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Molecular cloning and expression of *Bacillus cereus* xylanase gene in *E. coli* bl21

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ABSTRACT: Recombinant production of bacterial xylanases offers a cost-effective and industrially relevant alternative to native enzyme extraction. In this study, the xylanase gene from *Bacillus Cereus* (~1071 bp) was amplified by PCR and cloned into the pET-21c(+) expression vector (~5441 bp) for heterologous expression in Escherichia coli BL21. Ligation and transformation were confirmed through colony PCR and restriction digestion with BamHI, producing a distinct band of 6512 bp, consistent with the recombinant plasmid. The successful cloning and verification demonstrate the feasibility of generating functional recombinant xylanase in E. coli. Bacterial xylanases, unlike fungal enzymes, exhibit high stability across a broad pH range and elevated temperatures, enhancing their suitability for industrial applications such as pulp and paper processing, food, and biofuel production. This work establishes a foundation for large-scale recombinant xylanase production and provides a platform for further optimization of fermentation conditions to improve enzyme yield. Future studies will focus on optimizing expression levels, scaling up production, and evaluating the enzyme's performance under industrial process conditions.

KEYWORDS: Xylanase gene, Escherichia coli BL21, Polymerase Chain Reaction (PCR), Bacillus cereus, pET 21c(+)

INTRODUCTION

Xylan is one of the most abundant polysaccharides in nature and a major structural component of hemicellulose in plant cell walls. It consists primarily of β-1,4-linked xylose residues that are often substituted with side groups such as arabinose, glucuronic acid, and acetyl moieties, resulting in a structurally diverse polymer [1]. Xylan is widely distributed in hardwoods, softwoods, grasses, and agricultural biomass, making it an important renewable resource [2]. The enzymatic degradation of xylan is mediated by xylanases, which hydrolyze the xylan backbone into xylo-oligosaccharides and xylose. Complete hydrolysis requires the coordinated action of several accessory enzymes; however, endo-1,4-β-xylanase plays a central role by initiating the cleavage of internal xylosidic bonds [3] .

Xylanases have gained considerable industrial significance due to their applications in pulp and paper processing, animal feed improvement, food and beverage production, biofuel generation, and bio bleaching. The functional properties of xylanases vary depending on their microbial source [4]. Fungal xylanases generally exhibit optimal activity under acidic conditions and moderate temperatures, which limits their performance in industrial processes requiring alkaline pH and elevated temperatures [5]. In contrast, bacterial xylanases typically display broader pH tolerance and higher thermo stability, making them more suitable for industrial environments that involve harsh processing conditions [6].

Among bacterial producers, *Bacillus Cereus* is a Gram-positive, spore-forming bacterium known for its ability to secrete extracellular enzymes with desirable industrial properties [7]. Xylanases derived from Bacillus species often demonstrate alkaline stability, thermal resistance, and high catalytic efficiency [8]. These characteristics highlight *B. cereus* as a promising source of industrially relevant xylanases. However, enzyme production from native bacterial strains is often constrained by low yields, complex purification steps, and increased production costs [9].

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To overcome these limitations, recombinant DNA technology offers an efficient strategy for large-scale enzyme production. Escherichia coli BL21 is widely employed as a heterologous expression host due to its rapid growth, genetic stability, and compatibility with high-expression vectors such as pET-21c(+). In the present study, the xylanase gene from *Bacillus Cereus* was amplified, cloned into the pET-21c(+) vector, and expressed in E. coli BL21. The successful cloning and confirmation of recombinant constructs provide a foundation for efficient xylanase production and support the potential application of bacterial xylanases in industrial processes.

MATERIAL AND METHODS

Collection of bacterial strain and plasmid

Bacillus Cereus was obtained from the Culture Bank of the Department of Mycology and Plant Pathology, University of the Punjab, Lahore, Pakistan. Escherichia coli BL21 was used as the host strain for cloning and expression and was procured from the Industrial Biotechnology Institute culture bank, GCU Lahore. The expression vector pET-21c(+) was employed for cloning.

