# Comparing *Alpha-xylosidase* Enzyme in Different Species *in Silico* using Bioinformatics Tools

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***Abstract*—Alpha-xylosidase is an enzyme that catalyzes the release of α-xylose. This enzyme belongs to different families of plant, bacteria, fungi, archaea, and protist. Retrieved amino acid of alpha-xylosidase from NCBI was analyzed for multiple sequence alignment, cluster analysis, conserved motif, and their Pfam analysis using different bioinformatics tools. In MSA retrieved different conserved residue amino acid for each group except in protist, only two conserved residues were found. The cluster analysis showed the evolutionary closeness among species using neighbor-joining construct method that showed two major clusters based on closeness of the alpha xylosidase enzyme sequences of the source organisms. Fifteen conserved motifs belong to different families were assessed. These identified motifs showed the evolutionary closeness among species at the molecular level.**

***Index Terms*—Alpha-xylosidase, Cluster, Motif, Pfam, Sequence.**

## I. Introduction

Alpha xylosidase (EC 3.2.1.177) that has a recommended name (alpha-D-xyloside xylanohydrolase) is an enzyme that catalyzes the release of α-xylose from the non-reducing terminal side of α-xyloside substrate [1]. A-xylosidases as an enzyme have been characterized, but their structures and mechanisms have limited information. Therefore, the research and development activities involving α-xylosidases become very interesting in today's investigation. Recently, some studies have purified and characterized some α-xylosidases from microorganisms and plants but few of them have examined their amino acid sequences. For *Bacillus* sp. (No. 693–1), a α-d-xylosidase was purified by precipitation with ammonium sulfate and successive chromatography on DEAE-Toy pearl 650 and hydroxyapatite [2]. According to the study, α-d-xylosidase was highly specific for α-glycosidic linkages. In *Lactobacillus pentosus*, it has been shown that *xylQ*, encoding α-xylosidase, is responsible for the metabolism of isoprimeverose [3]. As a glycoside hydrolase family 31, the specificity of the aglycone-binding site of *Escherichia coli* α-xylosidase was characterized by examining the enzyme's transxylosylation-catalyzing property [4]. In enzymatic studies of α-xylosidase, the amino acid sequence explained from *Sulfolobus solfataricus* which was the first α-xylosidase described in archaea. It has shown optimal activity at 90°C and high hydrolytic activity on the disaccharide isoprimeverose and catalyzes the release of xylose from xyloglucan oligosaccharides [5]. Relating to the plant, α-xylosidase could be playing an important role in cell expansion. Few studies suggested that α-xylosidase contributes to maintain the mechanical integrity of the primary cell wall in the growing and pre-growing plant tissues [6]. It was also mentioned that the α-xylosidase activity is necessary to remove the xylose side chains that will block the degradation of the backbone [7,8]. Two Arabidopsis (*Arabidopsis thaliana*) mutant lines with insertions in the a-xylosidase gene AtXYL1 were characterized, and both lines showed a reduction to undetectable levels of a-xylosidase activity against xyloglucan oligosaccharides [7].

In addition, the α-xylosidase has now been purified to apparent homogeneity by a facile procedure involving lectin affinity chromatography [9]. From cabbage leaves, an alpha-xylosidase activity against xyloglucan oligosaccharides was purified and measured by pentose release [8]. In this purification, α-xylosidase activity is present in apoplastic extractions from Arabidopsis seedlings. Naturally, α-xylosidase was produced by the fungus *Aspergillus niger* and activities in the same temperature and pH ranges. A α-xylosidase (AxlA) was purified and characterized from a commercial enzyme preparation from *A. niger*, and the encoding gene was identified [10].

## II. Materials and Methods

### *A. Retrieval of the Sequence*

*All 59 sequences of amino acid of alpha-xylosidase from different groups* such as *archaea, protist, plant, fungi*, *and bacteria were retrieved from NCBI databases* (http://www.ncbi.nlm.nih.gov).

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*B. Multiple Sequence Alignment*

The multiple sequence alignment ClustalW of the individual groups was performed using MEGA6 software.

*C. Phylogeny Analysis*

Neighbor-joining tree approach implemented in the MEGA6 software was used to construct phylogenetic tree relationship among sequences.

*D. Conserved Motif Identification*

The motifs were identified using MEME server (multiple EM for Motif Elicitation).

*E. Identification of Conserved Motifs Family*

Pfam database server was used to identify motif conserved sequence.

