Isolation and Characterization of Xylanase-Producing *Geotrichum candidum* and Its Application in Bioethanol Production from Bajra (Pearl Millet)

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Abstract - The identification of robust enzyme-producing microorganisms remains a key step in improving lignocellulosic biomass conversion processes. In this study, xylanase-producing yeasts were isolated from multiple environmental sources including soil, compost, fruit waste, and sugarcane bagasse collected from Raipur, Chhattisgarh, India. Among these, fruit waste and compost samples showed comparatively higher yeast abundance, suggesting that carbohydrate-rich microenvironments favor the proliferation of xylan-degrading species. Following primary screening, several isolates demonstrated xylanolytic potential; however, *Geotrichum candidum* consistently exhibited the highest enzymatic activity under laboratory conditions. Optimization experiments indicated that xylanase production and activity were optimal at pH 5 and 40°C, reflecting suitability for mildly acidic biomass processing systems. The crude enzyme preparation was subsequently applied for the hydrolysis of bajra (pearl millet) biomass. The released fermentable sugars were subjected to anaerobic fermentation for ethanol production. After distillation, ethanol concentrations ranged between 20 and 24 g/L. Fourier Transform Infrared (FTIR) analysis confirmed the characteristic functional groups corresponding to ethanol, and High-Performance Liquid Chromatography (HPLC) showed a prominent ethanol peak at a retention time of approximately 4.39 minutes, indicating substantial purity of the distillate. The findings demonstrate the enzymatic efficiency of *G. candidum* in xylan degradation and support the utilization of bajra as a viable lignocellulosic substrate for bioethanol production. The study provides an integrated framework linking microbial enzyme production with downstream biofuel generation, contributing to sustainable biomass valorization strategies.

Keywords: Xylanase-producing yeast, *Geotrichum candidum*, Bajra (Pearl millet), Bioethanol production, Lignocellulosic biomass valorisation.

I. INTRODUCTION

The growing demand for sustainable energy has intensified efforts to identify alternatives to fossil fuels. Continued reliance on petroleum-based resources, coupled with rapid industrial expansion and population growth, has not only accelerated resource depletion but also amplified environmental challenges such as greenhouse gas accumulation and climate instability (Yemets et al., 2020). In response to these concerns, bioethanol has emerged as a practical and scalable renewable fuel option. Its biodegradability, relatively lower carbon emissions, and compatibility with existing fuel infrastructure make it an attractive substitute for conventional fuels. Among the various feedstocks available for bioethanol production, lignocellulosic biomass has received particular attention. Unlike first-generation biofuel substrates, lignocellulosic materials are abundant, cost-effective, and do not directly compete with food supplies (Khalaji et al., 2020). However, their structural complexity presents a significant technical barrier. The tight association between cellulose, hemicellulose, and lignin creates a recalcitrant matrix that resists enzymatic breakdown. Hemicellulose, which constitutes a substantial portion of this matrix, is rich in xylan — a branched heteropolysaccharide primarily composed of xylose units (Zerva et al., 2020). Efficient conversion of xylan into fermentable sugars is therefore essential for improving overall biomass saccharification efficiency. Xylanases (EC 3.2.1.8) play a central role in this process by cleaving the β-1,4-glycosidic linkages of xylan, generating xylo-oligosaccharides and xylose. Their activity enhances cellulose accessibility and reduces the intensity of chemical pretreatment required for biomass processing (Bouiche et al., 2020; Sharma et al., 2020). From a process engineering perspective, this reduction in pretreatment severity can lower operational costs and environmental impact, making enzymatic hydrolysis more attractive for industrial applications. Microbial systems remain the primary source of industrial xylanases. Although bacteria and filamentous fungi have been extensively investigated, yeasts are gaining interest for biomass conversion processes. Their rapid growth kinetics, relative ease of cultivation, and tolerance to fermentation-related stresses offer practical advantages in integrated bioprocessing systems (Jamaldheen et al., 2021). Additionally, certain yeast-derived enzymes exhibit functional stability across broader pH and temperature ranges, which is desirable for lignocellulosic hydrolysis. Natural habitats rich in decaying organic matter — such as compost, fruit residues, agricultural soils, and agro-industrial wastes — provide promising environments for isolating potent xylanase-producing yeasts (Blessie et al., 2021). Among reported species, *Geotrichum candidum* has demonstrated notable enzymatic versatility. Its adaptability to diverse substrates and environmental conditions suggests potential suitability for lignocellulosic biomass conversion. Nevertheless, systematic evaluation of *G. candidum*-derived xylanase in the context of bioethanol production from regionally available cereal biomass remains limited (Reddy & Reddy, 2021).

