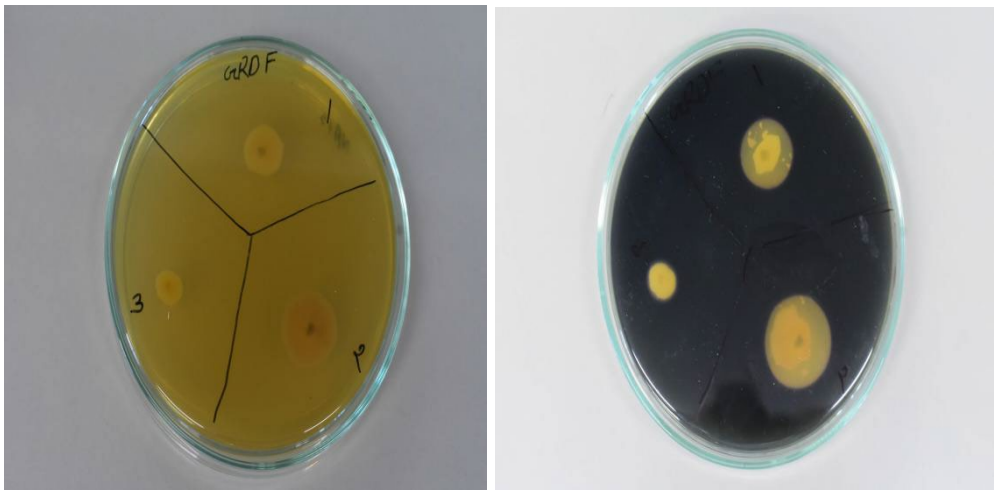

Figure 1. Starch Hydrolysis by Bacterial Isolates

Cellulase Activity

For cellulase activity determination, the pure isolates were point-inoculated onto carboxymethyl cellulose (CMC) agar plates and incubated at 37°C for 24 hours. The plates were then flooded with Gram's iodine to visualize the hydrolysis of CMC. The clear zones formed around the colonies were measured, and the Halo: Colony ratio was determined.

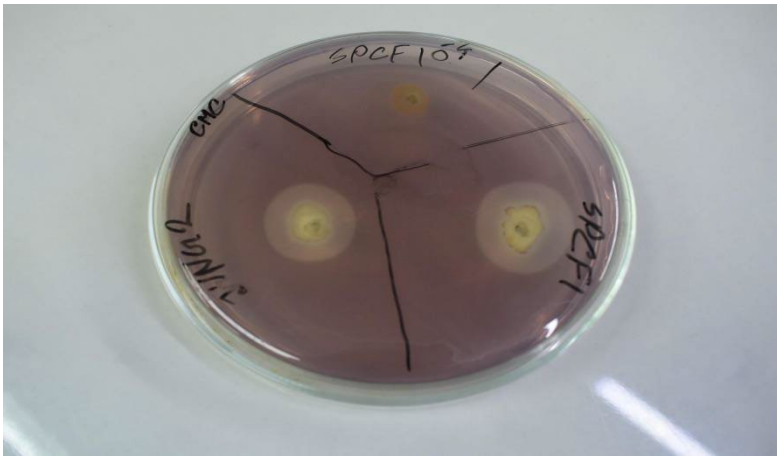

Figure 2. Cellulase Activity on CMC Agar

Protease Activity

Protease activity was evaluated by inoculating the bacterial isolates onto casein agar plates. After 24 hours of incubation at 37°C, the plates were flooded with Lugol's iodine solution. The formation of clear zones around the colonies indicated protease activity, which was measured by calculating the Halo: Colony ratio.

