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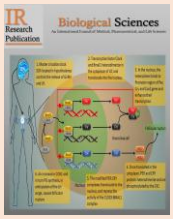

In silico identification of hemicellulase producing microorganisms found in animal manure

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Phylogenetic tree

ABSTRACT

To speed up biological processes in all living things, nature has produced enzymes. In industries like leather, textile, food, paper, detergents, medicines, and feed, a variety of enzymes act as industrial biocatalysts, and their usage in commercial chemical reactions is expected to improve significantly. This study aims to find out the high activity of xylanase-producing microorganisms to reduce the cost of raw materials that are used in several commercial industries like paper pulp bleaching, bioconversion of lignocellulosic biomass into biofuels, etc. This study was conducted to characterize and identify the most highly efficient hemicellulose degrading enzymes from bacterial and fungal species by using bioinformatics methodologies. in this study, 27 xylanase-producing bacterial sequences and 13 xylanase-producing fungal sequences were retrieved from the NCBI data bank (http://www.ncbi.org). The predicted microorganisms were further analyzed by retrieving protein sequences from uniprot. This study concluded that Geobacillus strearothermophilus and Claviceps purpurea, exhibit a high level of specific enzyme activity as compared to target bacterial sp. Myceliophthora thermophila and fungal sp. Aspergillus nigar respectively at the favorable temperature of 60◦C with optimal neutral pH. The identified enzymes will be helpful in the reduction of cost and energy required in the conversion of biomass or in various other industrial sectors for the processing of raw material.

Introduction

The research of enzymatic potential within organic leftovers such as animal manure has attracted substantial attention in the development of sustainable approaches to the conversion of biomass and the management of waste [1]. Hemicellulases, enzymes that degrade intricate polysaccharides found in the cell walls of plants, have enormous promise in a variety of commercial uses [2, 3]. The identification and characterization of these digestive enzymes using computational methods referred to as in silico analysis, has emerged as an effective means of unraveling the enzymatic diversity inside animal excrement [4]. Digestive enzymes are a natural source of enzymes

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in animals found in the alimentary tracts of primates (including humans) and carnivorous plants, where they aid in food processing [3]. They are a large variety of enzymes that are produced by fungi, bacteria, and algae, etc are involved in the production of xylose, the enzyme secreted by bacteria, fungus that is involved in the degradation of hemicellulose and to check out the activity of these enzymes [5].

Animal feed production followed by the agriculture, paper, and clothing sectors used xylanase for the first time in the 1980s. Cellulase, xylanase, and pectinases currently account for 20% of the global enzyme market [6]. The synthesis of the xylanase enzyme is mostly dependent on microorganisms. In recent years, the bioconversion of hemicellulose increased attention because of its practical applications in improving the digestibility of animal feedstock [3]. For efficient utilization/degradation of hemicellulose, enzymatic saccharification into fermentable sugars are focused attention [6]. Therefore, hemicellulases with outstanding enzyme properties are still a bottleneck. Over the last few years, the importance of xylanase has grown dramatically because of its biological activities in different manufacturing processes such as paper, food, and pulp sectors [7]. Microbial xylanases have received a lot of attention. Xylanases are regarded as "one of the most industrially significant enzymes". Xylan is the most common natural hemicellulose and has a variety of industrial uses, including food, textiles, cellulose pulp bleaching, seed germination, degumming, and agro-waste treatment [8]. Hemicelluloses make up nearly 20–35 percent of lignocellulosic biomass, making them the most abundant polysaccharides in nature. Lignocellulose is comprised of cellulose, hemicelluloses, and lignin [7, 8]. Among hemicellulose, xylans are commonly found in nature. Different enzymes, such as xylanases and xylosidase, break down xylan within hemicellulose [9]. Microorganisms are primarily responsible for the manufacture of xylanase enzymes. Moreover, wide-range manufacturing of fungal xylanase is problematic owing to the prolonged period and collaboration of an extremely glutinous polymer that inhibits oxygen transport [10].

