CHAPTER 5:

EXPLORING POSSIBILITIES OF APPLICATION OF THE ISOLATED ENZYMES WITH EMPHASIS ON ENHANCING WATERHYACINTH BIOCONTROL

5. EXPLORING POSSIBILITIES OF APPLICATION OF THE ISOLATED ENZYMES WITH EMPHASIS ON ENHANCING WATERHYACINTH BIOCONTROL

5.1 Introduction

Among the basic component of plant cell wall hemicelluloses, xylan constitute a major portion. The heterogeneous polymer is primarily composed of a linear β-(1,4)-D-xylan backbone partially acetylated and substituted with a variety of side chains, mainly including single α-D-glucuronosyl (and its 4-O-methyl ether) and α-L-arabinosyl units. The structural moiety of this polymer can be broken or completely degraded by the action of several hydrolases. Among the presence of several hydrolases, the key enzyme is xylanase or, more specifically, endo- β-(1,4)-xylanase (EC 3.2.1.8). The enzyme cleaves the internal β-(1,4) bonds in the xylan backbone at non-modified residues, yielding different chain length substituted xylooligosaccharides. Based on the structural and functional properties of these enzymes, the majority of the xylanases have been classified under the family 10 (formerly F, which consisted of high molecular mass xylanases with acidic pIs) and 11 (formerly G, which consisted of lower molecular mass xylanases with basic pIs) of the glycosyl hydrolases (GH) (Biely et al. 1997, Sapag et al. 2002). Xylanases have been reported, not only, in the biotechnology-oriented studies (Kulkarni et al. 1999) but also, in their phytopathogenic abilities to degrade plant cell walls (Walton 1994), along with induction of plant defence responsive mechanism (Lotan and Fluhr 1990).

Fungi, are known to, release an array of primary metabolites, often extracellular hydrolytic enzymes that are capable of degrading/damaging plant cell wall, collectively called cell-wall degrading enzymes (CWDEs). Several phytopathogenic fungi have been used as a promising source of range of CWDEs for industrial use as well (Giovannoni et al. 2021, Pietro et al. 2003). *Fusarium oxysporum*, well-known as a major crop phytopathogenic (Gómez et al. 2016) ascomycete whose genome encodes a complete xylanolytic degradative system, produces a stockpile of CWDEs which efficiently allows conversion of plant biomass (cellulose and xylan) into ethanol (Gómez-Gómez et al. 2002, Jaroszuk-Scisel and Kurek 2012). Enzymatic degradation of plant polysaccharides has been used in fuel and many other industries but no such literature of CWDEs associated with weed biocontrol, has been reported. Although a

lot of studies has been done using the fungi as control agent of the weed, yet no work has been done to study their primary metabolites (which embarks the breakdown of the plant cell wall during initiation of disease) as bioherbicides. Plant cell wall degradation and disintegration is crucial for pathogenesis and in wilt diseases as it initiates pathogen ingression into the host xylem and thereafter for fungal nutrition within the xylem (Jorge et al. 2005).

Endoxylanase (or Xylanase) (E.C. 3.2.1.8) from *F. oxysporum* sp. *lycopersici* 4287, have shown plant cell wall degradation (Ruiz et al. 1997, Ruiz-Roldan et al. 1999, Cardinale and Matta 2001, Gómez-Gómez et al. 2001, 2002). The overall high activity and efficiency at degrading plant cell-wall components make xylanase, from the fungi, a repertoire of strategic potential for the biofuel industries (Jaroszuk-Scisel and Kurek 2012). Nearly six different β-(1,4)-xylanases have been identified from the *F. oxysporum* genome belonging to the GH10 and GH11 families (Alconada and Martinez 1994, Christakopoulos et al. 1996a, 1996b, 1997, Gómez-Gómez et al. 2001, Kwon et al. 2007), which have been characterised and discussed for their role in pathogenesis. We have also found similar results where the strain of fungi have shown that the xylanase enzyme has one of the best biocontrol potential against the targeted weed, waterhyacinth (mentioned in the previous **chapter 4**).