Bajra (pearl millet, *Pennisetum glaucum*) represents one such underutilized biomass resource. Widely cultivated in arid and semi-arid regions of India, bajra is valued for its drought tolerance and low input requirements (Katiyar et al., 2021). Beyond its role as a staple crop, significant quantities of residual biomass are generated during cultivation and processing. This biomass contains appreciable amounts of hemicellulose, making it a promising substrate for bioethanol production. Valorization of bajra residues could therefore support both sustainable waste management and rural bioeconomy development. The bioconversion of lignocellulosic biomass into bioethanol typically involves pretreatment, enzymatic hydrolysis, fermentation, and product recovery. Within this sequence, efficient enzymatic hydrolysis directly influences fermentable sugar availability and subsequent ethanol yield (Melati et al., 2021). Following fermentation, analytical confirmation of ethanol production is essential. Techniques such as Fourier Transform Infrared (FTIR) spectroscopy and High-Performance Liquid Chromatography (HPLC) enable structural verification and quantitative assessment of ethanol purity (Melati et al., 2023).

Despite extensive research on lignocellulosic bioethanol, there remains a practical need for region-specific, integrated strategies that combine locally isolated enzyme producers with locally available biomass resources (Lamichhane et al., 2023). Such alignment can reduce dependency on commercial enzyme formulations and enhance process sustainability. Therefore, the present study aimed to isolate xylanase-producing yeasts from diverse environmental samples collected in Raipur, Chhattisgarh, India, identify the most efficient isolate, and evaluate its enzymatic potential. The study further investigated optimization of xylanase activity with respect to pH and temperature and assessed the applicability of the crude enzyme for hydrolysis of bajra biomass. The resulting hydrolysate was subjected to anaerobic fermentation for bioethanol production, followed by ethanol recovery and characterization using FTIR and HPLC analyses (Nanda et al., 2024; Fatima et al., 2024).

II. MATERIALS AND METHODS

Sample Collection:

Environmental samples were obtained from multiple sites within Raipur, Chhattisgarh, India, to isolate potential xylanase-producing yeasts. The selected sampling sources included agricultural soil, compost heaps, decomposing fruit waste, and sugarcane bagasse collected from local agro-processing areas. These substrates were chosen based on their high organic and carbohydrate content, which may support the growth of lignocellulose-degrading microorganisms. Approximately 100–200 g of each sample was collected using sterile spatulas and transferred into pre-labeled sterile polyethylene bags. Labels included sample code, collection site, date, and substrate type to ensure traceability during downstream analysis. The samples were transported to the laboratory under ambient conditions and processed within 24 hours to minimize microbial community shifts during storage (Harish et al., 2024).

Enrichment and Isolation of Yeasts:

For enrichment, approximately 1 g of each environmental sample was inoculated into 50 mL of liquid enrichment medium designed to promote yeast growth. The medium contained yeast extract and peptone as nutrient sources, while xylan was supplied as the primary carbon source to preferentially support xylan-utilizing microorganisms. The use of xylan also helped limit the overgrowth of non-target bacteria and filamentous fungi. The inoculated flasks were incubated at 30°C for 48–72 hours under static conditions to allow selective proliferation of yeast populations. After visible turbidity was observed, serial dilutions were prepared in sterile 0.85% (w/v) saline solution. Appropriate dilutions were spread onto yeast extract peptone dextrose (YPD) agar plates and incubated at 30°C for 24–48 hours. Distinct colonies displaying morphological characteristics typical of yeasts — including smooth texture, creamy appearance, and circular margins — were selected for further purification. Each isolate was subcultured by repeated streaking on fresh YPD agar plates until morphologically uniform colonies were obtained, indicating pure cultures (Banyal et al., 2024).

Enumeration of Yeast Population:

Yeast population density in each sample was estimated using the standard plate count technique. Following incubation, plates containing 30–300 well-separated colonies were selected for counting to ensure statistical reliability. The number of colonies was recorded and expressed as colony-forming units (CFU) per gram of original sample, taking the dilution factor into account. All analyses were carried out in triplicate to minimize experimental variation, and results were reported as mean values (Saïed et al., 2024).

Qualitative Screening for Xylanase Activity:

Qualitative Preliminary screening of xylanase production was carried out using xylan agar plates. The medium consisted of yeast extract, peptone, NaCl, agar, and beechwood xylan as the sole carbon source. The pH was adjusted to 5.0 prior to sterilization to create conditions favorable for yeast-derived xylanase activity. Each purified yeast isolate was spot-inoculated onto the surface of the xylan agar plates and incubated at 30°C for 48–72 hours. Following incubation, plates were gently flooded with 0.1% (w/v) Congo red solution and allowed to stand for 15 minutes to facilitate dye binding with undegraded xylan. Excess stain was removed by washing the plates with 1 M NaCl for approximately 10 minutes. The appearance of clear zones surrounding the colonies indicated hydrolysis of xylan. The diameter of these halo zones was measured in millimeters, and isolates were categorized based on relative clearing intensity as weak (+), moderate (++), or strong (+++) producers (Saharan et al., 2024).