The xylan molecule is converted into constituent sugars by the combined activity of all these enzymes. Fungi, bacteria, algae, yeast, snails, and seeds have all been found to synthesize xylanases [11]. Bacteria fungi and bacteria, on the other hand, synthesize the majority of these enzymes. Bacterial xylanases differ from fungal xylanases in several ways. As a result, Bacterial and actinomycete xylanases work in a broader range of pH values of between 5 and 9, and at temperatures of around 35 to 60 ^{o}C which makes them suitable for a wide range of manufacturing sectors including pulp [14]. On the other hand, Fungi are substantial producers because of higher xylanase activity (compared to bacteria and yeast), higher yields, and extracellular enzyme release [11].

A differentiating factor between xylanase-producing fungal and bacterial species is the existence of cellulose-producing cellulase enzyme there are only a few instances of fungal xylanases that lack the activity of cellulose [12]. The investigation's purpose was to gain insight into how isolates could produce xylanases. The use of xylanase in a variety of sectors i-e Fabric bioprocessing, pulp and paper biobleaching and other waste reprocessing, and biological conversion into more desirable items, feed, and food has grown considerably in recent years [4]. Researchers are discovering more microbial strains for xylanase synthesis as a result of increased interest in this field of research. The manufacture of enzymes, chemicals, and antibiotics for various industries has been a recent emphasis of biotechnology [13].

The biomass can be successfully degraded by linking numerous enzymes that hydrolyze complicated polymers into monomers that can be fermented in suitable industrial environments [14]. All of these enzymes need to be active in a variety of circumstances including a high degree of temperature, high pH, extreme osmotic level, and fluctuating pressure. However, the activity of hydrolytic enzymes can only withstand extreme temperatures [15]. This large variety of circumstances necessarily results in an extended period for effective biomass decomposition, which poses the risk of reactions to contamination [13]. These difficulties can be addressed by increasing the temperature at which the reaction occurs. As a result, there is a significant need for xylanases that operate with greater efficiency at much higher temperatures than are currently accessible economically [16]. High temperatures increase substrate and product solubility, shorten the process of hydrolysis, decrease the viscosity of the substrate, and limit the potential of contamination by microbes, proving thermostable xylanases useful for a variety of industrial practices [10, 17].

- To be evaluated for this market, the xylanase must meet the following criteria:
- To avoid cellulose fiber hydrolysis, they must be cellulolytic [16].
- To diffuse efficiently through pulp fibers, they must have a lower molecular mass[18].
- At high temperatures and pH conditions, they must be active and stable [19].
- Enzyme production should be high and inexpensive [18].

Because of its biological activities in various manufacturing procedures such as paper pulp and food industries, the importance of xylanase has increased considerably in recent years. Microbial xylanase has gotten a lot of interest [16]. The purpose of this study is to find out the high activity of xylanase-producing microorganisms to reduce the cost of raw materials that are used in several commercial industries like paper pulp bleaching, bioconversion of lignocellulosic biomass into biofuels, etc. This study was conducted to characterize and identify the most probably highly efficient hemicellulose degrading enzymes from bacterial and fungal species by using bioinformatics methodologies.

Materials and methods

Study Site Description and Duration

The research work and experimentation were take place in Microbiology and Molecular genetics lab of University of Okara from April 2023 to September 2023.

In silico identification of microorganisms

In this study the xylanase producing bacterial and fungal species that found in animal manure were identified by using different databases such as NCBI and CAZy.

Retrieval of sequences

After the identification of xylanase producing bacterial and fungal species, a total of 27 xylanase producing bacterial sequences and 13 xylanase producing fungal sequences were retrieved from ncbi gene data bank (http://www.ncbi.org).

Analysis of hemicellulase enzyme's producing bacteria and fungi

Retrieved protein sequences from uniprot.org were used to conduct additional analysis on the identified microbes. Subsequently, the sequence collection of all retrieved sequences was compared with already identified xylanase-producing microorganisms. then all sequences were blasted against the target species including xylanase-producing bacteria *Myceliophthora thermophila* (strain ATCC 42464 / BCRC 31852 / DSM 1799) and xylanase-producing fungi with aspergillus nigar in NCBI blast (ncbi.nlm.nih.gov/Blast.cgi) and the corresponding DNA sequences were obtained in FASTA file format.