Hence aiming to focus on the potentiality of such metabolites against this weed, the possibilities of studying the enzymes, purifying them and enhancing their activity through understanding the physiochemical and biotechnological studies that would help to improve their bioherbicidal potential against waterhyacinth.

5.2 Materials and methods

5.2.1. *Culture condition and enzyme production*

The fungus, *F. oxysporum* sp. *lycopersici* 4287, was cultivated at its optimized condition, of 27 ^{o}C for 72 hours incubation, using 1.5 % concentration of xylan in the growing medium at pH 4.0, for production of the enzyme at their best active condition.

[The fungus was itself grown in a similar nutrient medium composition when grown in agar solid plates, for maintenance of similar parity in growing conditions. The plates were incubated for 7 days at 28 ^{o}C for conidia production.]

5.2.2. *Enzyme extraction and purification procedure*

The enzyme xylanase, produced in the fermented culture media was filtered using a Whatmann (no. 1) cellulose filter paper (with 0.1 µm pore size), The filtrate was then subjected to centrifugation at 10,000 rpm for 10 min at 4 ^{o}C (to avoid enzyme denaturation), to remove the mycelia and other media components. The supernatant was then vacuum filtered using 0.2 µm cellulose filter discs to remove spores completely and the supernatant used as the crude enzyme.

The supernatant was subjected to fractionated ammonium sulphate (45-60 % w/v) precipitation in an ice-bath (**Annexure I-1.4**) followed by centrifugation (10,000 rpm for 25 mins at 4 °C) for partial purification of the enzyme. The pellet was suspended again, partly in

(i) 20 mM Tris HCl - 100 mM NaCl buffer (pH 8) and partly in,

(ii) 50 mM Acetate - 100 mM NaCl buffer (pH 4) (based on the pH of the media used)

They were refrigerated and stored overnight. The suspension was then dialyzed against the same buffer with PD10 gel-filtration chromatography (pre-packed with Sephadex G25) column (**Annexure I-1.5**), for desalting (Aygan et al. 2008). The salt-free, purified enzyme was spectrophotometrically observed at 290 nm to estimate the presence of protein and assayed for their enzyme activity.

The enzyme fraction, with the best xylanase activity, was then spun through Amicon® Ultra-15 Centrifugal Filter Units (Merck) (with a membrane of 30 kDa cut-off), attached with a Thermo Fisher Scientific Heraeus Multifuge X1R Swing out rotor centrifuge at 5000 rpm for 45 min at 4 ^{o}C.

5.2.3. *Electrophoresis and molecular mass determination*

Sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) (**Annexure I-1.6**) and Native-PAGE were carried out with 10 % polyacrylamide gel using Mini Protean II electrophoresis system (Bio-Rad, Richmond, Calif.) as described by Laemmli (1970). Proteins on the polyacrylamide gel were stained with 0.2 % Coomassie brilliant blue (CBB) R-250. Fermentas Page Ruler™ pre-stained protein

ladder Plus (SM 1811) of the low molecular weight range (Bio-Rad) including 10, 15, 25, 35, 55, 70, 100, 130, 250 kDa marker proteins were used as standards for estimation of molecular weight of the purified xylanase.

Enzyme assay: At all purification steps, enzyme activity was measured by DNSA assay method (Bernfeld, 1955).

Protein determination: The protein content of the enzyme solution at every step of purification was estimated according to Lowry et al. (1951) with Bovine serum albumin (BSA) as standard (**Annexure I-1.7**).

5.2.4. *Biocontrol potential of the enzymes and their observation at tissue level*

Untreated and unaffected waterhyacinth leaves were transversely sectioned with a fine sterile blade. The sections kept in distilled water in watch glasses (to prevent dehydrating of the sections) to which prepared Safranin stain was added and kept for 1 - 2 mins (depending on the concentration). It was followed by double dilution by washing with distill water for 5 mins each. The section was observed under the microscope to observe the section of waterhyacinth leaf in the control condition.