## III. Result and Discussion

*A. Retrieval of the sequence*

The amino acid sequence of alpha-xylosidase for all groups of organisms which include bacteria, fungi, protist, and archaea was retrieved from NCBI protein database which is listed with their accession number, species name, and source (Table I).

*B. Multiple sequence alignment*

MSA showed the presence of some conserved residues in all alpha-xylosidase sequences from different sources (bacteria, plant, fungi, and archaea), in case of protist sequences we found only two conserved residues in M (methionine) and G (glycine), and in all groups, when we did MSA for SG sequence, there was only one residue to be conserved and it was M (methionine).

*C. Phylogeny analysis*

Two major clusters were obtained from cluster analysis of plant, bacteria, fungi, archaea, and protist. Cluster A consisted of forty one species of (bacteria, fungi and archaea) while cluster B consisted of 18 species of (plant and protist)(Fig. 1).

Cluster analysis of archaea profile (Fig. 2) showed a major clusters containing the species (*Halorubrum litoreum*, *Halorubrum terrestre*, *Halorubrum arcis*, *Halorubrum sodomense*, *Halorubrum persicum*, *Halobellus rufus*, *Halalkalicoccus paucihalophilus, Halococcus agarilyticus*, *Halobellus clavatus,* and *Haloquadratum walsbyi*), whereas *S. solfataricus* P2 were out grouped from clusters.

While cluster analysis of bacteria contains all species except *Mesoplasma florum* was out grouped from cluster (Fig. 3).

Cluster analysis of plant contained a single major cluster. *A. thaliana*, *Capsicum chinense*, *Dario zibethinus*, *Gossypium arboreum*, *Cajanus cajan*, *Glycine soja*, *Cucurbita moschata*, *Pinus pinaster*, *Triticum urartu,* and *Apostasia schenzenica* except *Quercus suber* were out grouped (Fig. 4).

Cluster analysis of fungi profile showed two major clusters contained all species except *Hypsizygus marmoreus* and *Aspergillus luchuensis* (Fig. 5).

**TABLE I** Sequence for bacteria, archaea, plant, fungi, and protist

| Source | Names of organisms | ID. No. |
|---|---|---|
| Archaea | *Halobellus clavatus* | WP_089764400.1 |
| Archaea | *Halococcus agarilyticus* | WP_049898176.1 |
| Archaea | *Haloquadratum walsbyi* | WP_014555711.1 |
| Archaea | *Halobellus rufus* | WP_049986155.1 |
| Archaea | *Sulfolobus solfataricus P2* | CAB99206.1 |
| Archaea | *Halorubrum persicum* | PHQ38378.1 |
| Archaea | *Halorubrum sodomense* | WP_092919868.1 |
| Archaea | *Halalkalicoccus paucihalophilus* | WP_084383827.1 |
| Archaea | *Halorubrum arcis* | WP_007996116.1 |
| Archaea | *Halorubrum terrestre* | WP_007345443.1 |
| Archaea | *Halorubrum litoreum* | WP_008367079.1 |
| Protist | *Galdieria sulphuraria* | EME27948.1 |
| Protist | *Entamoeba dispar SAW760* | EDR29357.1 |
| Protist | *Monosiga brevicollis MX1* | EDQ90437.1 |
| Protist | *Entamoeba histolytica KU27* | EMD47715.1 |
| Protist | *Reticulomyxa filosa* | ETO30872.1 |
| Protist | *Cyanidioschyzon merolae strain 10D* | XP_005535776.1 |
| Protist | *Phytophthora sojae* | EGZ29380.1 |
| Plant | *Arabidopsis thaliana* | BAS22093.1 |
| Plant | *Quercus suber* | POE62868.1 |
| Plant | *Cajanus cajan* | KYP32436.1 |
| Plant | *Triticum urartu* | EMS58120.1 |
| Plant | *Glycine soja* | KHN24274.1 |
| Plant | *Pinus pinaster* | AAL40352.1 |
| Plant | *Apostasia shenzhenica* | PKA63523.1 |
| Plant | *Cucurbita moschata* | XP_022961483.1 |
| Plant | *Capsicum chinense* | PHU02026.1 |
| Plant | *Durio zibethinus* | XP_022734472.1 |
| Plant | *Gossypium arboreum* | KHG12090.1 |
| Fungi | *Talaromyces islandicus* | CRG89788.1 |
| Fungi | *Diplocarpon rosae* | PBP20223.1 |
| Fungi | *Verticillium alfalfae VaMs. 102* | XP_003001749.1 |
| Fungi | *Aspergillus luchuensis* | GAT20945.1 |
| Fungi | *Colletotrichum chlorophyti* | OLN85299.1 |
| Fungi | *Penicillium chrysogenum* | KZN85482.1 |
| Fungi | *Hypsizygus marmoreus* | KYQ39752.1 |
| Fungi | *Madurella mycetomatis* | KXX78646.1 |
| Fungi | *Valsa mali* | KUI69639.1 |
| Fungi | *Aspergillus lentulus* | GAQ07024.1 |
| Fungi | *Neonectria ditissima* | KPM38392.1 |
| Fungi | *Phialophora attae* | KPI40962.1 |
| Fungi | *Beauveria bassiana* | PMB64514.1 |
| Fungi | *Tolypocladium paradoxum* | POR32450.1 |
| Fungi | *Escovopsis weberi* | KOS22613.1 |
| Bactria | *Enterococcus gallinarum* | WP_061053204.1 |
| Bactria | *Klebsiella aerogenes* | AMQ60125.1 |
| Bactria | *Escherichia coli* | WP_058589855.1 |
| Bactria | *Thermoanaerobacterium aotearoense* | WP_014758167.1 |
| Bactria | *Streptomyces malaysiensis* | PNG97797.1 |
| Bactria | *Shigella boydii* | WP_039067018.1 |
| Bactria | *Confluentibacter citreus* | WP_100615348.1 |
| Bactria | *Clavibacter capsici* | WP_053775917.1 |
| Bactria | *Nitrospirillum amazonense* | WP_100085476.1 |
| Bactria | *Mesoplasma florum* | WP_097351472.1 |
| Bactria | *Pseudoalteromonas atlantica* | WP_096740340.1 |
| Bactria | *Alteromonas macleodii* | OZB97055.1 |
| Bactria | *Salmonella enterica* | GAS75352.1 |
| Bactria | *Paenibacillus bovis* | ANF96431.1 |
| Bactria | *Zunongwangia mangrovi* | WP_092543950.1 |