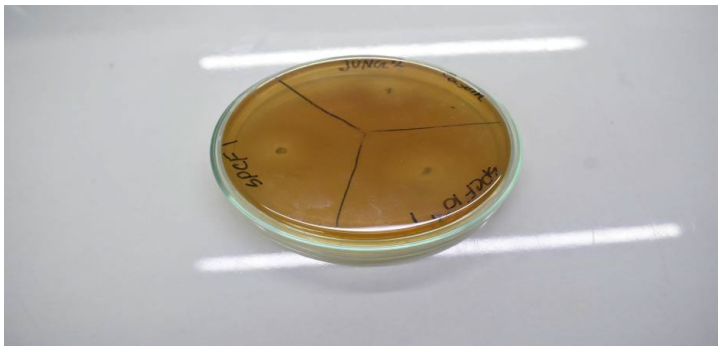

Figure 3. Protease Activity on Casein Agar

Pectinase Activity

To assess pectinase activity, pure isolates were inoculated onto pectin agar plates and incubated at 37°C for 24 hours. The plates were treated with Lugol's iodine solution to visualize the hydrolysis of pectin. The diameters of the clear zones were measured, and the Halo: Colony ratio was recorded.

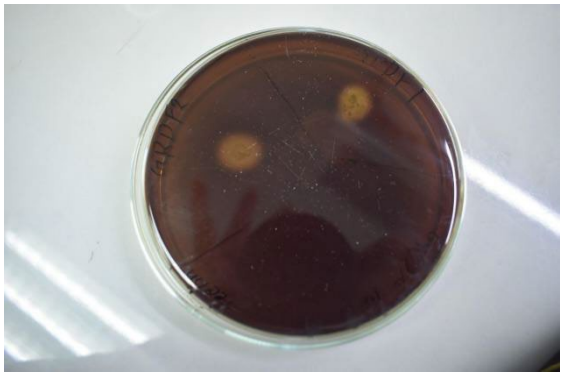

Figure 4. Pectinase Activity on Pectin Agar

Lipase Activity

Lipase activity was determined by inoculating the bacterial isolates onto lipase agar plates and incubating them at 37°C for 24 hours. After incubation, the plates were flooded with Lugol's iodine solution to visualize the formation of clear zones around the colonies. The Halo: Colony ratio was measured to determine lipase activity.

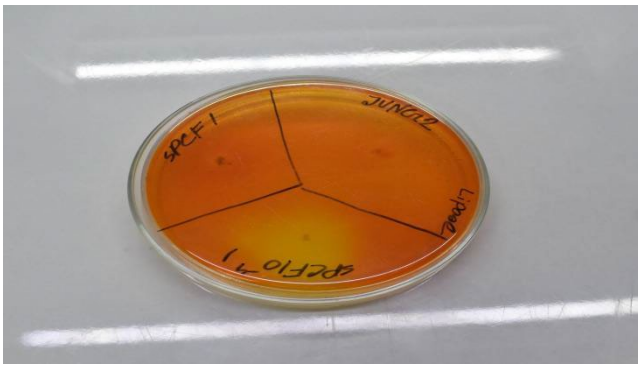

Figure 5. Lipid Hydrolysis by Bacterial Isolates

2.5. Extracellular enzyme activities

The extracellular enzyme activities (amylase, cellulase, protease, and lipase) of the isolates were also evaluated in liquid culture media. Each bacterial isolate with the highest Halo: Colony ratio was cultured in liquid broth specific to the enzyme being tested (e.g., starch liquid broth for amylase, CMC liquid broth for cellulase, casein liquid broth for protease, and lipase liquid broth for lipase) and incubated at 37°C with continuous shaking at 200 rpm for 4 days. After incubation, the supernatant was obtained by centrifugation at 10,000 g for 15 minutes at room temperature. The clear supernatant was used for further enzyme assays.