The purified enzyme, xylanase (the activity of which was previously measured by DNSA method), in their optimised condition, were further analysed by observing their action, at a regular interval (after application of the enzyme), by adding on the transverse tissue section of pre-stained untreated waterhyacinth leaves, under a microscope (Nikon Trinocular Research Model E with Nikon DSLR model 5100) and images of change were recorded accordingly. Observations were also made after overnight incubation (over 20 hours) of the tissue section, in microscopic slide, at 27 ^{o}C, with the addition of the enzyme.

5.2.5. *Confirmation of the presence of active genes of the potent fungal enzyme via expression profiling by RT-PCR*

5.2.5.1. **Preparation of the fungal cDNA**

Substrate-confined fungi inoculated and substrate-less fungi inoculated mat were both collected, weighed and cryopreserved at -80 ^{o}C, adding liquid N_2. Inoculation of the

fungi was performed based on optimised condition, as previously, for xylanase production. RNA was isolated, from the filtered cryopreserved samples, following the protocol provided by Nucleospin® RNA Plant and Fungi Kit (**Annexure I-1.8**) and cDNA prepared using BioRad iScript cDNA Synthesis Kit (1708890) (**Annexure I-1.9**) respectively.

5.2.5.2. **Primers and PCR amplification of the xylanase gene (Xylanase expression profiling)**

The GenBank accession number for glycoside hydrolases family (here, GH9, GH10, GH11), confirming the presence of the FO (*F. oxysporum* sp. *lycopersici* 4289), of the potent enzymes (here, xylanases 2, xylanase 3 and xylanase 5) were recorded from NCBI (https://www.ncbi.nlm.nih.gov/) and CAZy (http://www.cazy.org/) databases. From the FASTA sequences obtained respective primers were designed using the online primer designing tool, Primer 3 (https://bioinfo.ut.ee/primer3-0.4.0/) (**Annexure I-1.13**). With the different sets of primer expression profile of both the substrate-treated and untreated cDNA was analysed to confirm the presence of active gene component of the carbohydrate-active enzyme responsible for the cell-wall degradation.

Primer (**Annexure I-1.12**) used:

Xylanase 2 (XYL2)	F: 5'>GCTCAATCGGACAAACCAAT<'3
	R: 5'>GTCGGTGATGGTTCCGTAGT<'3
Xylanase 3 (XYL3)	F: 5'>ATCTTCGCTGAGGACGGTAA<'3
	R: 5'>GGCAAGCGTTCATGACAGTA<'3
Xylanase 5 (XYL5)	F: 5'>TGGTAAGGGTTGGTCTCCTG<'3
	R: 5'>GCTGGAAGGTCTGAGTACCG<'3

The PCR reaction mixtures contained 5 µl of PrimeSTAR® Max DNA Polymerase (by Takara Bio), 1 µl of each of the forward and reverse primers of the respective xylanase gene (Bioreserve-Labdeals), 1 µl of prepared template fungal cDNA, and 2 µl of nuclease free water to make in a total volume of 10 µl. The PCR started with a 6- min

denaturation at 98 ^{o}C, followed by 32 cycles of 30 seconds at 98 ^{o}C, 20 seconds at 55 ^{o}C, and 45 seconds at 72 ^{o}C. A final extension of 3 min at 72 ^{o}C, 12 ^{o}C for 15 mins was performed, ending at 4 ^{o}C standby. The amplified PCR products were analyzed by agarose gel electrophoresis (**Annexure I-1.10**), where the aliquots of the PCR-end products were mixed and loaded with the loading dye and ran with 1X TAE Buffer, until 70 % run coverage. It was then stained with EtBr (Ethidium Bromide) for 15 mins in a gel rocker (for uniform mixing and staining)

The above PCR was repeated with 22, 24, 26, 28, 30 cycles and the gene expression was assessed, by visualizing the width and clarity of the band observed. β-actin (a housekeeping gene) was used as control.