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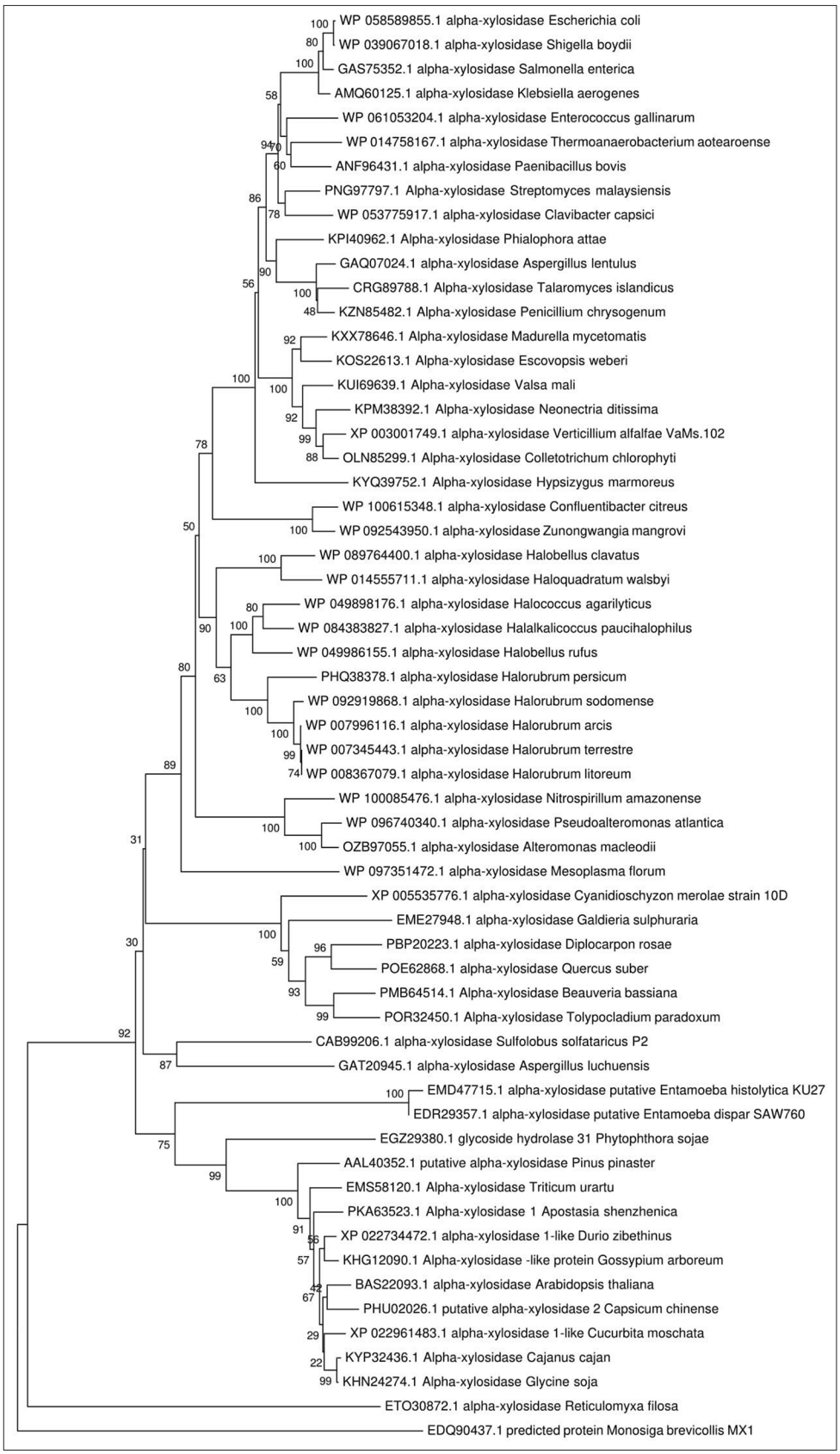


