

BIOPROSPECTING FOOD INDUSTRY EFFLUENTS FOR ENZYME-PRODUCING BACTERIA: MOLECULAR CHARACTERIZATION AND PRODUCTION ENHANCEMENT

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DOI: <https://doi.org/10.5281/zenodo.18691079>

Keywords

Enzyme production, food industrial effluents, *Pseudomonas granadensis*, *Bacillus cereus*, *Enterobacter cloacae*, phylogenetic analysis, optimization.

Article History

Received: 20 December 2025

Accepted: 04 February 2026

Published: 19 February 2026

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Abstract

Industrial effluents are known reservoirs of diverse microorganisms with valuable biotechnological potential. There has not been much research done in Pakistan on using bacteria from wastewater to produce enzymes. In this work, food industry effluents were collected using the direct sampling (DS) method at the Hayatabad Industrial Estate in Peshawar in order to identify, filter, and optimize bacterial strains that produce industrially significant enzymes. Out of the 23 bacterial isolates that were collected, hydrolytic zone formation demonstrated that 12 (52.17%) had protease activity, 7 (31.13%) had cellulase activity, and 6 (26.08%) had pectinase activity. Both Gram-positive and Gram-negative isolates were found by microscopic and Gram staining, and biochemical analysis showed that *Bacillus cereus*, *Pseudomonas granadensis*, and *Enterobacter cloacae* were strong makers of enzymes. 16S rRNA sequencing was used to establish the molecular identity, and phylogenetic analysis revealed 99% similarity to reference strains. According to optimization experiments, *Pseudomonas granadensis* displayed peak cellulase activity at 45 °C (0.41 absorbance), *Bacillus cereus* reached maximum protease activity at 37 °C (0.51 absorbance), and *Enterobacter cloacae* displayed optimal pectinase activity at 30 °C (0.41 absorbance). At neutral pH 7.0, all isolates showed the highest enzyme yields; production peaked after 48 hours of incubation and then began to decline. Citrus pectin increased pectinase activity (0.39 absorbance), starch modestly supported cellulase synthesis (0.26 absorbance), and glucose was the most efficient carbon source for protease generation (0.52 absorbance) among the substrates examined. Peptone facilitated the highest levels of cellulase (0.39) and protease (0.51) activity among nitrogen sources, but yeast extract enhanced the formation of pectinase (0.42). These results demonstrate the encouraging potential of bacterial isolates from wastewater as long-term sources of industrial enzymes when cultured under ideal circumstances.

Introduction

Enzymes are basically Proteins, which carry out advance vital metabolic reactions more quickly, making them biological catalysts. Throughout the food sector, textile

manufacturing, pharmaceutical development, waste treatment, and biofuel production processes, they play crucial roles. Because companies are currently looking for ecologically friendly production methods, the worldwide

enzyme market has developed steadily (Liu and Kokare, 2023). Proteases, cellulases, and pectinases are examples of industrial enzymes that are used in a variety of industries, including bioremediation, food and beverage, pulp and paper, animal feed, and textiles. According to Niyonzima (2019), they are essential components because of their propensity to reduce activation energy, provide specialized roles, and be reusable. Reduced energy requirements, safe operation, reasonable costs, and environmental protection are only a few benefits of enzyme-mediated processes. Researchers created modified microbes that could be grown in industrial production environments by combining recombinant DNA technology with protein engineering (Singh et al., 2016). Enzymes that people derived from natural sources were essential to traditional food production as well as manufacturing operations. Before learning that microbes produced better results due to their higher output, lower costs, year-round availability, and ability to generate enzymes without ethical dilemmas, people relied mostly on plants and animals for their enzyme needs. Because of their rapid growth, ease of cultivation, ability to produce goods through large-scale fermentation processes, and ability to have their genes modified, bacteria are the organism of choice for research (Sen et al., 2021). The majority of commercial enzymes are hydrolytic enzymes. Proteases dominate the enzyme market, followed by carbohydrases, which include cellulases and amylases (Kirk et al., 2002). Through genetic engineering techniques, enzyme properties such as solvent tolerance, substrate selectivity, and thermal stability are improved. Extremophilic bacteria generate extremozymes that function in harsh conditions, such as extremely high or low pH, extremely high or low pressure, and extremely high or low temperature. The lignocellulolytic and amylolytic enzymes produced by thermophilic prokaryotes have significant economic significance (Elleuche et al., 2014).

Because industrial wastewater discharge disturbs microbial habitats, it causes serious pollution issues. Acids, metals, and hydrocarbons are among the complex chemical combinations

released by the effluents (Ilyas et al., 2019). Because they transform contaminants into useful carbon sources, microorganisms are able to thrive in harmful settings. According to Selvarajan et al. (2018), microbial diversity exhibits dependence on dissolved oxygen levels, food availability, heavy metal concentrations, and the pH and salinity conditions these pollutants produce.

Due to their rapid growth, short generation time, ability to be maintained on low-cost media, and the fact that their enzymes are produced outside of cells, which facilitates processing, bacteria provide a number of advantages for the manufacture of enzymes (Deng et al., 2025). While the genetic system allows for the generation of strains with improved production capabilities, bacterial enzymes operate across wide pH and temperature ranges. Because they can grow on a variety of industrial waste sources, bacteria can be used to produce enzymes at a minimal cost (Nair et al., 2024).

Industrial enzymes reduce 700 million kg of CO₂ emissions annually and serve as environmentally acceptable alternatives to synthetic chemicals (Fasim et al., 2021). According to Thapa et al. (2019), the industrial enzyme market grew from \$0.31 billion in 1960 to \$6 billion in 2020 and is projected to reach \$9 billion by 2027. The worldwide food enzyme market is expected to reach USD 3.3 billion by 2026, with the food industry using over 55% of industrial enzymes to support baking, beverage, dairy, and juice processing processes (Okpara et al., 2022).