2.6. Shake flask experiments

For the determination of enzyme activity, bacterial isolates were subcultured in specific enzyme-producing broths. Amylase production was supported in a medium containing 10 g/L soluble starch, 10 g/L peptone, 20 g/L yeast extract, and 20 g/L NaCl. Cellulase production was encouraged using a medium composed of 0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, 0.2% CMC sodium salt, and 0.02% peptone. Pectinase activity was induced in a medium containing 5 g/L pectin, 0.5 g/L K_2HPO_4, 0.1 g/L $MgSO_4$, 0.2 g/L NaCl, 0.2 g/L $CaCl_2$, 0.01 g/L $FeCl_3$, and 1 g/L yeast extract. Lipase production was carried out in a medium composed of 10 g olive oil, 1 g phenol red, 1 g $CaCl_2$, and 1 mL Tween 80, while protease production was supported using a medium containing 10 g casein, 1 g KH_2PO_4, and 2 g $MgSO_4$. All bacterial cultures were incubated at 37°C with continuous shaking at 200 rpm for four days. Following incubation, the supernatant was collected by centrifugation at 10,000 g for 15 minutes and used for enzyme activity assays[12].

2.7. Statistical analysis

Enzyme activity data were presented as the average Halo: Colony ratios for each bacterial isolate. The results of the shake flask experiments were evaluated on the basis of changes in the clear zone diameters over the incubation period (days 1-5). Data analysis was performed to identify bacterial strains with the highest enzyme production potential.

3. Results

Table 1. Extracellular Enzyme Activities of Bacterial Isolates

Bacterial Strain	Amylase (mm)	CMC (mm)	Pectin (mm)	Lipase (mm)	Casein (mm)
SPCF 1	10	23	8	–	15
SPCF 10^{-4} 1	10	–	–	22	22
JUNG 2	12	20	14	–	10
GRDF 1	20	29	11	–	21
GRDF 2	25	30	12	–	21
GRDF 10^{-4} 1	25	–	–	–	20
PEDF 3	22	25	7	3	20
PEDF 10^{-3} 1	25	32	8	25	23
PLW 1AH	9	21	21	10	–
PLW 2AH	–	–	–	–	–
PLW 2BH	–	–	–	–	–
PLW 2CH	20	30	13	–	–
PLW 4AH	28	32	24	–	4
PLW 4BH	13	19	12	11	–
PLW 4CH	11	–	–	–	–
PLW 5AH	–	–	–	–	9
PLW 5BH	–	–	–	–	–
PLW 5CH	17	25	20	–	12
PLW 5EH	40	40	20	–	5
PLW 5FH	30	46	20	–	12
PLW 5GH	35	30	26	–	6
PLW 5IH	–	–	–	–	6
PLW 6AH	–	–	–	–	14
PLW 6BH	23	28	22	–	5
PLW 6CH	33	17	14	–	–
PLW 6DH	14	29	24	–	5
PLW 6EH	–	–	–	–	5
PLW 6EH W	–	–	–	–	10
PLW 6EH Y	–	–	–	–	10

Bacterial Strain	Amylase (mm)	CMC (mm)	Pectin (mm)	Lipase (mm)	Casein (mm)
PLW 6FH W	–	–	–	–	10
PLW 6FH Y	–	–	–	–	10
PLW 1E	–	–	–	–	–
PLW 3D	–	–	–	12	15
PLW 3E	–	–	–	–	–
PLW 4C	–	–	–	–	–
PLW 5A	–	–	–	–	–
PLW 5C	–	–	–	–	–
PLW 6B	–	–	–	–	–
PLW 6C	–	–	–	–	–

Table 1. (*Continued*)

This table 1 summarizes the primary screening results of extracellular enzyme activities in all bacterial isolates. Clear zones (halo diameters, mm) indicate enzyme activity on respective substrates. Strong amylase and cellulase producers included PLW 5EH, PLW 5FH, GRDF 2, and PEDF 10^{-3} 1. Moderate to weak activities were observed for pectinase, lipase, and protease. These results were used to select potential strains for secondary (shake-flask) enzyme production experiments.