Production of Crude Xylanase:

The isolate exhibiting the highest xylanolytic activity during preliminary screening was selected for enzyme production. For this purpose, the culture was inoculated into a xylanase-inducing liquid medium supplemented with beechwood xylan as the principal carbon source to stimulate enzyme synthesis. The inoculated flasks were incubated at 30°C for 72 hours under shaking conditions at 120 rpm to ensure adequate aeration and uniform nutrient distribution. At the end of the incubation period, the culture broth was subjected to centrifugation at 10,000 × g for 10 minutes at 4°C to separate the biomass from the extracellular enzyme fraction. The resulting clear supernatant was carefully collected and used directly as the crude xylanase preparation for subsequent enzymatic assays (Ramesh et al., 2025).

Quantitative Estimation of Xylanase Activity:

Xylanase activity was determined by measuring the release of reducing sugars using the 3,5-dinitrosalicylic acid (DNS) method. Briefly, 0.5 mL of crude enzyme extract was mixed with 0.5 mL of 1% (w/v) beechwood xylan prepared in the appropriate buffer system. The reaction mixture was incubated at 30°C for 10 minutes to allow enzymatic hydrolysis of the substrate. The reaction was subsequently stopped by the addition of 1 mL DNS reagent. The tubes were then placed in a boiling water bath for 5 minutes to facilitate color development resulting from the reaction between DNS and released reducing sugars. After cooling to room temperature, absorbance was recorded at 540 nm using a UV–Visible spectrophotometer. A standard calibration curve was constructed using known concentrations of xylose, and enzyme activity was calculated accordingly. One unit (U) of xylanase activity was defined as the amount of enzyme required to release 1 µmol of reducing sugar per minute under the specified assay conditions (Mgeni et al., 2025).

Effect of pH on Xylanase Activity:

To examine the influence of pH on enzyme performance, xylanase activity was measured across a pH range of 3.0 to 10.0. A series of 50 mM buffer systems was employed to maintain stable reaction conditions: citrate buffer for pH 3–6, phosphate buffer for pH 6–8, and glycine–NaOH buffer for pH 9–10. For each pH value, the standard DNS assay was performed at a constant temperature of 30°C to ensure that observed variations in activity were attributable solely to changes in pH. Enzyme reactions were conducted under identical substrate and incubation conditions to maintain comparability across treatments. All assays were performed in triplicate, and the results were expressed as mean enzyme activity values (Emmanuel et al., 2025).

Effect of Temperature on Xylanase Activity:

The thermal profile of the enzyme was evaluated by conducting activity assays at temperatures between 20°C and 70°C, while maintaining the previously determined optimum pH. Prior to initiating the reaction, substrate-buffer mixtures were allowed to equilibrate at the selected temperature to ensure stable assay conditions. The enzymatic reaction was started by adding the crude enzyme extract, followed by incubation under the respective temperature conditions. Reducing sugar release was quantified using the DNS method as described earlier. Enzyme activity at each temperature point was calculated based on the corresponding standard curve values. All experiments were carried out in triplicate, and the final activity values were reported as mean ± standard deviation of three independent measurements (Naveena et al., 2025).

Identification of the Potent Yeast Isolate:

The isolate exhibiting the highest xylanase activity during screening was subjected to detailed identification. Preliminary characterization was carried out based on colony morphology, microscopic observation, and general phenotypic traits. Microscopic examination helped confirm typical yeast-like cellular features and growth patterns. For molecular identification, genomic DNA was extracted from the selected isolate using standard protocols. The ribosomal gene region was amplified through PCR, and the resulting amplicon was purified and sequenced. The obtained nucleotide sequence was analyzed using BLAST against reference sequences available in the NCBI GenBank database to determine phylogenetic similarity. Based on sequence homology and morphological consistency, the isolate was identified as *Geotrichum candidum*. The corresponding sequence data had been previously reported by our group (Jamaldheen et al., 2021).

Pretreatment and Enzymatic Hydrolysis of Bajra:

Bajra (Pearl millet) grains were initially cleaned to remove surface impurities and foreign particles, followed by drying at ambient laboratory conditions. The dried grains were ground using a laboratory grinder to obtain a fine powder, thereby increasing surface area for subsequent processing. To enhance enzymatic accessibility, the powdered biomass was subjected to thermal pretreatment. The treated substrate was then allowed to cool before being suspended in the appropriate buffer system under optimized pH conditions. Crude xylanase enzyme was added to the suspension, and hydrolysis was carried out under previously standardized temperature and incubation parameters to facilitate the breakdown of hemicellulosic components. After completion of enzymatic hydrolysis, the reaction mixture was filtered to remove residual solid material. The clarified hydrolysate, containing released fermentable sugars, was collected and directly utilized for downstream fermentation studies (Khalaji et al., 2020).