Biofuel is created when cellulases hydrolyze cellulose to convert it to glucose (Srivastava et al., 2018). Their capacity to soften fruits and clear fluids while extracting tastes enhances food processing (Jayasekara et al., 2019). Cellulases are used in the textile industry for both biopolishing and biostoning. Cellulases are used in detergents to keep fabrics bright and in the paper industry to increase brightness while conserving 20–40% of energy (Bhardwaj et al., 2021). By weakening cell walls, cellulases enhance fruit juice processing by achieving complete liquefaction, reduced viscosity, and improved filtration outcomes (Ejaz et al., 2021).

Approximately 60% of all industrial enzymes produced worldwide are proteases (Mamo et al., 2018). Generally speaking, microbial proteases are preferable to those found in plants or animals (Raveendran et al., 2018). Produce protein hydrolysates, tenderize meat, and work on food to modify proteins (Razzaq et al., 2019). Proteases aid in enzyme-based hair removal during the leather-making process, which is a more environmentally friendly alternative to harsh chemicals (Song et al., 2023). Medical work involves investigating cancer, managing biofilms, and healing wounds (Ahmad et al., 2019). Lipases aid in the breakdown of triglycerides and are really utilized in the production of margarine and cheese tastes (Chandra et al., 2020).

Achieving optimal enzyme levels requires achieving optimal dietary and physical conditions. Microbial metabolism is significantly impacted by temperature, and *Bacillus cereus* appears to produce a good amount of proteases at temperatures between 35 and 40°C (Patel et al., 2023). The majority of microorganisms appear to prefer a neutral to possibly slightly alkaline environment, and maintaining a medium pH helps balance out charged ions. Hydrolytic enzymes appear to function best after 24 to 72 hours, and the length of time you incubate things dictates how long your culture develops before nutrients run out (Bharathi et al., 2019). In fact, the choice of carbon source has a significant impact on the creation of enzymes; certain substrates aid in their synthesis, whereas readily fermentable sugars largely inhibit it. The essential amino acids are provided by nutrient sources, however organic sources typically somewhat increase production (Ja'afar et al., 2021).

Enzyme synthesis is made economically feasible for commercial quantities by optimization. The amount you get back is actually greatly influenced by factors such as pH, temperature, incubation duration, mixing intensity, and nutrient levels (Singh et al., 2016). Industries begin to experience decreased productivity and possibly even higher costs in the absence of optimization. Certain statistical techniques, including Response Surface Methodology (RSM), aid in determining the ideal circumstances, increasing

yields and reducing expenses. Process optimization promotes sustainable food production and yields consistent results (Haleem et al., 2023).

The food industry in Pakistan is expanding rapidly, producing a large amount of organic waste and untreated wastewater. Reducing pollution and obtaining some renewable enzymes are two benefits of really managing those wastewaters to separate beneficial microbial strains. In fact, there has not been much recent effort to isolate, perform molecular testing, and optimize enzyme-producing bacteria from Pakistan's food sector waste. By providing native strains capable of producing high-value enzymes that are both cost-effective and well-optimized, closing this gap could benefit nearby industry.

METHODOLOGY

Study Design

The research study used an experimental laboratory-based study design. The purpose of this study was to isolate and identify enzyme-producing bacterial strains from food industry effluents and evaluate their potential through closely watched laboratory testing. The study was carried out at the Sarhad Institute of Allied Health Sciences, SUIT, Peshawar, Microbiology Laboratory.

Study Site/period

Samples of wastewater from food processing firms that release effluents were gathered for the investigation. The entire study project took ten months to finish, from October 2024 to June 2025.

Sample Collection

Several food industries located in Peshawar's Hayatabad Industrial Estate provided samples of their industrial effluents. These samples were utilized for both later physicochemical analysis and bacterial isolation. Samples were collected in sterile 50 mL screw-capped plastic containers. After that, the containers were taken to the lab and sealed in sterile bags. Following appropriate labeling, the samples were sent to Sarhad University of Science and Information

Technology's Microbiology Laboratory in Peshawar, where they were kept at 4 °C until they could be processed further.

Serial dilution

Serial dilution was used to reduce the microbial load of the food industrial effluent samples that were collected and produce isolated colonies of bacterial strains. A 1 mL aliquot of each effluent sample was aseptically transferred into 9 mL of sterile distilled water in a test tube to achieve a 10^{-1} dilution. This process was carried out one after the other to prepare dilutions up to 10^{-5} . 0.1 mL of each dilution was equally spread out onto nutrient agar plates using a glass spreader that had been sterilized. For 24 to 48 hours, the plates were incubated at 37°C. In order to isolate and purify the bacterial strain, well-isolated colonies from the higher dilutions (usually 10^{-2} to 10^{-5}) were selected based on distinctive morphological traits and subcultured.

Detection of enzyme Activities of Bacteria

All purified bacterial isolates were screened for cellulase, protease, and pectinase enzyme production.

Cellulase Activity Assay

The cellulose agar plate assay was used to assess the synthesis of cellulase in all pure bacterial isolates. Cellulase agar plates, which were created by fortifying minimum media with 1% (w/v) cellulose, were spot streaked with each bacterial isolate. For forty-eight hours, the plates were incubated at 37 °C. Following incubation, Gram's iodine solution was added to the plates, and they were left to sit at room temperature for five minutes. Positive cellulase activity was shown by the formation of discrete hydrolysis zones surrounding the bacterial colonies on a dark blue background (Mg et al., 2015).

Protease Activity Assay

Skimmed milk agar plates were used to measure the bacterial isolates' production of protease. In order to serve as the protein supply, 1% (w/v) skim milk was added to minimal medium. Under aseptic conditions, each isolate was spot streaked over the agar surface and cultured for 24 to 48 hours at 37 °C. Proteolytic hydrolysis of the

skimmed material was demonstrated by the development of discrete clear zones surrounding the bacterial colonies, hence verifying the presence of active protease enzymes. (Sinha and others, 2013).