Table 2. Amylase Activity during Shake-Flask Fermentation

Strain	Day 1	Day 2	Day 3	Day 4	Day 5	Day 5C
PLW 1AH	24	18	30	30	28	23
PLW 5CH	0	0	0	25	29	24
PLW 5EH	50	0	23	27	38/34	24
PLW 6CH	0	18	0	19	35/30	17
PLW 2CH	35	0	18	0	0	0
PLW 4AH	32	20	25	18	0	0
PLW 4BH	21	25	35	0	0	0
PLW 5FH	26	24	30	17	0	0
PLW 5GH	2.5	2	0	3	–	–
PLW 6BH	3	2.5	1.5	0	–	–
PLW 6DH	2	2	2	3	–	–

Amylase production varied significantly across strains and incubation days. The maximum activity was noted in PLW 5EH (50 mm on Day 1), followed by PLW 1AH and PLW 5CH. The drop after Day 2 suggests nutrient depletion or enzyme degradation. Strains maintaining activity through later days (PLW 1AH, PLW 5CH) are promising for industrial starch hydrolysis (Table 2).

Table 3. Cellulase Activity during Shake-Flask Fermentation

Strain	Day 1	Day 2	Day 3	Day 4	Day 5
PLW 1AH	4	7	0	6	17
PLW 5CH	0	0	7	0	19
PLW 5EH	0	11	0	10	22
PLW 6CH	0	5	0	6	0
PLW 2CH	0	0	0	0	0
PLW 4AH	20	20	4	5	5
PLW 4BH	18	19	3	4	4
PLW 5FH	18	19	4	5	5
PLW 5GH	2.5	2	0	3	–
PLW 6BH	3	2.5	1.5	0	–
PLW 6DH	2	2	2	3	–

PLW 5EH exhibited the highest cellulase activity (22 mm on Day 5), followed by PLW 5CH and PLW 1AH. Consistent halos from PLW 4AH and 4BH indicate stable enzyme secretion. These results confirm the potential of selected isolates in cellulose degradation, relevant for bioconversion and biofuel applications (Table 3).

Table 4. Pectinase Activity during Shake-Flask Fermentation

Strain	Day 1	Day 2	Day 3	Day 4
PLW 1AH	0	0	0	0
PLW 5CH	3	2	2	3
PLW 5EH	0	0	0	0
PLW 6CH	5	3	3	4
PLW 2CH	0	0	0	0
PLW 4AH	3	2	2	3
PLW 4BH	0	0	0	0
PLW 5FH	5	3	3	4
PLW 5GH	0	0	0	0
PLW 6BH	0	0	0	0
PLW 6DH	0	0	0	0

Pectinase activity was generally low to moderate. Notable degradation halos were observed for PLW 6CH, PLW 5FH, and PLW 5CH, with a maximum of 5 mm. This indicates potential for mild pectinolytic activity, possibly useful in fruit processing or textile applications (Table 4).

Table 5. Lipase Activity during Shake-Flask Fermentation

Strain	Day 1	Day 2	Day 3	Day 4	Day 5
PLW 1AH	11	10	13	10	0
PLW 5CH	12	11	11	9	2
PLW 2CH	10	12	13	14	5

Strain	Day 1	Day 2	Day 3	Day 4	Day 5
PLW 4BH	15	0	14	9	3
PLW 3D	0	0	0	0	0

Table 5. (*Continued*)

Lipase-producing strains included PLW 2CH and PLW 1AH, with maximum activity of 14 mm and 13 mm, respectively. These isolates demonstrate moderate lipolytic potential, useful in biodegradation and detergent enzyme research (Table 5).

Table 6. Protease Activity during Shake-Flask Fermentation

Strain	Day 1	Day 2	Day 3	Day 4
PLW 1AH	0	0	0	0
PLW 5CH	0	0	0	0
PLW 5EH	0	0	0	4
PLW 6CH	0	0	0	0
PLW 3D	0	0	0	4
PLW 6AH	0	0	0	3

Protease activity (casein hydrolysis) was weak, observed only in PLW 5EH and PLW 3D (4 mm halos). The limited activity suggests low protease secretion compared to carbohydrate-degrading enzymes. However, these strains may express protease under different nutrient or pH conditions (Table 6).