Genomic DNA isolation and molecular identification

Genomic DNA of *Bacillus Cereus* was extracted using the cetyltrimethylammonium bromide (CTAB) method as previously described by Naveed et al [10]. The quality and integrity of the extracted DNA were evaluated by electrophoresis on a 1% agarose gel. Molecular identification of the bacterial isolate was performed through PCR amplification of the 16S rRNA gene using universal primers depicted in Table 1 under standard thermal cycling conditions, following the methodology outlined by Naveed et al [11].

Primer design and PCR amplification of xylanase gene

Gene-specific primers were designed to amplify the xylanase gene from *B. cereus*, incorporating NcoI and BamHI restriction sites at the 5′ ends of the forward and reverse primers, respectively. PCR amplification was carried out using genomic DNA as a template under optimized cycling conditions. The amplified product (~1071 bp) was confirmed by agarose gel electrophoresis and purified prior to cloning [12].

Restriction digestion and ligation

The purified xylanase gene and pET-21c(+) vector were double-digested with NcoI and BamHI. Digested products were purified and ligated using T4 DNA ligase under standard conditions. The ligation mixture was incubated overnight to ensure efficient recombinant plasmid formation.

Table 1. Primers for 16sRNA and xylanase gene

Gene	Primers	Sequence	Product size (bp)	Primer Melting Temperature(°C)
16S9F	Forward	GAGTTTGATCCTGGCTCAG	1490	54
	Reverse	GGCTACCTTGTTACGA		
Xylanase gene	Forward	CCATGGGGCTTGAAAAGT	1071	54
	Reverse	GGATCCTCCTTGAATTGCCTT		

Table 2. Components of ligation mixture

Constituents	Quantity
Product which was amplified	6 µL
pET-21c(+)	2 µL
T4 DNA ligase	2 µL
Ligase buffer	2 µL
Distilled water	9 µL
Total	21 µL

Preparation of competent cells and transformation

Chemically competent E. coli BL21 cells were prepared using the calcium chloride method. The ligated plasmid was transformed into competent cells by heat shock. Transformed cells were recovered in LB medium and plated on LB agar containing ampicillin, IPTG, and X-gal. Plates were incubated overnight at 37 °C.

Screening and confirmation of positive clones

White colonies were selected as putative positive clones. Colony PCR was performed to confirm the presence of the xylanase gene. Recombinant plasmids were isolated using the

alkaline lysis method [13] and further confirmed by restriction digestion with BamHI, followed by agarose gel electrophoresis [14].

RESULTS

Genomic DNA isolation and molecular identification

High-quality genomic DNA was successfully isolated from Bacillus cereus, as confirmed by the presence of intact high-molecular-weight DNA bands on a 1% agarose gel (Figure 1). PCR amplification of the 16S rRNA gene yielded a distinct amplicon of approximately 1500 bp (Figure 2). Subsequent Sanger sequencing and sequence analysis confirmed the identity of the isolate as Bacillus cereus, showing high sequence similarity with reference strains deposited in public databases (Figure 3).

Amplification of the xylanase gene

PCR amplification of the xylanase gene from B. cereus genomic DNA resulted in a clear and specific band of approximately 1071 bp, corresponding to the expected size of the target gene (Figure 4). The successful amplification confirmed the presence of the xylanase gene and its suitability for downstream cloning procedures.

Isolation and restriction of expression vector

The pET-21c(+) expression vector was successfully isolated and verified by agarose gel electrophoresis, yielding a plasmid band corresponding to its expected size of approximately 5441 bp as shown in Figure 5. Double digestion of the vector using NcoI and BamHI produced linearized plasmid fragments, confirming successful restriction and readiness for ligation (Figure 6).