Pectinase Activity Assay

Pectin agar plates, which were made by mixing 1% (w/v) citrus pectin with minimal medium, were used to measure pectinase activity. After being spot-applied to the pectin agar plate surface, the bacterial isolates were incubated for 24 to 48 hours at 37 °C. Gram's iodine solution, which stains un-hydrolyzed pectin, was added to the plates after they had been incubated. Pectin hydrolysis and, consequently, pectinase activity were shown by the development of clear zones surrounding the colonies (Bibi et al., 2019).

Crude Enzyme Assay

Crude enzyme activity assays were used to find the most effective bacterial isolates for additional characterisation prior to biochemical testing. Dinitrosalicylic Acid (DNS) was used to measure the crude enzyme activity. The crude enzyme-containing supernatant was obtained by centrifuging the culture broth at 7000 rpm for 10 minutes after bacterial cultures were cultivated in production media under normal conditions.

Enzyme Activity Measurement

The amount of reducing sugars released as a result of enzymatic hydrolysis was measured using the DNS method. In short, 50 mM phosphate buffer (pH 7.0) was combined with 0.5 mL of crude enzyme solution and 0.5 mL of 1% (w/v) substrate solution (specific to the enzyme being tested). For half an hour, the reaction mixture was incubated at the ideal temperature. Following incubation, the tubes were boiled for five minutes and 1 mL of DNS reagent was added to halt the reaction. A UV-Vis spectrophotometer was used to measure the absorbance at 540 nm after the sample had cooled to room temperature.

Microscopic Identification

Following the guidelines in Bergey's Manual of Determinative Bacteriology, Gram staining and a

few biochemical assays, including those for catalase, oxidase, indole, and motility, were carried out.

Phylogenetic Analysis

Pure culture plates of certain bacterial isolates were made, packaged, and shipped to Macrogen Inc. in South Korea for 16S rRNA gene sequencing in order to perform phylogenetic analysis. To guarantee sequence quality, FinchTV was first used to view and edit the obtained raw chromatogram files. CodonCode Aligner was used to further clean, assemble, and align the sequences in order to provide consensus sequences. To find homologous sequences and identify the closest relatives, these sequences were run using the NCBI database's BLAST program. A phylogenetic tree was built in MEGA 11 software by following standard procedures: importing aligned FASTA sequences, choosing the best-fit nucleotide substitution model, and building the tree using the neighbor-joining (NJ) method with 500 bootstrap replications to test the robustness of the inferred evolutionary relationships. ClustalW was used to perform multiple sequence alignment of the obtained sequences with reference strains.

Optimization of culture conditions for optimum enzyme production

The one-variable-at-a-time (OVAT) method was used to apply controlled modifications to a selection of bacterial isolates. To guarantee reproducibility, each experiment was carried out in triplicate, and the DNS approach was used to measure enzyme activity in each instance.

Temperature Optimization

After being injected into production media, the bacterial isolates were cultured for 48 hours at various temperatures (25°C, 30°C, 37°C, and 45°C). After centrifuging the culture supernatants for ten minutes at 7000 rpm, the DNS test was used to measure the enzyme activity. For the synthesis of enzymes, the temperature that produced the maximum DNS

absorbance was thought to be ideal.

PH Optimization:

Prior to sterilization, the manufacturing medium's pH was changed using 1N HCl or 1N NaOH to 5.0, 6.0, 7.0, 8.0, and 9.0. After being inoculated, each flask was incubated for 48 hours at the ideal temperature that had been previously established. The DNS method was used to measure the enzyme activity in the culture supernatant, and the ideal pH was defined as the one that produced the greatest amount of reducing sugar release.

Incubation Period Optimization:

Samples were taken out of the flasks after 12, 24, 48, and 72 hours of incubation at the ideal pH and temperature. The DNS assay was used to measure the enzyme activity following centrifugation. The optimal time for maximal enzyme yield was determined to be the incubation period with the highest DNS absorbance.

Carbon source optimization

The basal medium was supplemented with glucose, sucrose, lactose, starch, and maltose (1% w/v) in order to identify the optimal carbon source. Flasks that had been inoculated were incubated at 150 rpm for the ideal duration, temperature, and pH. After incubation, the supernatants were gathered, and the DNS method was used to measure the enzyme activity. The ideal carbon source was determined to be the one that produced the highest level of enzyme activity.

Optimization of Nitrogen source

Peptone, yeast extract, ammonium sulfate, urea, and sodium nitrate (0.5% w/v) were all added separately to the optimized medium with the optimum carbon source in order to optimize the nitrogen source. The DNS test was used to measure the enzyme activity in the supernatant after the cultures were incubated under previously ideal physical conditions. The most advantageous nitrogen supply for the synthesis of enzymes was found to support the highest DNS

absorbance value.

Analysis of Data

This research study's data analysis was carried out using Microsoft Excel 2016. The software was used to create graphical representations of enzyme activity under various optimization circumstances, create bar charts, and arrange

experimental results.

RESULTS

Sample Collection

Bacterial isolation from wastewater samples obtained from food manufacturers in Peshawar's Hayatabad Industrial Estate was accomplished with success. There were 23 different bacterial colonies in all.

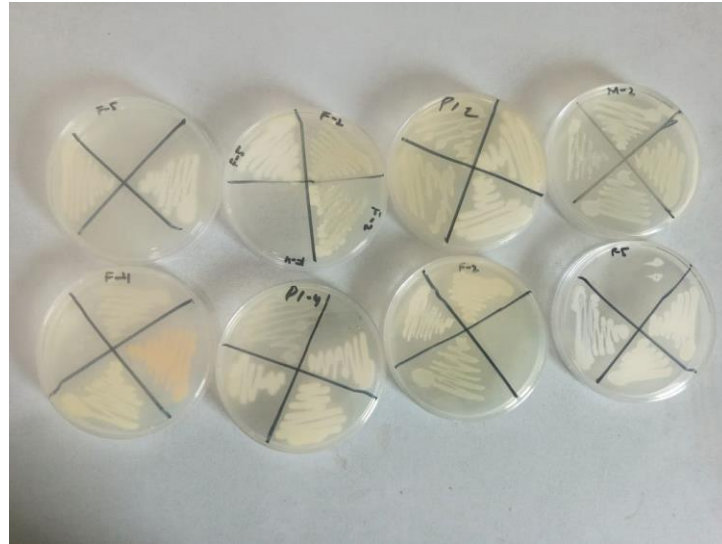


Fig 1: Microbiological Investigation of Bacterial Isolates from Food Processing Effluents