4. Discussion

The present study evaluated the extracellular enzyme activities of various bacterial isolates through primary screening (Table 1) and subsequent shake-flask fermentation experiments (Tables 2–6). The enzymes investigated included amylase, cellulase (CMC), pectinase, lipase, and protease, reflecting the isolates' potential for carbohydrate, lipid, and protein degradation.

Primary screening revealed considerable variation among bacterial isolates in their extracellular enzyme production (Table 1). Amylase and cellulase activities were strongest in isolates PLW 5EH, PLW 5FH, GRDF 2, and PEDF 10^{-3} 1, with halo diameters reaching up to 40–46 mm. These results suggest that these strains are highly capable of starch and cellulose hydrolysis, making them promising candidates for industrial applications in food processing, biofuel production, and bioconversion of plant biomass. Pectinase, lipase, and protease activities were moderate to weak across most strains. Pectinase activity was most noticeable in PLW 6CH, PLW 5FH, and PLW 5CH, while lipase production was highest in PLW 2CH and PLW 1AH. Protease activity was generally low, with only PLW 5EH and PLW 3D producing measurable halos. These findings indicate that while some isolates have potential for polysaccharide and lipid degradation, proteolytic activity may require optimized culture conditions or induction by specific substrates[13].

Shake-flask fermentation experiments revealed dynamic changes in enzyme activity over time (Tables 2–6). PLW 5EH produced the highest amylase activity on Day 1 (Table 2), but this declined in subsequent days, likely due to nutrient depletion or enzyme degradation. Strains such as PLW 1AH and PLW 5CH maintained significant activity over multiple days, suggesting stable enzyme production suitable for prolonged fermentation. Low or delayed amylase production in other strains highlights the importance of strain-specific enzyme regulation and culture conditions in maximizing yield[14].

Cellulase activity followed a similar trend (Table 3), with PLW 5EH again showing the highest activity by Day 5. PLW 5CH and PLW 1AH also demonstrated appreciable cellulolytic activity, while PLW 4AH, 4BH, and 5FH displayed moderate, consistent activity throughout fermentation. Several strains, including PLW 2CH and PLW 6CH, exhibited negligible cellulase production, indicating potential limitations or repression by medium components. High cellulase producers identified here may be leveraged for lignocellulosic biomass degradation and biofuel production[15].

Pectinase activity was generally low across all strains (Table 4), with mild hydrolysis observed in PLW 6CH, PLW 5FH, and PLW 5CH. This suggests limited but potentially useful pectinolytic activity. The overall low pectinase production indicates that these strains either do not constitutively express pectinases or that the shake-flask conditions were suboptimal for induction. Further studies with modified media or inducer supplementation could enhance pectinase secretion[16].

Lipase production was moderate (Table 5), with PLW 2CH and PLW 1AH being the most active. Other strains showed minimal or no activity, suggesting that lipase secretion is highly strain-specific. These isolates demonstrate potential for applications in biocatalysis, biodetergents, and waste lipid degradation. Protease activity was weak (Table 6), detected only in PLW 5EH, PLW 3D, and PLW 6AH. The limited proteolytic activity may be due to substrate specificity, requirement of inducers, or regulatory constraints, which could be addressed by optimizing culture conditions[17].

Overall, strains PLW 5EH, PLW 5FH, and PLW 5CH emerged as multi-enzyme producers, showing high amylase and cellulase activity with moderate pectinase production (Tables 2–4). PLW 1AH and PLW 2CH are promising for lipase production (Table 5), while protease activity remained limited across isolates (Table 6). The variability in enzyme profiles highlights the importance of selecting appropriate strains for targeted industrial applications.

5. Conclusion

Bacterial isolates exhibited diverse extracellular enzyme activities, with PLW 5EH, PLW 5FH, and PLW 5CH showing strong amylase and cellulase production, and PLW 1AH and PLW 2CH demonstrating notable lipase activity. Pectinase and protease activities were generally low, indicating the need for optimized conditions. These findings highlight the potential of selected strains for industrial enzyme applications and provide a basis for further enzyme characterization and strain improvement.

Conflict of interest

The authors declare no conflict of interest

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