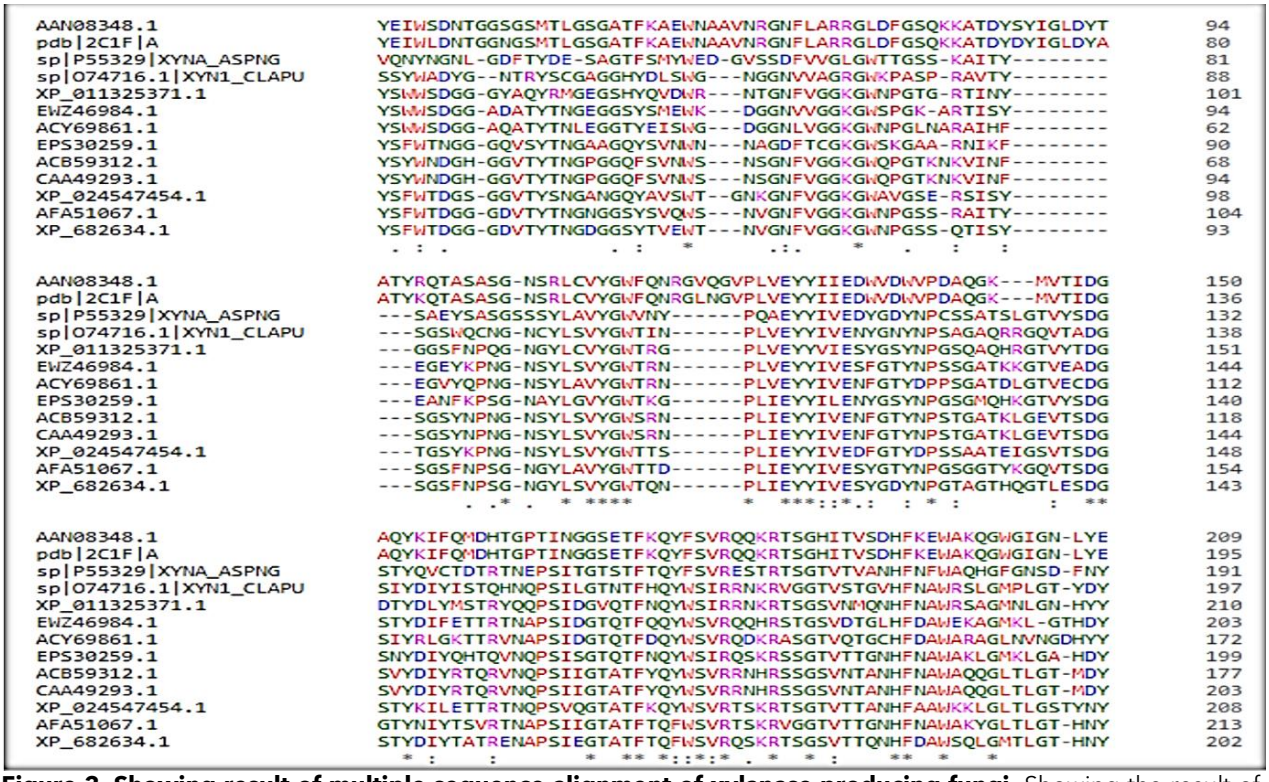

Figure 3. **Showing result of multiple sequence alignment of xylanase producing fungi**. Showing the result of alignment of multiple sequences of target fungal strain which retrieved from the uniprot with other xylanase producing fungal strains. Colors are used in order to highlight the conserved regions. According to multiple sequence alignment, the matching location was 31 to 36 and exhibited 8.094% identity, and claviceps purpurea shared a high degree of relationship with the target fungal strain *Aspergillus nigar* accession numbers O74716 and P55329 respectively.