Screening for Enzyme Production

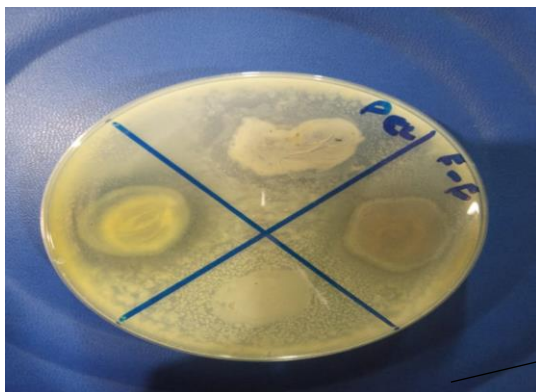
Differential synthesis of the enzymes cellulase, protease, and pectinase was found by assessing the enzyme activity of bacterial isolates. Four (17.39%) of the 23 isolates that were tested had positive cellulase activity, with clear zones that ranged from 11 ± 0.4 mm to 13 ± 0.6 mm. Six

isolates (26.08%) exhibited protease activity, resulting in hydrolysis zones ranging from 14 ± 0.4 mm to 20 ± 0.7 mm. In contrast, pectinase activity was found in just three isolates (11.04%), with zone widths ranging from 10 ± 0.3 mm to 11 ± 0.4 mm.

Table 1: Measurement of Hydrolysis Zones (mm) in Cellulase, Protease, and Pectinase Producing Bacterial Isolates

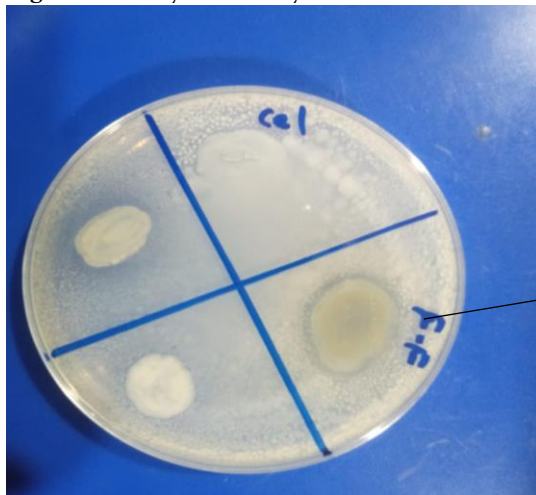
S.no	Cellulase Zone (mm)	Protease Zone (mm)	Pectinase Zone (mm)
B1	12 ± 0.5	18 ± 0.6	–
B2	11 ± 0.4	–	–
B3	–	16 ± 0.5	–
B4	–	–	–
B5	–	–	–
B6	–	19 ± 0.6	–
B7	12 ± 0.5	–	–
B8	–	15 ± 0.5	10 ± 0.3
B9	–	14 ± 0.4	–
B10	–	–	–

B11	-	16 ± 0.5	-
B12	-	-	-
B13	-	-	-
B14	-	-	-
B15	13 ± 0.6	17 ± 0.5	11 ± 0.4
B16	-	-	-
B17	-	-	-
B18	-	-	-
B19	11 ± 0.2	-	-
B20	-	-	-
B21	-	17 ± 0.6	-
B22	-	-	-
B23	-	20 ± 0.7	10 ± 0.3
	Positive Isolates 4 (31.13%)	Positive Isolates 6 (52.17%)	Positive Isolates 3 (26.08%)



Strain name: *B. cereus*
Strain no: B3
Zone: 16mm

Fig 2: Proteolytic activity in the bacterial isolates.



Strain name: *P. granadensis*
Strain no: B2
Zone: 11mm

Fig 3: Bacterial colonies exhibited detectable cellulase activity.
Gram Staining

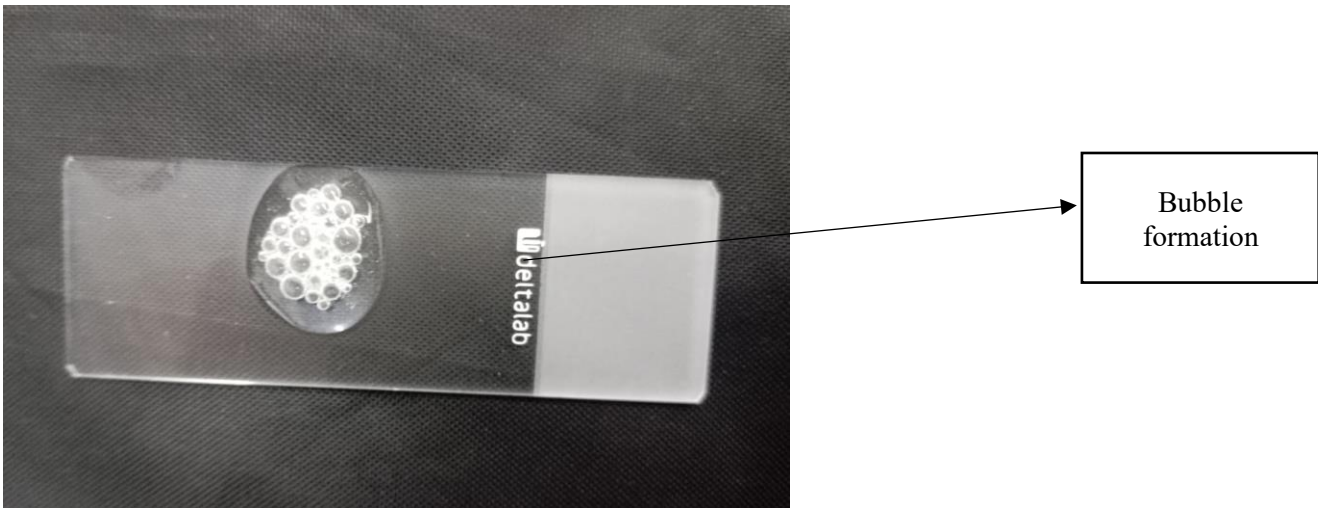


Fig 4: Microscopic examination following Gram staining confirmed a Gram-negative isolate.