Bioethanol Production:

The enzymatic hydrolysate obtained from bajra biomass was used as the fermentation substrate without further modification. Prior to inoculation, the hydrolysate was adjusted to the desired pH and transferred into sterile fermentation flasks. Actively growing yeast culture was introduced under aseptic conditions, and the system was maintained under anaerobic conditions to promote ethanol production. Fermentation was carried out at 30°C for a defined period, during which samples were withdrawn at regular intervals to monitor residual sugar concentration and ethanol formation. The progression of fermentation was assessed by observing a decline in fermentable sugars along with a corresponding increase in ethanol levels. Upon completion of fermentation, the broth was subjected to distillation for recovery of bioethanol. The distilled fraction was collected and preserved for subsequent analytical characterization (Blessie et al., 2021).

FTIR Analysis for Bioethanol production

Fourier Transform Infrared (FTIR) spectroscopy was performed to verify the presence of characteristic functional groups corresponding to ethanol in the recovered distillate. Prior to analysis, the distilled bioethanol fraction was filtered to remove any residual particulate matter and allowed to equilibrate to room temperature to ensure stable spectral acquisition. A small aliquot of the sample was subjected to FTIR analysis over the mid-infrared region. The obtained spectrum was examined for absorption bands typically associated with ethanol, including O–H stretching vibrations, C–H stretching, and C–O functional group signatures. The observed peaks were compared with standard ethanol spectra to confirm chemical identity (Reddy & Reddy, 2021).

HPLC Analysis for Bioethanol production

High-performance liquid chromatography (HPLC) analysis was carried out to determine ethanol concentration and assess the purity of the bioethanol obtained from bajra hydrolysate fermentation. Prior to injection, the distilled sample was passed through a 0.22 µm membrane filter to remove residual particulates and prevent column blockage. Filtered samples were injected into the HPLC system under appropriate chromatographic conditions. Ethanol was identified based on its characteristic retention time by comparison with a standard ethanol solution analyzed under identical conditions. Quantification was performed using a calibration curve generated from known ethanol concentrations. The chromatographic profile was examined for the presence of additional peaks that might indicate impurities. A single dominant peak corresponding to ethanol, with minimal secondary peaks, was considered indicative of satisfactory purity (Saharan et al., 2024).

III. RESULTS

Yeast Population in Environmental Samples

Yeast populations varied significantly among different environmental samples collected from Raipur, Chhattisgarh. Compost and fruit waste samples showed markedly higher yeast densities compared to soil and sugarcane bagasse samples. The highest colony-forming units (CFU/g) were recorded in fruit waste sample S11, indicating rich fermentable substrates favoring yeast proliferation.

Table 1. Yeast population (CFU/g) isolated from different environmental samples.

S. No.	Sample ID	Sample Type	Mean CFU ($\times 10^{-5}$ CFU/g)
1	S1	Soil	0.23
2	S2	Soil	0.19
3	S3	Soil	0.038
4	S4	Soil	0.62
5	S5	Compost	32.00
6	S6	Compost	109.33
7	S7	Compost	4.77
8	S8	Compost	72.67
9	S9	Fruit waste	210.00
10	S10	Fruit waste	58.00
11	S11	Fruit waste	820.00
12	S12	Fruit waste	15.00
13	S13	Sugarcane bagasse	1.01
14	S14	Sugarcane bagasse	4.20
15	S15	Sugarcane bagasse	0.068

Qualitative Screening of Xylanase-Producing Yeasts

Xylanase activity was assessed qualitatively by observing clear halo zones on xylan agar plates after Congo red staining. Several isolates demonstrated moderate to strong xylan-degrading ability. The isolate YSB2 exhibited the highest halo diameter, indicating strong xylanolytic potential.

Table 2. Qualitative xylanase activity of yeast isolates on xylan agar

Isolate	Mean Halo Zone (mm)	Qualitative Rating
YC1	21.0	+++
YC8	22.3	+++
YFW8	21.3	+++
YFW11	19.3	+++
YSB2	23.3	+++ (Highest)

3.3 Quantitative Xylanase Activity

Quantitative estimation using the DNS method confirmed xylanase production by the selected isolates. The enzymatic activity varied among isolates, reflecting differences in substrate utilization efficiency.

Table 3. Quantitative xylanase activity expressed as absorbance values (540 nm) using DNS assay.