Cloning and screening of recombinant plasmid

Following incubation for 24 hours at 37 °C, successful transformation was indicated by the appearance of white colonies on selective LB agar plates. To confirm the presence of the xylanase gene, six white colonies were randomly selected for colony PCR using gene-specific primers. Agarose gel electrophoresis of the PCR products revealed a clear band of approximately 1071 bp, corresponding to the expected size of the xylanase gene (Figure 7). Lane 1 shows the DNA ladder, and lane 3 displays the amplified xylanase gene, confirming the successful incorporation of the gene into the pET-21c(+) vector.

Restriction analysis of recombinant pET-21c(+)-xylanase plasmid

A single restriction digestion of the recombinant pET-21c(+)-xylanase plasmid was performed using BamHI to confirm the presence of the inserted gene. Agarose gel electrophoresis revealed a distinct band of approximately 6512 bp, corresponding to the combined size of the vector and the xylanase insert (Figure 8). Lane 1 shows the DNA ladder, while lanes 2–5 display the digested recombinant plasmids. Positive recombinant plasmids were confirmed by the presence of the expected band in lanes 2 and 4, whereas the negative control lanes showed no amplification, verifying the successful cloning of the xylanase gene.

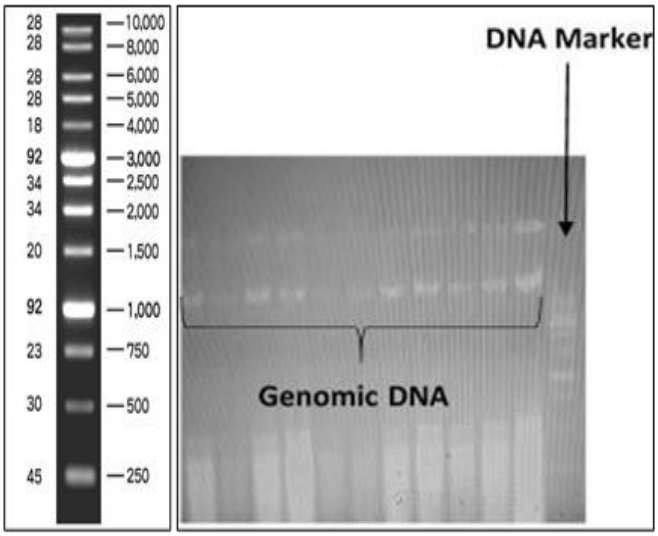

Figure 1. *Bacillus Cereus* genomic DNA quantification.

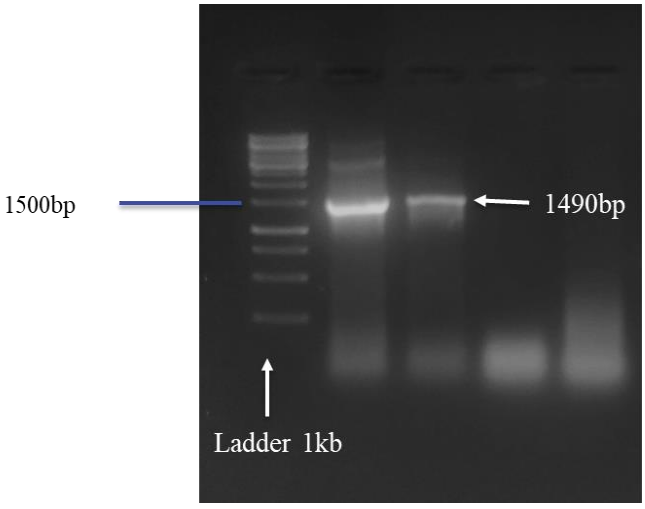

Figure 2. 16SrRNA gene amplification.