Biochemical Tests

Table 2: Characterization of Selected Bacterial Isolates Based on Biochemical Tests

Organism	Catalase	Indole	Motility	Oxidase
<i>Bacillus cereus</i>	+	-	+	-
<i>Pseudomonas granadensis</i>	+	-	+	+
<i>Enterobacter cloacae</i>	+	-	+	-



Bubble
formation

Fig 5: Catalase Test Results of the Bacterial Isolates

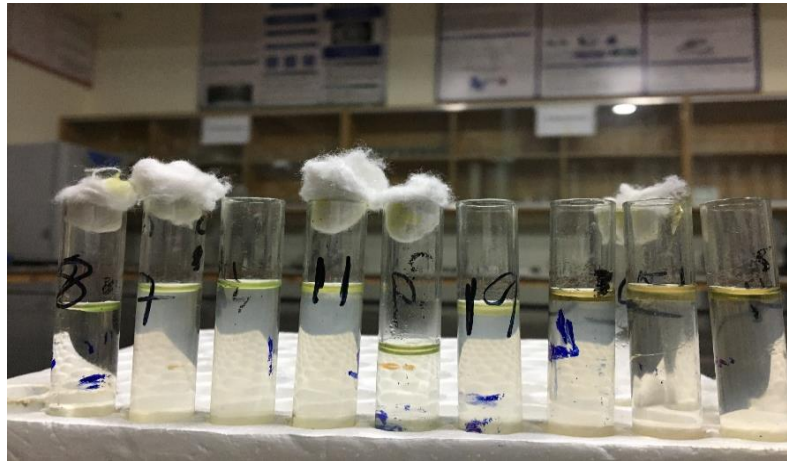


Fig 6: Diagrammatic demonstration of Indol test



Fig 7: Motility test results demonstrated by selected isolates

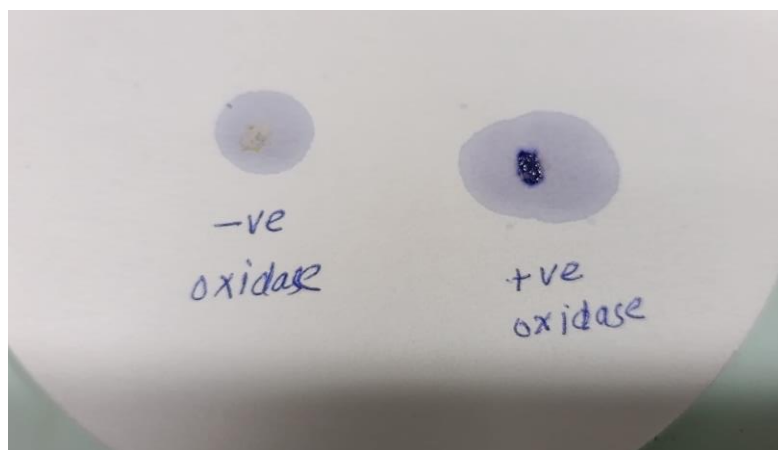


Fig 8: Oxidase test of the targeted isolates.

Phylogenetic Analysis

Six *P. granadensis*, three *Bacillus cereus*, and three *Enterobacter cloacae* were among the twelve bacterial isolates that were collected. 16S

rRNA sequencing was performed on one representative isolate from each of the three bacterial species based on this distribution. According to the 16S rRNA sequence analysis,

there was 99% similarity with the *Enterobacter cloacae*, *Bacillus cereus*, and *P. granadensis*

shown in figures 4.5, respectively.

Primer Information

Sequencing Primer Name Primer Sequences						PCR Primer Name Primer Sequences			
785F 5' (GGA TTA GAT ACC CTG GTA) 3'						27F 5' (AGA GTT TGA TCM TGG CTC AG) 3			
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'						1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'			

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
LT629778.1	<i>Pseudomonas granadensis</i>	5943170	465629	467098	0	2654	0.0	1459/1470	99

Kingdom	Family	Genus	Species
Bacteria	Pseudomonadaceae	<i>Pseudomonas</i>	<i>Pseudomonas granadensis</i>

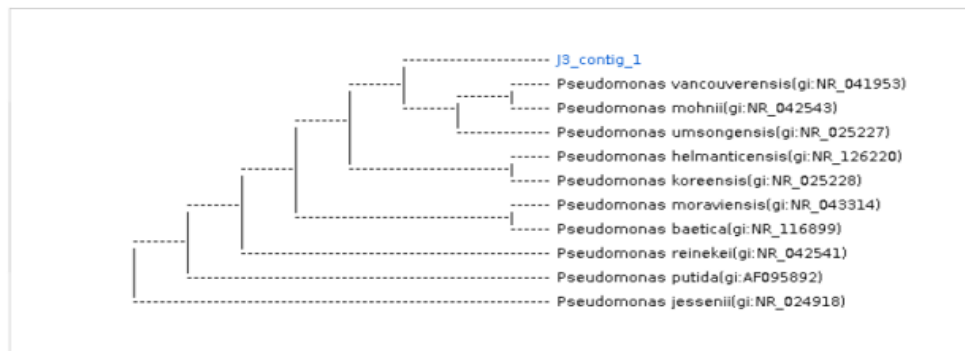


Fig 9: Phylogenetic placement of J3-contig showing close resemblance with *Pseudomonas granadensis*.