Sample	Mean Absorbance
YS9	0.881
YS14	0.651
YS20	0.923
YS23	0.730
YS28	0.963

Effect of pH on Xylanase Activity

Xylanase activity was evaluated across a pH range of 3–10 at 30°C. Maximum activity was observed at pH 5, indicating that the enzyme functions optimally under mildly acidic conditions.

Table 4. Effect of pH on xylanase activity of Geotrichum candidum

pH	Mean Activity ($U \cdot mL^{-1}$)
3	0.85
4	1.65
5	3.30
6	2.85
7	1.95
8	1.05
9	0.48
10	0.19

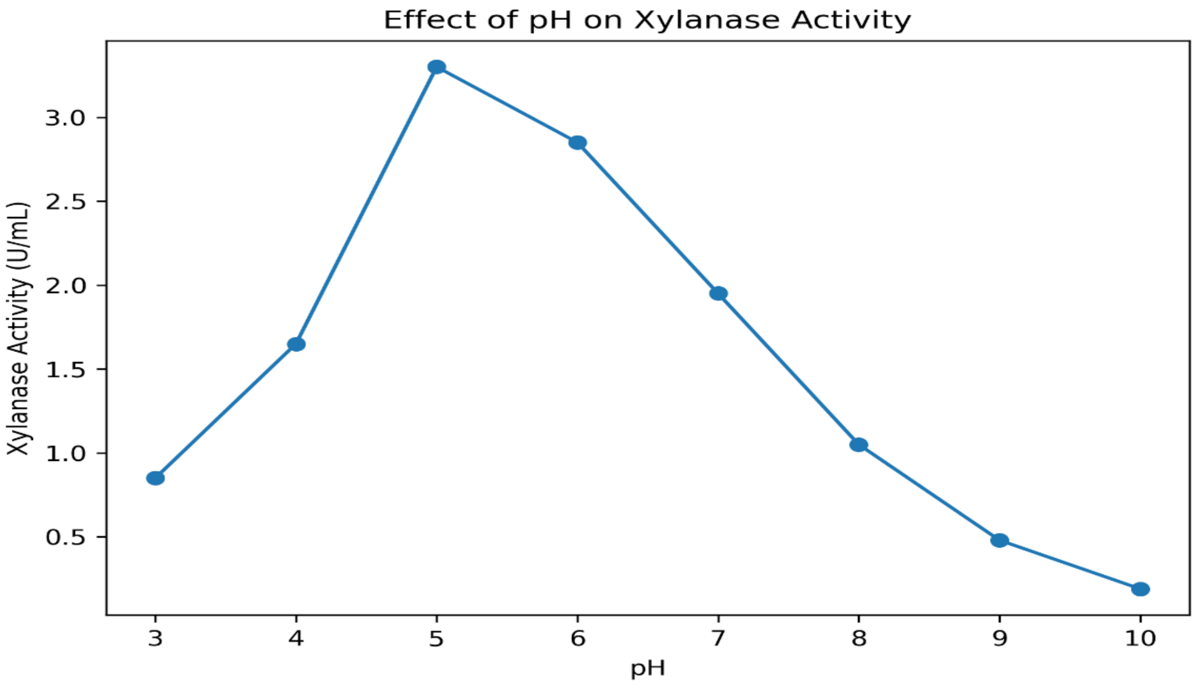

Figure 1. Effect of pH on xylanase activity produced by Geotrichum candidum. Maximum enzyme activity was observed at pH 5, indicating optimal activity under mildly acidic conditions.

Effect of Temperature on Xylanase Activity

Temperature profiling showed that xylanase activity increased with temperature up to 40°C, beyond which a gradual decline was observed.

Table 5. Effect of temperature on xylanase activity of Geotrichum candidum.

Temperature (°C)	Mean Activity (U·mL⁻¹)
20	0.62
30	1.85
35	2.65
40	3.45
45	3.05
50	2.05
60	0.88
70	0.29