5.2.6. *Phylogenetic analysis of the active genes of the potential fungal enzyme*

Four sets of complete full-length CDS which encodes the glycoside hydrolase family 11 (GH11) endoxylanases, with known enzyme structure and sequences, were retrieved using *F. oxysporum* sp. *lycopersici* 4287 endo-β-1,4-xylanase- XYL2, XYL3 and XYL5 genes, respectively from the NCBI database were selected and send to nBLAST. Only complete CDS were chosen and chromosomal, bacterial and unidentified sequences were ignored. In addition, hit sequences (of about 250 sequences) were then narrowed, to remove the identical pairs and the N-terminal peptide leader sequence was trimmed (analysed with the FASTA sequences obtained). The remaining sequence pool containing unique sequences, restricted to *Fusarium* isolates, were arranged and aligned using default parameters, group wise according to the respective gene and gone for multiple alignment.

As the xylanase gene is proportionate to genes for the enzyme, therefore the nucleotides sequences were converted into protein sequences. The sequences were then further analyzed and aligned by multiple alignment and then phylogenetic tree were prepared with the maximum-likelihood to reconstruct their phylogenetic relations and understand the evolutionary lineage.

Sequence analysis, amino acid alignment and phylogenetic tree analysis for the carbohydrate active enzyme, of the selected fungal species, was carried out using CLC sequence viewer software version 8.0 (Qiagen, Germantown, MD, USA). This were

done to understand the relatedness among the different enzymes produced from different species of the selected fungi, grouped into the several categories of the active genes responsible for the cell-wall degradation.

5.3 Results

5.3.1. *Enzyme purification and molecular weight determination*

Xylanase on partial purification by ammonium sulphate precipitation were suspended partly in 20 mM Tris HCl - 100 mM NaCl buffer (pH 8) and partly in 50 mM Acetate - 100 mM NaCl buffer (pH 4). On analyzing the enzyme activity in both conditions, it was found that the suspension in 50 mM Acetate - 100 mM NaCl buffer (pH 4), showed better activity in comparison to the other and among the 2 cut percentages, 60 % w/v (187.33 U/ml) had better activity, with respect to 45 % (157.42 U/ml), highlighted well in **Table 5.1**.

Table 5.1: Enzyme activity of the partially purified xylanase, after ammonium sulphate precipitation

Buffer condition where pellets were suspended	**Enzyme Activity (U/ml) (at 45 % w/v cut)**	**Enzyme Activity (U/ml) (at 60 % w/v cut)**
50 mM Acetate - 100 mM NaCl buffer (pH 4)	A_{45}= 134.93	A_{60}= 187.33
20 mM Tris HCl - 100 mM NaCl buffer (pH 8)	TH_{45}= 119.86	TH_{60}= 157.42

The purified protein (Acetate buffer fraction used henceforth) showed a single band on CBB-stained SDS-PAGE at around 24 kDa (**Figure 5.1**). The enzyme produced by FO seems to be a monomer, which was further confirmed by the single band on native gel.

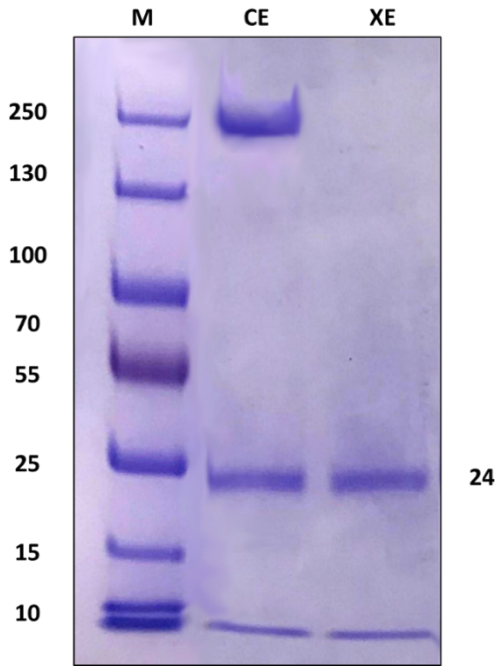


Figure 5.1: SDS-PAGE of purified xylanase from *Fusarium oxysporum* sp. *lycopersici* 4287. CE: crude enzyme; XE: purified xylanase enzyme; M: Molecular weights markers (kDa).