Primer Information

Sequencing Primer Name Primer Sequences						PCR Primer Name Primer Sequences			
785F 5' (GGA TTA GAT ACC CTG GTA) 3'						27F 5' (AGA GTT TGA TCM TGG CTC AG) 3			
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'						1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'			

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
CP001918.1	<i>Enterobacter cloacae</i>	5314581	1015780	1017255	0	2704	0.0	1473/1477	99

Kingdom	Family	Genus	Species
Bacteria	Enterobacteriaceae	<i>Enterobacter</i>	<i>Enterobacter cloacae</i>

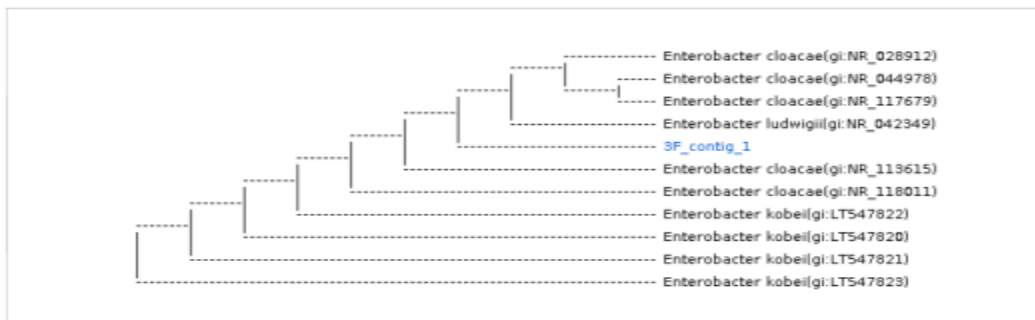


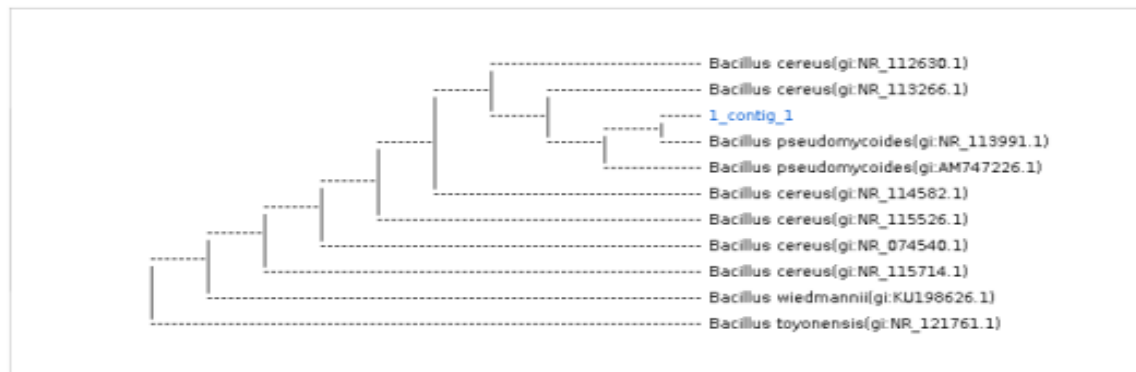
Fig 10: Phylogenetic placement of 3F-contig showing close resemblance with *Enterobacter cloacae*.

Primer Information

Sequencing Primer Name	Primer Sequences	PCR Primer Name	Primer Sequences
785F	5' (GGA TTA GAT ACC CTG GTA) 3'	27F	5' (AGA GTT TGA TCM TGG CTC AG) 3'
907R	5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R	5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
NR_074540.1	Bacillus cereus	1512	25	1503	97	2721	0.0	1477/1479	99

Kingdom	Family	Genus	Species
Bacteria	Bacillaceae	Bacillus	Bacillus cereus



Phylogenetic placement of 1-contig-1 showing close resemblance with *Bacillus cereus*.

Optimization of Culture Conditions

Temperature Optimization

The four selected isolates' enzyme activity was examined at 25, 30, 37, and 45 degrees Celsius. The findings show that 37°C (0.51 absorbance)

was the ideal temperature for *Bacillus cereus* to produce protease, 45°C (0.41 absorbance) for *Pseudomonas granadensis* to produce cellulase, and 30°C (0.41 absorbance) for *Enterobacter cloacae* to produce pectinase.

Table 3: Temperature optimization of selected bacterial isolates

Bacteria	Temperature: 25 °C	Temperature: 30 °C	Temperature: 37 °C	Temperature: 45 °C
<i>B. cereus</i> (Protease)	0.22ug/ml	0.36ug/ml	0.51ug/ml	0.41ug/ml
<i>P. granadensis</i> (Cellulase)	0.18ug/ml	0.28ug/ml	0.33ug/ml	0.41ug/ml
<i>E. cloacae</i> (Pectinase)	0.22ug/ml	0.41ug/ml	0.36ug/ml	0.30ug/ml

PH Optimization

At pH 7.0, the enzyme activity of all three bacterial isolates was at its peak. At this pH, the highest absorbance (0.41) was observed by *Bacillus cereus* (Protease), the highest activity

(0.36) by *Pseudomonas granadensis* (Cellulase), and the highest activity (0.28) by *Enterobacter cloacae* (Pectinase). At both acidic and alkaline pH values, activity declined.