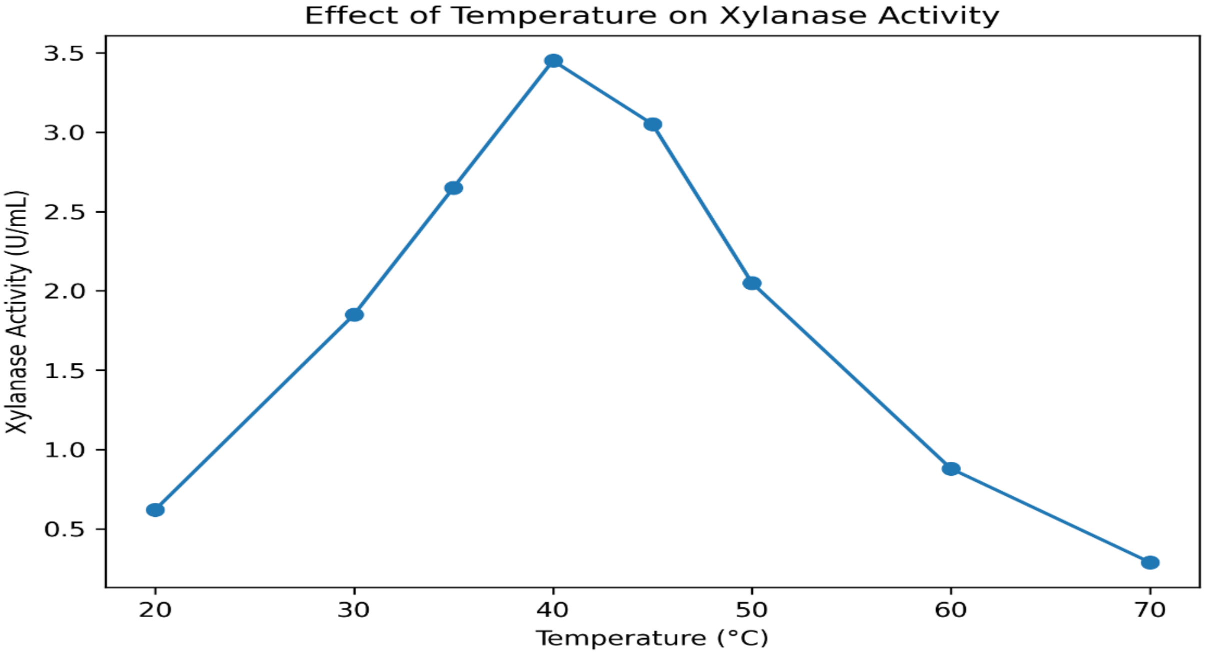

Figure 2. Effect of temperature on xylanase activity produced by Geotrichum candidum. The enzyme exhibited maximum activity at 40 °C, followed by a gradual decline at higher temperatures.

Bioethanol Production from Bajra (Pearl Millet)

Pretreated bajra biomass was subjected to enzymatic hydrolysis using crude xylanase, followed by fermentation under anaerobic conditions. Efficient conversion of fermentable sugars into ethanol was observed during fermentation. The distillation of fermented broth yielded bioethanol, confirming the feasibility of bajra as a substrate for bioethanol production. The ethanol concentration obtained from the fermented bajra hydrolysate after distillation was 20–24 g/L.

FTIR Analysis of produced Bioethanol:

FTIR spectroscopy was performed to confirm the chemical identity of the bioethanol recovered from bajra fermentation. The FTIR spectrum recorded in the range of 4000–400 cm^{-1} showed characteristic absorption bands corresponding to ethanol, confirming successful bioethanol production. A broad and strong absorption band observed in the region of ~3300–3400 cm^{-1} corresponds to the O–H stretching vibration, which is a characteristic feature of alcohols and indicates the presence of hydroxyl groups in ethanol. The absorption bands detected around ~2970–2870 cm^{-1} are attributed to C–H stretching vibrations of methyl and methylene groups, confirming the aliphatic nature of ethanol. The prominent peak observed near ~1050–1100 cm^{-1} corresponds to C–O stretching vibrations, a diagnostic band for ethanol molecules. Additional minor bands observed in the lower wavenumber region are associated with bending vibrations of C–H bonds and further support the presence of alcohol functional groups.