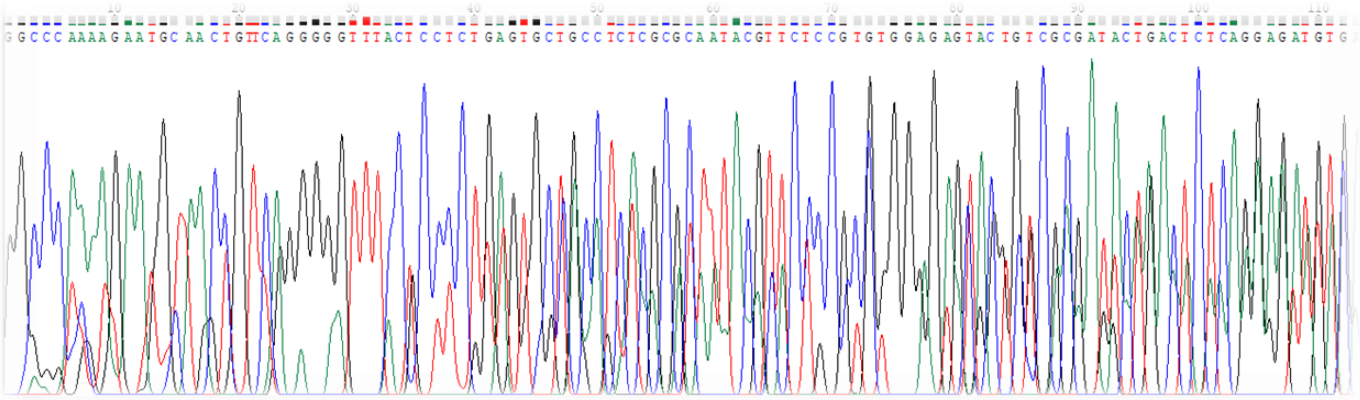

Figure 3. Chromatogram of 16rs RNA sequence of Bacillus cereus.

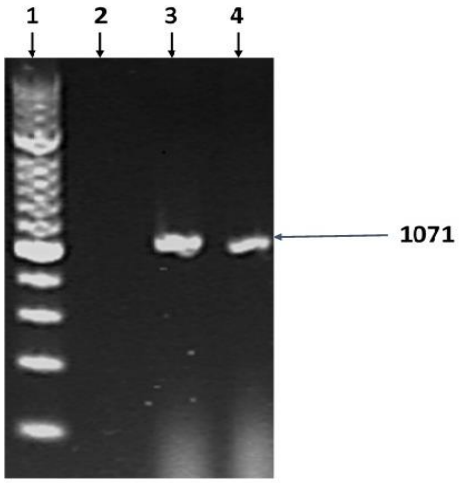

Figure 4. Agarose gel electrophoresis showing PCR amplification of the xylanase gene from Bacillus cereus. Lane 1: DNA ladder; Lanes 2 and 3: amplified xylanase gene (~1071 bp).

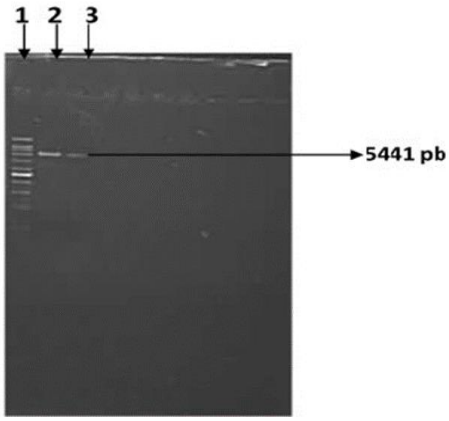

Figure 6. Agarose gel electrophoresis of the pET-21c(+) plasmid after double digestion with NcoI and BamHI. Lanes 2 and 3 show the linearized plasmid fragments, confirming successful restriction and purification of the vector for ligation with the xylanase gene. Lane 1 represents the DNA ladder.