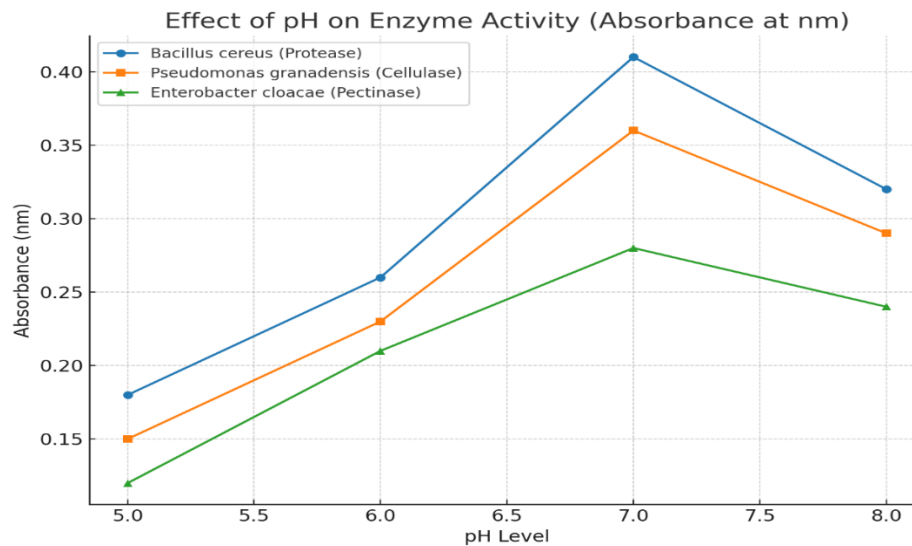


Fig 13: Effect of pH optimization on Enzyme Activity of bacterial isolates

Incubation Period Optimization

After 48 hours of incubation, the three bacterial isolates showed their highest levels of enzyme synthesis. The highest activity was reported by *Bacillus cereus* (Protease) at 0.41, followed by *Pseudomonas granadensis* (Cellulase) at 0.36,

and *Enterobacter cloacae* (Pectinase) at 0.28. After 48 hours, there was a slow drop in enzyme activity, with less production at 72 and a significant drop at 96 hours. This was probably caused by feedback inhibition and food depletion.

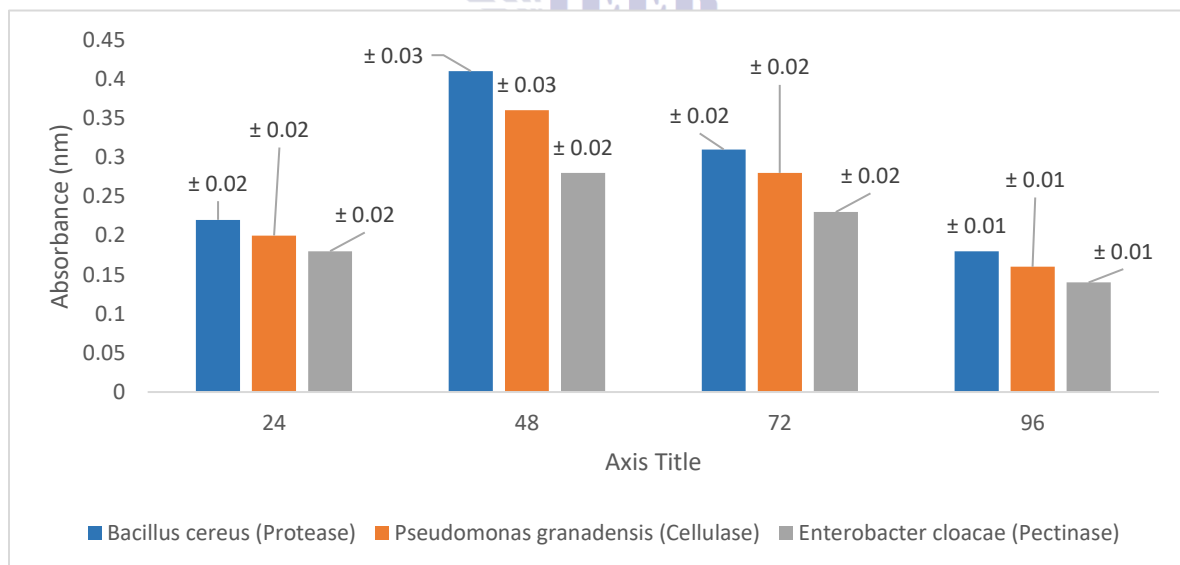


Fig 14: Effect of Different incubation periods on Enzyme Activity of bacterial isolates measured through absorbance at 540 nm.

Effect of Carbon Source

Bacillus cereus (Protease) demonstrated the highest absorbance (0.52) among the carbon sources that were evaluated, and glucose

supported the highest enzyme production. When cultivated on starch, *Pseudomonas granadensis* (Cellulase) produced the least amount of enzyme (0.26), but *Enterobacter cloacae* (Pectinase) on

citrus pectin showed moderate activity (0.39). This suggests that among the studied isolates,

glucose is the most advantageous carbon source for the highest enzyme output.

Table 6: Enzyme activities of *Bacillus cereus*, *Pseudomonas granadensis* & *Enterobacter cloacae* utilizing different carbon sources.

Isolate	Carbon Source	Enzyme	Absorbance (540 nm)
<i>Bacillus cereus</i>	Glucose	Protease	0.52
<i>Enterobacter cloacae</i>	Citrus pectin	Pectinase	0.39
<i>Pseudomonas granadensis</i>	Starch	Cellulase	0.26

Effect of Nitrogen Source

Ammonium chloride, peptone, and yeast extract were among the nitrogen sources whose effects were assessed. Maximum cellulase and protease

production was enhanced by peptone, but pectinase activity was best promoted by yeast extract.

Table 7: Effect of different nitrogen sources on enzyme production of *Bacillus cereus*, *Pseudomonas granadensis* & *Enterobacter cloacae*.