The results of the purification procedure referred to 200 ml of culture filtrate, in several stages of purification are summarised in **Table 5.2**.

Table 5.2: Purification of a major xylanase from xylan culture filtrates of *F. oxysporum* sp. *lycopersici*

Buffer condition where pellets were suspended	Enzyme Activity (U/ml)	Total Protein (mg/ml)	Specific Activity (U/mg)
Crude extract	382.31	11.59	32.99
After ammonium sulphate precipitation	187.33	5.63	33.27
After Gel-filtration chromatography	134.93	3.23	41.77

5.3.2. *Biocontrol potential of the enzyme and their observation at tissue level*

The microscopic image of a transverse tissue section of waterhyacinth leaves shows slow degradation and dissolution of the cell layers (**Figure 5.2**, under 10 X magnification) few hours after incubation with the xylanase, hinting at the potency of the enzyme for cell wall degradation and biocontrol of the waterhyacinth. On leaving the tissue section overnight, a secondary infection appeared that was clearly visible with the presence of mycelial development on the tissue section (**Figure 5.3**, under 10 X magnification).

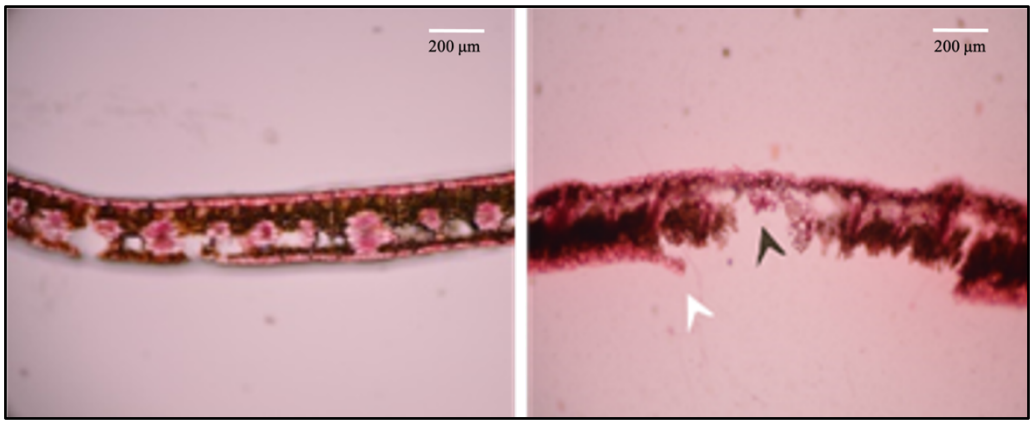


Figure 5.2: Slow dissolution and disintegration of the cell membranes, when the tissue was incubated with xylanase enzyme from *F. oxysporum* sp. *lycopersici* 4287

Figure 5.3: Secondary mycelial outgrowth on the outer epidermal layer of the waterhyacinth leaf, because of effect of xylanase treatment, after overnight incubation (over 20 hours)

The epidermal layers of transverse section waterhyacinth leaf, shows a peeling off effect because of xylanase treatment (**Figure 5.4**, under 40 X magnification).