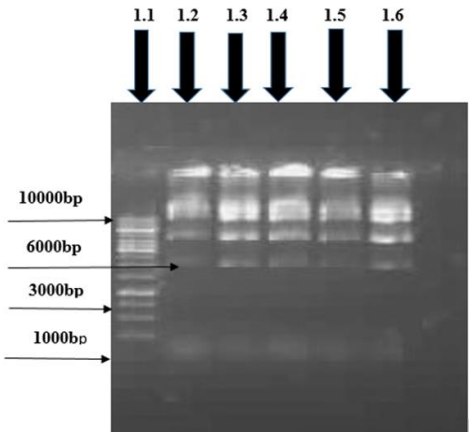

Figure 5. Agarose gel electrophoresis showing the isolation of the pET-21c(+) expression vector. A single distinct band corresponding to the expected plasmid size (~5441 bp) confirms successful plasmid extraction, ready for restriction digestion and downstream cloning.

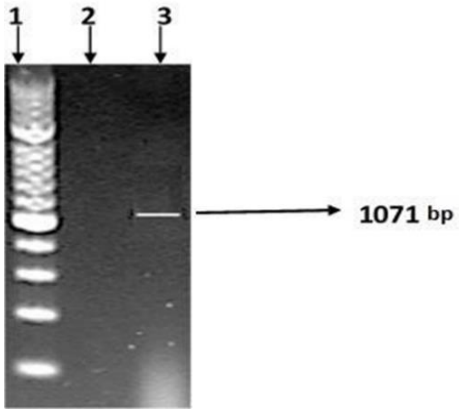

Figure 7. Agarose gel electrophoresis of colony PCR products confirming the presence of the xylanase gene in recombinant E. coli BL21 clones. Lane 1: DNA ladder; Lanes 2–6: PCR-amplified xylanase gene (~1071 bp) from six randomly selected white colonies; Lane 7: negative control. The distinct bands confirm successful incorporation of the gene into the pET-21c(+) vector.

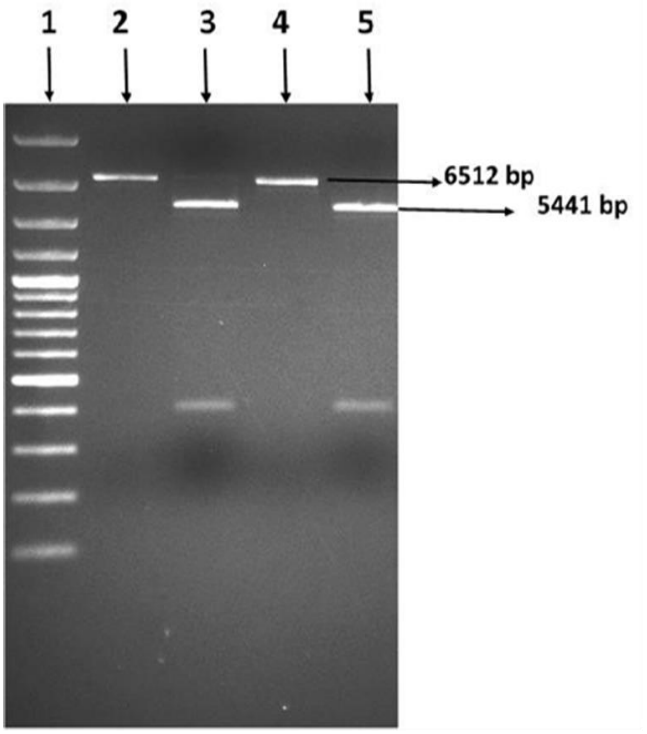

Figure 8. Agarose gel electrophoresis of purified recombinant pET-21c(+)-xylanase plasmids after single digestion with BamHI. Lane 1: DNA ladder; Lanes 2–5: digested plasmids from selected E. coli BL21 colonies. Bands at approximately 6512 bp in lanes 2 and 4 confirm the presence and correct insertion of the xylanase gene, validating successful cloning of the recombinant construct

DISCUSSION

Xylanases play a central role in the degradation of hemicellulose, catalyzing the conversion of xylan into xylose and xylo-oligosaccharides [15]. These enzymes are widely distributed among microorganisms, plants, and some higher organisms, and have been exploited in various industrial processes, including biofuel production, food processing, and pulp and paper industries [16]. In this study, the xylanase gene from Bacillus cereus was successfully cloned into the pET-21c(+) vector and heterologously expressed in Escherichia coli BL21, providing a reliable platform for recombinant enzyme production.