Fig. 1. Phylogenetic tree for bacteria, archaea, plant, fungi, and protest.

Cluster analysis of protist profile consists of two major clusters. Cluster A contained four species (*Entamoeba histolytica* KU27, *Entamoeba dispar* SAW760, *Phytophthora sojae,* and *Reticulomyxa filosa)*. Cluster B contained three species (*Monosiga brevicollis* MX1, *Galdieria sulphuraria,* and *Cyanidioschyzon merolae strain* 10D) (Fig. 6).

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**TABLE II** MOTIF AND MOTIF FAMILY

| Motif | Family | Source |
|---|---|---|
| PKWTFGVWMSRLSYZSREELESVAEELREREIPCDVLHVD | Glyco hydro 31 | Archaea |
| HWGGDPDTTFEGMAANLKGGLSLSLSGFPFWATDIGGFRGTPSEELYVRW | Glyco hydro 31 | Archaea |
| PWHFGEEAEAIVREYAKLRYRLLPYLYSYAERAARTGLPVWRPLVLEFZD | Glyco hydro 31 | Archaea |
| LRYQFIPYVYKKNYKLVLTALPVIRPLYL | NOT FOUND | Protist |
| YEWYRFMSKISPVLTLWADYNEPSSYFSNEMTLFRNAQHKIYEHREIHNC | NOT FOUND | Protist |
| FHEPFNKMPFNFFSTNYIILNFDEVGNPTTFNSFQHNDFKCDSJLFK | NOT FOUND | Protist |
| RPAPMPYWAFGFHQCRWGYHNLSVVEDVVENYYKKAKIPLDVIWNODDHMD | Glyco hydro 31 | plant |
| AQVWPGAVNFPDFLNPKTVSWWGDEIRRFHELVPVDGLWIDMNEASNFCS | Glyco hydro 31 | plant |
| PTEELCNRWIEVGAFYPFSRDHANYYSPRQELYQWESVAKSARNALGMRY | Glyco hydro 31 | plant |
| GERFGAFNKVGQSVSJWNEDGGTSSEQAYKNISFYLSSRGYGVFIBTPGK | Gal mutarotas 2 | fungi |
| WSYGLWLTTSFTTNYDEKTVNSFLEGMKERDIPVDVFHYDCFWMKAFQWC | Glyco hydro 31 | fungi |
| LGLSGFSFWSVDIGGFEGYPPPWJYKRWVAFGLLCSHSRLHGSSSYRVPW | Glyco hydro 31 | fungi |
| NYDEATVNSFIDGMAERBLPLSVFHFDCFWMKEFQWCDFEW | Not found | bacteria |
| VDFTNPDAVEWYADKLKGLVDMGVDCFKTDFGERIPTDVVY | Not found | bacteria |
| RSASVGSQRFPVHWGGDCYATYESMAESLRGGLSJGLSGFGFWSHDIGGF | Glyco hydro 31 | bacteria |

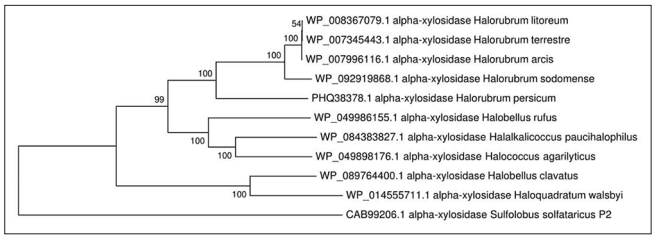


Fig. 2. Phylogenetic tree for archaea.