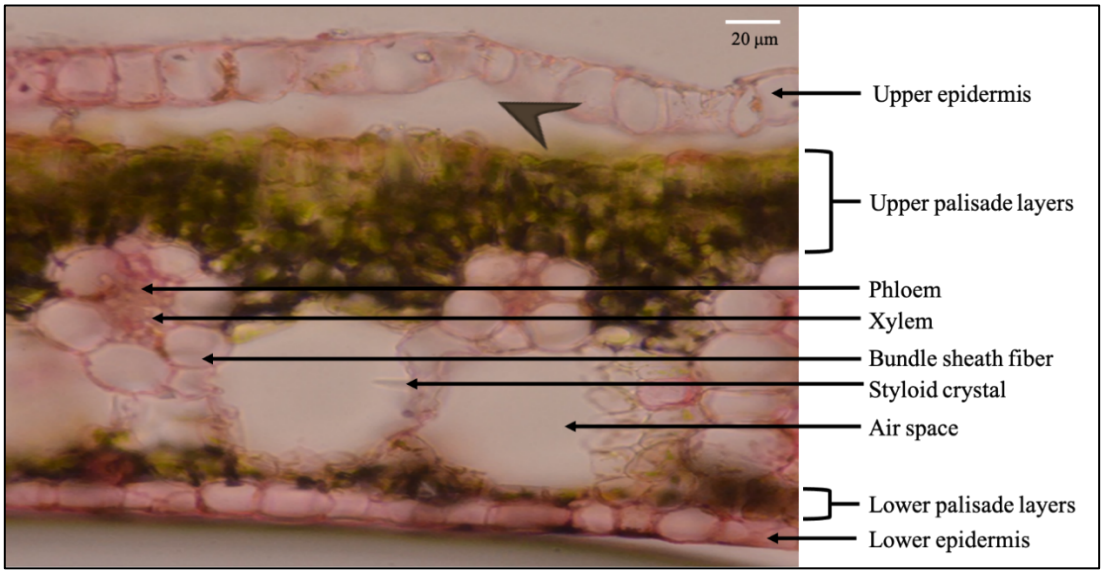


Figure 5.4: Peeling-off of the outer epidermal layer of the waterhyacinth leaf, because of effect of xylanase treatment

5.3.3. *Confirmation of the presence of active genes of the potent fungal enzyme via expression profiling by RT-PCR*

Optimising the expression profiling of the RNA derived cDNA of the xylanase genes (**Annexure I-1.11)**, it was found that on running the PCR for a loop of 24 cycles, best and optimised result (in the form of differentiation of the band width) was visibly observed, after gel electrophoresis. The xylan-inoculated media grown fungal mat showed higher expression (for XYL2, XYL3 and XYL5, from FO) than no substrate induced growing media, with the former having a thicker band intensity difference than the later one (**Figure 5.5**), hinting at the activation of a particular enzyme producing genes (XYL2, XYL3 and XYL5), in presence of xylan induced growing media.