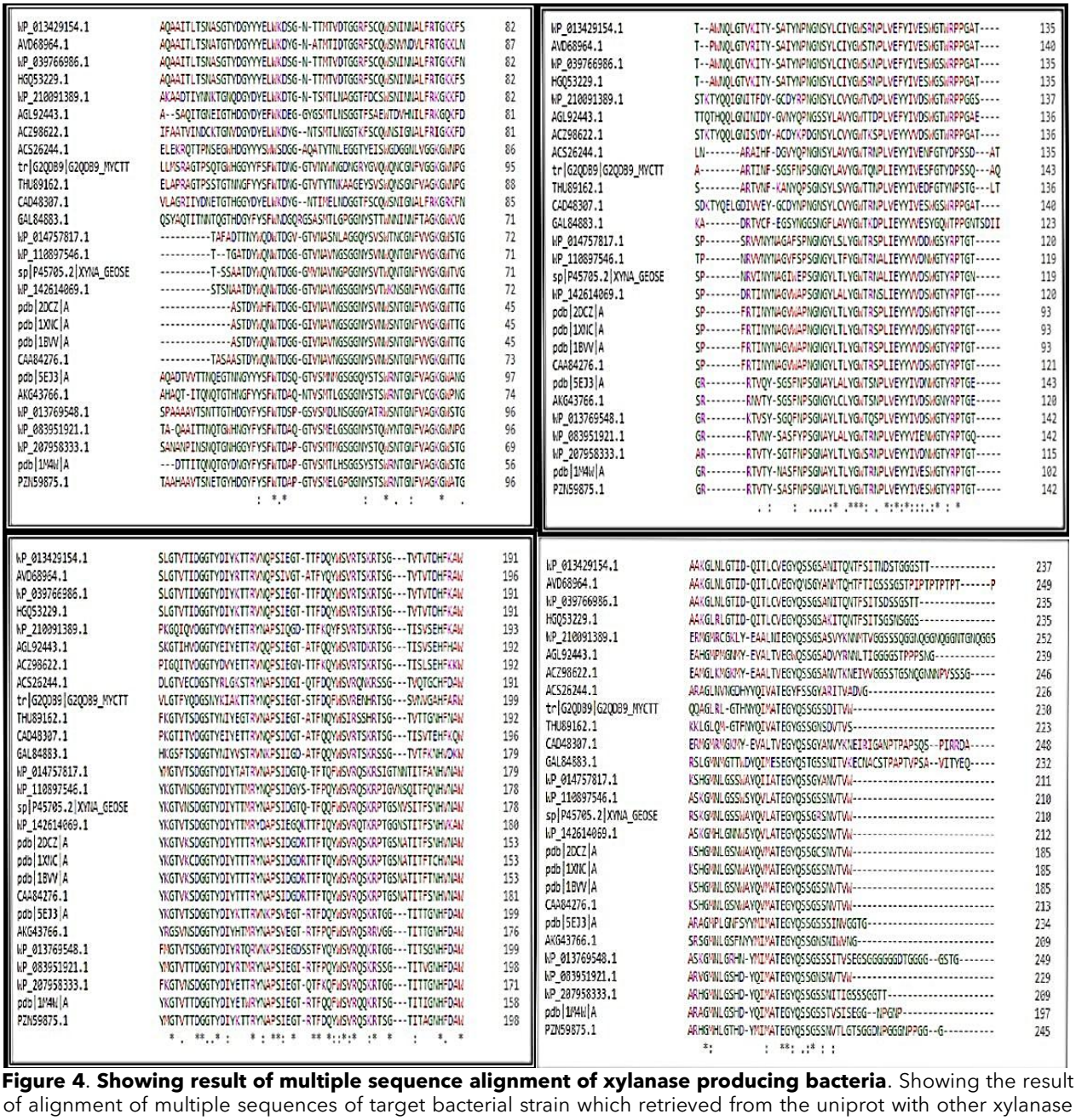

Figure 4. **Showing result of multiple sequence alignment of xylanase producing bacteria**. Showing the result of alignment of multiple sequences of target bacterial strain which retrieved from the uniprot with other xylanase producing bacterial strains. Colors are used in order to highlight the conserved regions. According to multiple sequence alignment, the matching location was 34 and 44 similar position and exhibited 3.205% identity, and Geobacillus stearothermophilus (P45705) shared a high degree of relationship with the target bacterial strain *Myceliophthora thermophila*.