The amplification of the xylanase gene (~1071 bp) and successful ligation into the pET-21c(+) vector were confirmed through colony PCR and restriction digestion, yielding the expected recombinant plasmid size (~6512 bp). These results are consistent with prior studies on bacterial xylanase cloning, such as Sun et al. (2009), who reported successful cloning in pGEM vectors [17], though expression levels were not optimized for large-scale applications. In contrast, the use of the pET-21c(+) vector and E. coli BL21 in the current study supports higher-level gene expression, which enhances the potential scalability of xylanase production [18], aligning with findings by Wakelin (2009).

Comparison with fungal xylanases highlights a key advantage of bacterial enzymes. Fungal xylanases typically show optimal activity under acidic pH (4–6) and moderate temperatures (<50°C), limiting their use in alkaline and high-temperature industrial processes [19]. In contrast, xylanases from *B. cereus* are known to tolerate broader pH ranges and elevated temperatures, which may offer improved stability under industrial conditions. However, claims regarding large-scale industrial applicability should be interpreted cautiously, as further studies are required to optimize fermentation parameters, including pH, temperature, and substrate concentration, and to evaluate pilot-scale production efficiency [20].

This study demonstrates the successful recombinant production of a functional bacterial xylanase, providing a platform for future optimization and comparative studies. The novelty of this work lies in the use of B. cereus xylanase with pET-21c(+) expression in E. coli BL21, which may enable higher expression yields than previous bacterial cloning attempts. Future research should focus on detailed characterization of enzyme kinetics, thermostability, and process performance under industrially relevant conditions to fully assess the practical utility of this recombinant xylanase.

Furthermore, this research provides a foundation for exploring genetic and process engineering strategies to enhance xylanase yield and activity. Directed evolution or site-directed mutagenesis could be applied to improve enzyme stability, substrate specificity, or catalytic efficiency. Additionally, integration with fermentation optimization and bioreactor scaling could facilitate cost-effective industrial production. These approaches not only broaden the applicability of bacterial xylanases but also contribute to sustainable biotechnological solutions for the bio-based production of chemicals, fuels, and food additives.

CONCLUSION

In this study, the xylanase gene from Bacillus cereus was successfully cloned and expressed in the E. coli BL21 (DE3) system, establishing a reliable platform for recombinant enzyme production. The approach demonstrated here provides a cost-effective and scalable method for generating functional bacterial xylanase, with potential advantages over conventional extraction from native strains. The results confirm that bacterial xylanases can offer enhanced stability and catalytic efficiency, supporting their suitability for

industrial applications. This work extends previous research by combining the robust pET-21c(+) expression system with E. coli BL21, highlighting the practical utility of recombinant DNA technology for enzyme production. Future studies focusing on fermentation optimization, process scaling, and enzyme characterization will further enhance yield and applicability, advancing the use of bacterial xylanases in sustainable industrial and biotechnological processes.

DECLARATION

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Authorship contributions

Concept and Writing: Ayesha Nawaz, Ali Irtaza Rizvi, Nimra Hanif, Rabail Afzal; Data Collection and Experiments: Muhammad Asim, Syed Ali Hamza, Tazmeen Shahid; References and Citations: Urooj Rasheed, Maryam Manzoor; Manuscript Drafting and Editing: Nawal Batool, Zainab Aqsa

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Ethics approval and consent to participate

All experimental procedures were conducted following institutional guidelines and standard laboratory practices; as the study did not involve human or animal subjects, formal ethical approval was not required.

Competing interests

The authors declared that there is no conflict of interest.

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