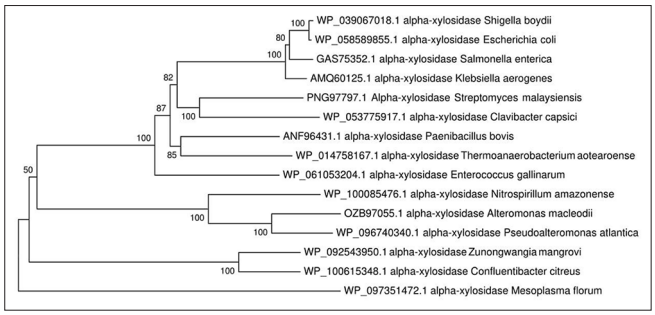


Fig. 3. Phylogenetic tree for bacteria.

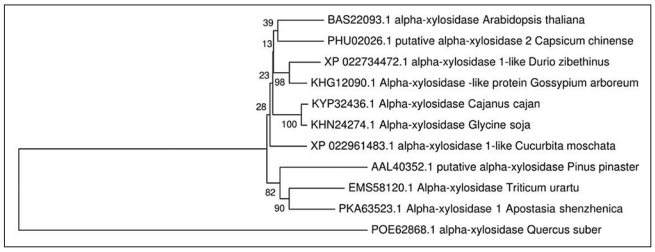


Fig. 4. Phylogenetic tree for plant.

### *D. Identify conserved motif*

In analysis sequence profile for plant, bacteria, fungi, archaea, and protist, 15 conserved motifs were obtained. Three conserved motifs were observed in each group (Table II).

In archaea and plant, profiles showed that all three conserved motif identification belonged to Glyco hydro 31 domain family. In fungi, profile showed that two of conserved motif identify belonged to Glyco hydro 31 domain

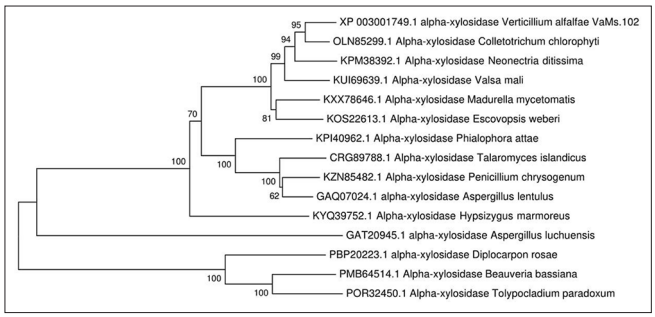


Fig. 5. Phylogenetic tree fungi.

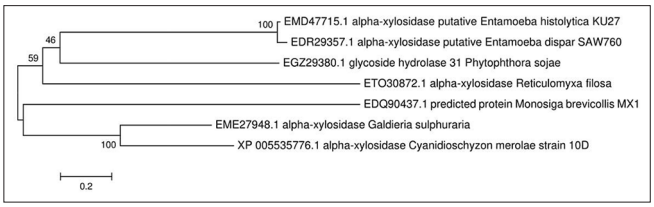


Fig. 6. Phylogenetic tree for protest.

family, whereas one conserved that motif identify belonged to Gal mutarotas 2 domain family. In bacteria, profile showed one conserved motif identify belonged to Glyco hydro 31 domain family, whereas the Pfam entry to other two bacteria conserved motif was not found. In protest, conserved motif Pfam was not grouped.

## IV. CONCLUSION

Our results revealed that alpha-xylosidase sequence in archaea, bacteria, plant, and fungi have many conserved residues area, but in protist, just two conserved residues were found. In clustering, we found that archaea, fungi, and bacteria close to each other all were in the same clusters, whereas plant and protist were in the other cluster. We obtained 15 different conserved motifs in plant, bacteria, fungi, archaea, and protist alpha xylosidase sequences profile.

Three conserved motifs were observed in each group. All groups bacteria, plant, archaea, and fungi conserved motif belong to glyco hydro 31 domain family. One motif sequence

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belongs to Gal mutarotas 2 domain family in fungi species, whereas in protist conserved motif Pfam was not grouped.

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