Results

The sequences were aligned using CLUSTAL W and omega for multiple sequence alignment to identify conserved domains and confirm the protein's functionality. On the basis of a JTT matrix-based model, a phylogenetic tree of all 27 bacterial xylanases and 13 fungal xylanases protein sequences was developed employing the highest-likelihood method. MEGA7 programme was used to comprehend the evolutionary gaps between the proteins. To comprehend the evolutionary lineages, several groupings or splits of xylanase-producing bacteria were carefully investigated.

In this present study,NCBI & CAZy databases were used to identify xylanase producing bacteria and fungi. After the identification of xylanatic organisms, clustal omega software was used to align multiple sequences of these organisms. A total 27 xylanase producing bacterial sequences and 13 xylanase producing fungal sequence were retrieved from ncbi gene data bank (http://www.ncbi.org). All of these sequences were selected on the basis of the quality factors of xylanse synthesis. Bacterial multiple sequence alignment result showed that the highlighted area was the conserved portion, and

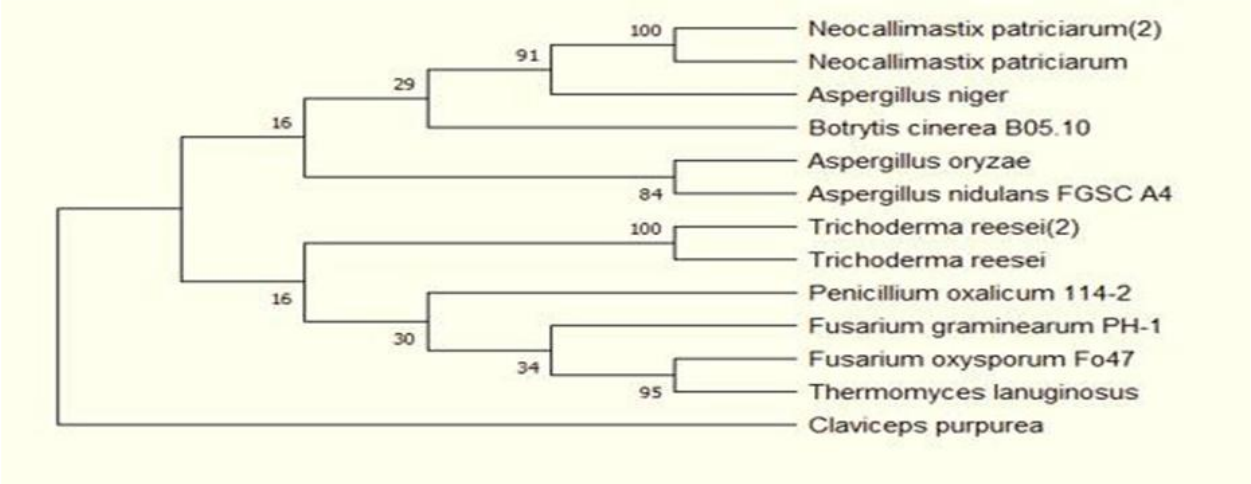

Figure 5. **Showing the phylogenetic tree of xylanase producing fungi by Maximum Likelihood method**. The phylogenetic relationship of Aspergillus nigar with other xylanase-producing fungal strains in the Genbank database. The neighbor-joining tree revealed the most similarity to claviceps purpurea xylanase. This study included 13 amino acid sequences. The final dataset contained 373 locations in total. MEGA X was used for phylogenetic research.