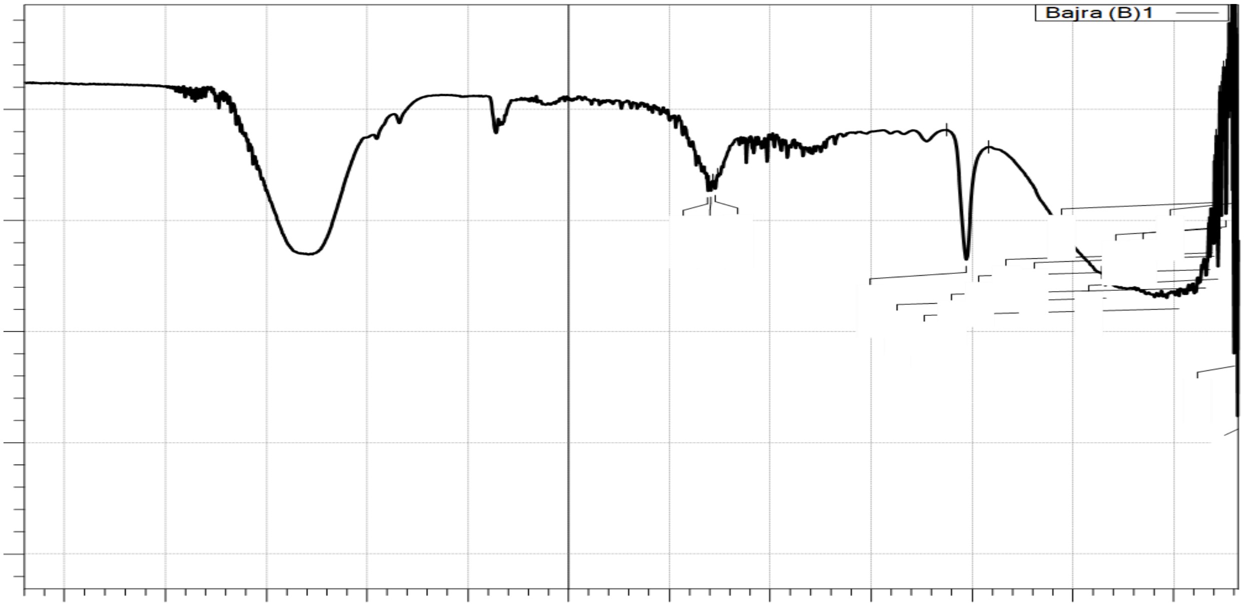

Figure 3. FTIR spectrum of bioethanol produced from bajra showing characteristic O–H, C–H, and C–O stretching vibrations confirming ethanol formation.

HPLC Analysis of Produced Bioethanol:

High-performance liquid chromatography (HPLC) was employed to quantify ethanol and assess the purity of bioethanol produced from bajra hydrolysate. The chromatographic analysis was carried out at 254 nm, and the resulting chromatogram of the bioethanol sample (Sample B) revealed multiple peaks corresponding to different components present in the fermented distillate. A major dominant peak was observed at a retention time of 4.388 min, accounting for 89.77% of the total peak area, indicating that ethanol was the predominant compound present in the sample. The high area percentage and peak height (90.55% height contribution) confirm the successful production of ethanol with high relative purity. Several minor peaks were detected at retention times ranging from 1.862 to 9.742 min, each contributing less than 4% individually to the total area. These minor peaks likely correspond to trace impurities, residual sugars, fermentation by-products, or other volatile compounds formed during fermentation and distillation.

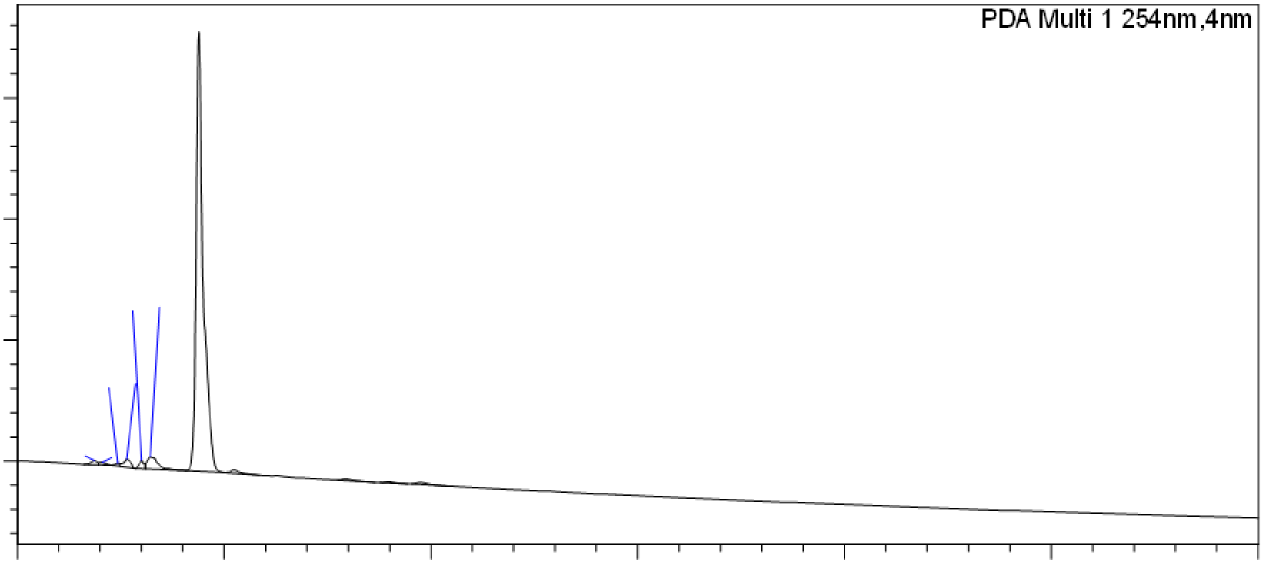

Figure 4. HPLC chromatogram of bioethanol produced from bajra showing a major ethanol peak at a retention time of ~4.39 min, indicating high relative purity of the fermented distillate

IV. DISCUSSION

The findings of the present investigation confirm that diverse environmental substrates can serve as effective reservoirs of xylanase-producing yeasts. Among the sampled niches, compost and fruit waste harbored noticeably higher yeast populations than soil and sugarcane bagasse. This trend is not unexpected. Organic residues rich in simple sugars and partially degraded plant polymers provide a favorable microenvironment for yeasts capable of utilizing complex carbohydrates. The particularly high population recorded in fruit waste sample S11 underscores the industrial relevance of nutrient-dense agro-wastes as potential sources of robust enzyme-producing strains.