Isolate	Nitrogen Source	Enzyme	Absorbance (540 nm)
<i>Bacillus cereus</i>	Peptone	Protease	0.51
<i>Pseudomonas granadensis</i>	Peptone	Cellulase	0.39
<i>Enterobacter cloacae</i>	Yeast Extract	Pectinase	0.42

DISCUSSION

The goal of the current study was to investigate, describe, and improve bacterial strains that produce enzymes that were separated from food industry effluents. The organic matter-rich effluents released by food processing businesses create an ideal habitat for the development of a variety of microbial communities with possible biotechnological uses. Because they can manufacture a variety of industrially useful enzymes, including cellulases, pectinases, and proteases, enzyme-producing bacteria have become more important among them. The most promising isolates were identified by molecular characterisation, and optimization experiments were conducted to increase their enzyme production in controlled environments. The results of this study shed light on the biochemical performance of the bacterial isolates from industrial effluents as well as the impact of several environmental and cultural factors on enzyme yield. They also demonstrate the potential of

these isolates as a sustainable source of enzymes. Twelve (52.17%) of the 23 different bacterial isolates that were obtained produced protease, nine (31.13%) produced cellulase, and six (26.08%) produced pectinase. The high frequency of protease-producing bacteria is in line with Gimenes et al.'s (2021) findings that proteolytic bacteria predominate in samples of industrial waste. Likewise, Anwar et al. (2020) discovered that the presence of complex organic materials in industrial effluents results in a variety of enzyme producers. The nature of food industry waste in this study area may be richer in polysaccharides as well as proteins than pectin-rich substrates, which could explain the lower proportion of pectinase producers here, in contrast to Haile et al. (2022), who discovered that pectinase producers were more prevalent in fruit processing effluents.

Protease, cellulase, and pectinase were found to be strongly produced by the strains *Pseudomonas granadensis*, *Bacillus cereus*, and *Enterobacter*

cloacae, respectively. These results are consistent with those of Kućuk et al. (2024), who found that *Bacillus* species produce a lot of proteases. *Pseudomonas granadensis*'s cellulase activity confirms the findings of Bakri et al. (2021), who identified *Pseudomonas* strains as effective cellulase makers. While other research, such as Riseh et al. (2024), have described *Bacillus* and *Aspergillus* species as more dominant pectinase producers than *Enterobacter* spp., it is interesting to note that *Enterobacter cloacae* is exhibiting substantial pectinase activity coincides with Patel et al. (2023). Environmental changes or variations in the substrate availability in the effluent sample could be the cause of this discrepancy.

Each isolate had a different ideal temperature for enzyme activity: *Pseudomonas granadensis* produced the most cellulase at 45°, *B. cereus* displayed the highest protease activity at 37°C, and *Enterobacter cloacae* produced the most pectinase at 37°C (3.2 U/mL). These results are consistent with those of Ullah et al. (2022), who found that *Bacillus cereus* is mesophilic and produces proteases best at temperatures between 35 and 40°C. Similarly, Gomez et al. (2021) showed optimal cellulase synthesis at higher mesophilic to lower thermophilic ranges (40–50°C), which is consistent with *Pseudomonas* species exhibiting increased cellulase output at 45°C. The optimal temperature for *Pseudomonas* cellulase, according to certain research like Moyo et al. (2022), is 37°C, suggesting that strain-specific variations or adaptation to local conditions may exist. The temperature at which *Enterobacter cloacae* produced pectinase was 30°C (0.41 absorbance). This is consistent with findings from Alkurtany et al. (2023), who discovered that some *Enterobacter* strains produced pectinase most effectively at 30°C, presumably as a result of variations in the medium composition or incubation circumstances. At neutral pH 7.0, all isolates produced their maximum amount of enzymes. This result confirms the findings of Khaswal et al. (2024), who found that the optimal pH range for bacterial cellulase and protease activity is neutral to slightly alkaline. Additionally, *Bacillus* cultures

flourish at neutral pH for optimal protease synthesis, according to Le Marc et al. (2021). Nonetheless, research has indicated that certain species of *Bacillus* and *Enterobacter* can withstand a slightly acidic pH in order to produce pectinase. Compared to strains investigated in fruit juice or silage fermentation contexts, these isolates appear to be less acidophilic based on the decreased activity seen at pH 5.0 in our investigation.

For all isolates, the enzyme activity maximum at 48 hours and then started to drop. This is in line with Inan Bektas et al. (2023), who found that *Bacillus* and *Enterobacter* strains produced the most enzymes in 48–72 hours as a result of ideal growth & nutrient availability. According to Guta et al. (2024), the decline in yields after 72 hours is probably caused by nutrient depletion and the buildup of metabolic byproducts, which leads in feedback inhibition. Nonetheless, Sanjaya et al. (2024) found that some thermophilic *Bacillus* strains maintained high enzyme output for up to 96 hours, indicating that the length of incubation might vary greatly depending on the strain type and medium composition.

Peptone was shown to be the most efficient nitrogen source for increasing the synthesis of both cellulase and protease. In particular, *Pseudomonas granadensis* showed increased cellulase production with an absorbance of 0.39 when cultured on peptone, but *Bacillus cereus* shown significant protease activity with an absorbance of 0.51. On the other hand, *Enterobacter cloacae* demonstrated a significant absorbance of 0.42, indicating that yeast extract was the most advantageous for pectinase synthesis. These results are consistent with recent research highlighting the significance of organic nitrogen sources in the synthesis of enzymes. For example, a study conducted in 2021 showed that peptone greatly promotes the development of protease in fungi, but inorganic sources such as ammonium sulfate can actually inhibit it (Sun et al., 2021).

Conclusion

The paper emphasizes how food industry

effluents were successfully used to isolate, characterize molecularly, and optimize bacterial populations that produce enzymes. Protease, cellulase, and pectinase production was shown to be significantly possible in *Pseudomonas granadensis*, *Bacillus cereus*, and *Enterobacter cloacae*. Optimization tests revealed that culture conditions had a significant impact on enzyme yields. With glucose and peptone serving as the best carbon and nitrogen sources, protease activity peaked at 37°C. When starch and peptone were present, cellulase synthesis peaked at 45°C. Citrus pectin and yeast extract were used to increase pectinase synthesis at 37°C. For all three enzymes, a 48-hour incubation period and a neutral pH of 7.0 were ideal. It proves that these wastewaters are a good source of a variety of microorganisms that can make important enzymes for industry. The enzyme yield was greatly increased by improving growth conditions, demonstrating these strains' useful potential for economical and sustainable commercial enzyme production. These results demonstrate that waste streams from the local food industry can provide important supplies of bacteria that produce relevant enzymes for industry. The viability of employing these isolates in upcoming biotechnological and industrial applications is further supported by their emphasis on how simple parameter optimization may greatly increase enzyme yields.

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