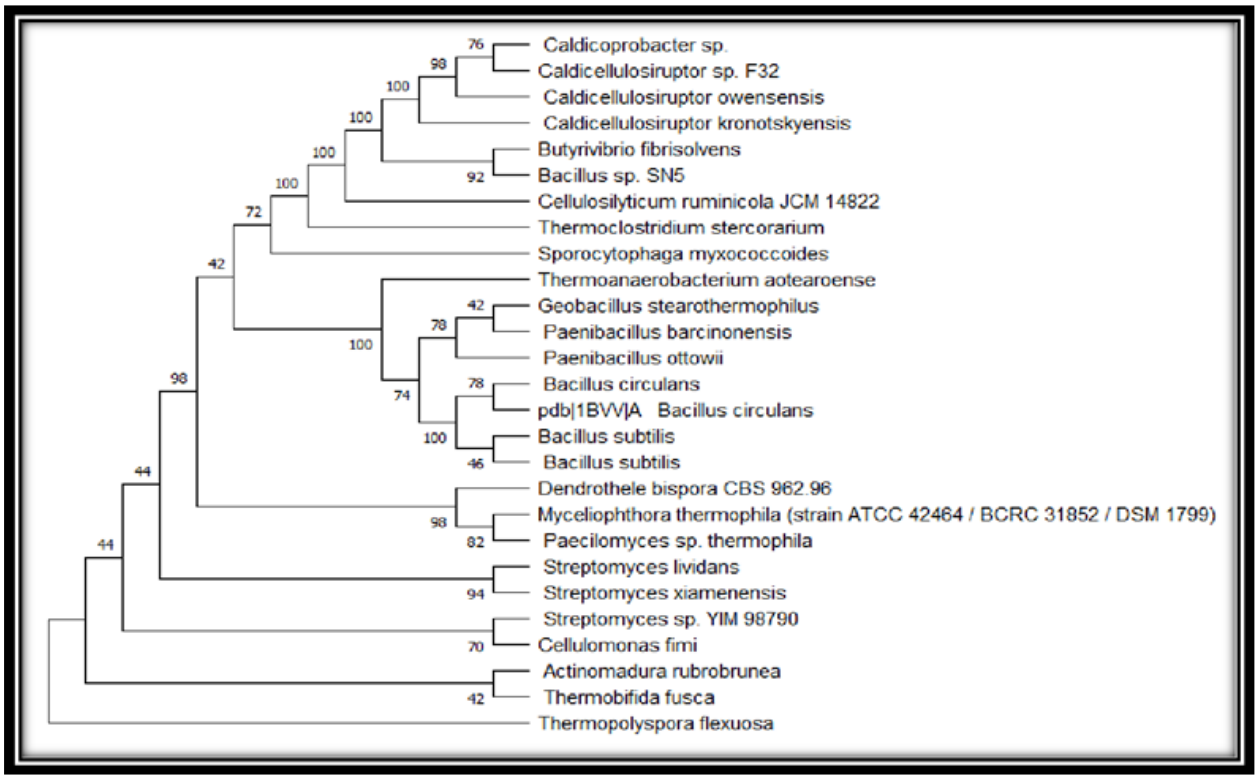

Figure 6. **Showing phylogenetic tree by using Maximum Likelihood method**. Phylogenetic tree of Myceliophthora thermophila xylanase with other xylanase-producing bacteria in the NCBI database The neighbor-joining tree revealed the greatest similarity to Geobacillus stearothermophilus. This study included 27 amino acid sequences. The final dataset contained a total of 880 locations. MEGA X was used for the construction of phylogenetic tree.

steric exhibits homology in all of these sequence alignments. Fig 4 showed that according to these alignment *Geobacillus stearothermophilus* (accession number P45705) was found to be very closely related to the targeted organism *Myceliophthora thermophila*. In the bacterial aligned sequence, 34 identical sites and 44 comparable locations indicated 3.205% identity Figure 4). The highlighted section of the xylanatic fungus alignment was a conserved area, and steric exhibited homology in all aligned sequences. Based on the alignment of multiple sequences, the identical location was 31-36 and showed 8.094% identity and claviceps purpurea (O74716) exhibited a high similarity with the targeted species Aspergillus nigar accession numbers P55329. Shown in (Figure 3).

Evolutionary analysis of fungi

The Maximum Likelihood approach and the JTT matrix-based model were used to build the phylogenetic tree. The figure shows the tree with the highest log likelihood (-4889.29). The initial tree(s) for the search for heuristics were generated automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances predicted using the JTT model and then choosing the topology with the highest log likelihood value. The fraction of places in each descendent clade where at least one unambiguous base is present in at least one sequence is given next to each internal node in the tree. This study included 13 amino acid sequences. The final dataset contained 373 locations in total. In, evolutionary analyses were carried out. Phylogenetic tree constructed by using MEGA X. According to phylogenetic analysis of xylanatic fungus it was observed that Fusarium oxysporum Fo47, *Fusarium graminearum* PH-1 showed 60% evouloutionary relationship. Penicillium oxalicum 114-2 showed 59% relationship. Claviceps purpurea, Botrytis cinerea B05.10 showed 61% relationship. Claviceps purpurea, Aspergillus niger showed 57% relationship. *Neocallimastix patriciarum* showed 63 % relationship.