Preliminary screening on xylan agar clearly differentiated the isolates in terms of their hydrolytic capacity. The formation of distinct halo zones after Congo red staining confirmed active xylan degradation. Isolate YSB2, which produced the largest clearance zone, demonstrated superior extracellular enzyme secretion relative to the other candidates. Differences in halo diameter likely reflect variations in enzyme diffusion, secretion efficiency, and substrate affinity. Such heterogeneity is commonly observed among environmental isolates, where metabolic capabilities are shaped by ecological adaptation. Subsequent quantitative analysis using the DNS method strengthened these observations. Differences in absorbance at 540 nm directly corresponded to variations in reducing sugar release, indicating strain-dependent enzymatic performance. The combined use of plate-based screening and spectrophotometric quantification proved essential for narrowing down the most promising isolate. Relying on qualitative assessment alone would not have provided sufficient resolution for selecting the highest producer. Enzyme activity profiling revealed a clear dependence on pH and temperature. Maximum xylanase activity was observed at pH 5, suggesting that the enzyme functions optimally under mildly acidic conditions. This characteristic is particularly advantageous for lignocellulosic processing, as many pretreatment strategies generate acidic reaction environments. The decline in activity at higher pH levels may be associated with alterations in the ionization state of amino acid residues involved in catalysis, ultimately affecting substrate binding and enzyme stability. Temperature optimization further demonstrated that enzymatic activity increased gradually up to 40°C. Beyond this point, activity declined, most likely due to progressive thermal destabilization of the enzyme structure. The moderate temperature optimum observed in this study is industrially favorable. Operating at extreme temperatures often increases energy demands and processing costs, whereas moderate conditions support both catalytic efficiency and economic feasibility. Molecular and phenotypic analyses identified the potent isolate as *Geotrichum candidum*. This yeast-like fungus is widely recognized for its enzymatic versatility and adaptability across substrates. Its demonstrated ability to produce functionally active xylanase reinforces its suitability for integration into biomass conversion systems. The practical applicability of the enzyme was validated through hydrolysis and fermentation of bajra biomass. Bajra remains underexploited as a bioethanol feedstock despite its abundance and resilience as a crop. Enzymatic treatment facilitated the release of fermentable sugars, which were subsequently converted into ethanol under anaerobic conditions. The ethanol yield obtained after distillation (20–24 g/L) reflects efficient bioconversion and supports the feasibility of utilizing bajra residues within a renewable energy framework. Analytical confirmation strengthened these findings. FTIR spectra displayed characteristic absorption bands corresponding to O–H, C–H, and C–O functional groups typical of ethanol. In parallel, HPLC analysis revealed a dominant peak at approximately 4.39 minutes, accounting for nearly 90% of the total chromatographic area. The minimal presence of secondary peaks indicates limited impurity formation and suggests that both fermentation and downstream recovery steps were effective. Taken together, the study demonstrates that locally isolated *G. candidum* can serve as an efficient xylanase producer and can be successfully integrated into a biomass-to-bioethanol workflow using bajra as substrate. The results highlight the value of combining indigenous microbial resources with regionally available agricultural biomass to develop cost-effective and sustainable bioenergy strategies.

V. CONCLUSION

This study demonstrated that environmental niches in Raipur, Chhattisgarh can serve as valuable reservoirs of xylanase-producing yeasts, particularly compost and fruit waste, which showed comparatively higher microbial abundance. Among the isolates obtained, *Geotrichum candidum* emerged as the most promising strain based on both plate screening and quantitative enzyme assays. The enzyme displayed optimal activity at pH 5 and 40°C, conditions that align well with typical lignocellulosic biomass processing environments. The applicability of the produced xylanase was further established through its use in the hydrolysis and fermentation of bajra (pearl millet) biomass. The ethanol concentration achieved after distillation (20–24 g/L) indicates effective conversion of released sugars into bioethanol. Analytical validation using FTIR and HPLC confirmed the presence and relative purity of ethanol in the final product. The integration of locally isolated *G. candidum* with an underutilized cereal substrate highlights a practical and regionally adaptable approach for bioethanol production. Further studies focusing on process scale-up and long-term operational stability would provide additional insight into its industrial potential.

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