Evoloutionary analysis of bacteria

The phylogenetic tree was constructed by using the Maximum Likelihood method and JTT matrix-based model. The tree with the highest log likelihood (-15292.63) is shown in Fig. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and Bio NJ algorithms to a matrix of pairwise distances estimated using the JTT model and then selecting the topology with superior log likelihood value. This analysis involved 27 amino acid sequences. There were a total of 880 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. According to phylogenetic analysis *Bacillus subtillis*, *Bacillus cereus*, *Bacillus circulans* Showed 78 to 98 % evouloutionary relationship and 3 strains of Caldicoprobacter sp. Showed 76 to 100% evouloutionary relationship. Streptomyces sp. Showed 70 to 92% evoloutionary relationship. *Cellulosilyticum ruminicola* JCM 14822, *Thermoclostridium stercorarium, Thermoclostridium stercorarium (Clostridium stercorarium), Hungateiclostridium thermocellum* JW20, *Dictyoglomus thermophilum, Butyrivibrio fibrisolvens,* Bacillus sp. SN5 showed 92% evolutionary relationship. Streptomyces sp. YIM 98790, *Cellulomonas fimi*, showed 70% *Thermobifida fusca, Thermopolyspora flexuosa*, showed 42%. paecellomyces sp. thermophila, *Myceliophthora thermophila* strain ATCC 42464, showed 42% to 78% and Geobacillus strearothermophilus showed 98% similarity with target *Myceliophthora thermophila* strain ATCC 42464 (Fig 6).

Discussion

A comprehensive review of the literature and biological database entries found a plethora of intriguing xylanase sequence information [6]. Gene sequencing databases for a wide range of bacterial and fungal genomes are readily available for xylanase protein sequence analysis [20]. Clustal omega and BLAST (National Centre for Biotechnology Information) algorithms were used to align bacterial and fungus xylanase sequences against target species Myceliopathora Thermophila and Aspergillus nigar, respectively. The identity and query coverage of the species under consideration were investigated [7]. To investigate further, 27 bacteria and 13 fungi were employed, and a more precise result was seen based on a study of currently available biological sequences data in biological databases (NCBI, UniProt). In silico sequence analysis indicated that bacteria, fungi, and actinomycetes produce xylanases [21]. The phylogenetic tree was constructed using the neighbor-joining (NJ) method revealing the xylanatic organism lineage. Using clustal omega, multiple sequence alignment was conducted. According to the results of 27 xylanatic bacterial alignment sequences, the highlighted area was the conserved portion and steric exhibits similarity in all of these sequence alignments. Fig 4 According to these results, one xylanase-producing bacteria, Geobacillus stearothermophilus (accession number P45705), resembled the target bacterial strain *Myceliophthora thermophila*. 34 identical locations and 44 comparable places in the bacterial-aligned sequence indicated 3.205% identity. The colored section of the xylanatic fungus alignment was a conserved region, and steric exhibited similarity in all aligned sequences. On the basis of the multiple sequence alignments, the matching location was 31-36 and exhibited 8.094% identities, and *Claviceps purpurea* shared a high degree of identity with the target species Aspergillus nigar accession numbers O74716 and P55329 respectively.

Conclusion

At the present time, several xylanases from various fungal sources have been identified. Two novel endo-1,4-xylanases were found in the present study from various xylanase-producing bacteria and fungi. However, in this study we found that *Geobacillus strearothermophilus* and *Claviceps purpurea*, demonstrating an increased level of particular enzymatic activity as compared to target bacterial and fungal sp. *Myceliophthora thermophila* and Aspergillus nigar respectively at a suitable temperature of 60 degrees Celsius and with a 7.0

pH. Because of their limited effect on saccharification, they are future options for commercial practices and biofuel generation.

Contribution of authors

None

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None

Conflict of interest

The author declare that they have no competing interest.

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