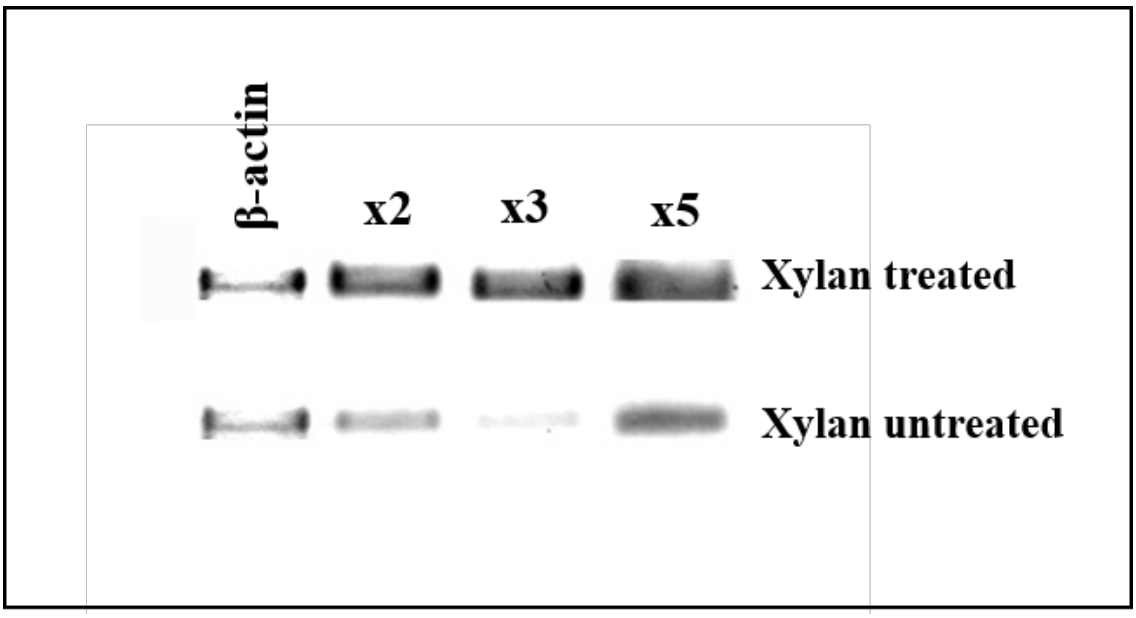
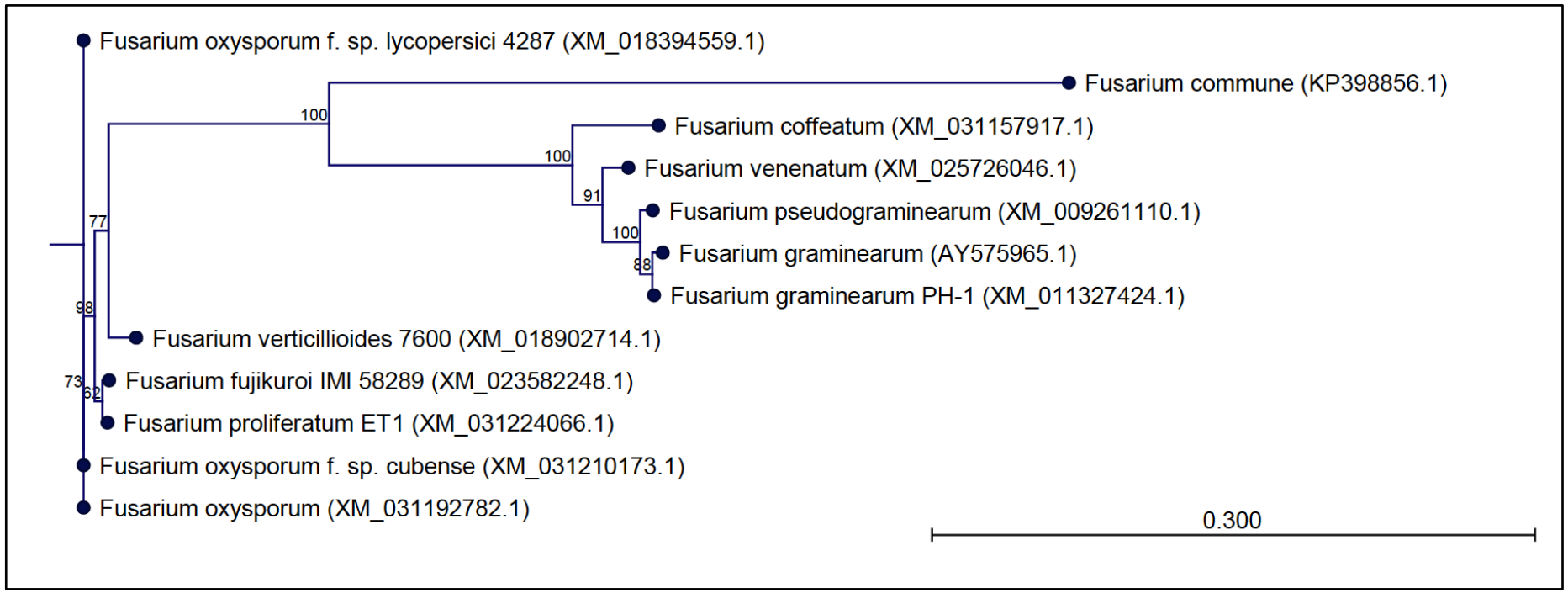


Figure 5.5: Amplification of the cDNA of xylanase family (XYL2, XYL3 and XYL5) enzyme, produced by *Fusarium oxysporum* sp. *lycopersici* 4287, observed on both treated and untreated xylan-inoculated growth media, with reference to β-actin (house-keeping gene) as control

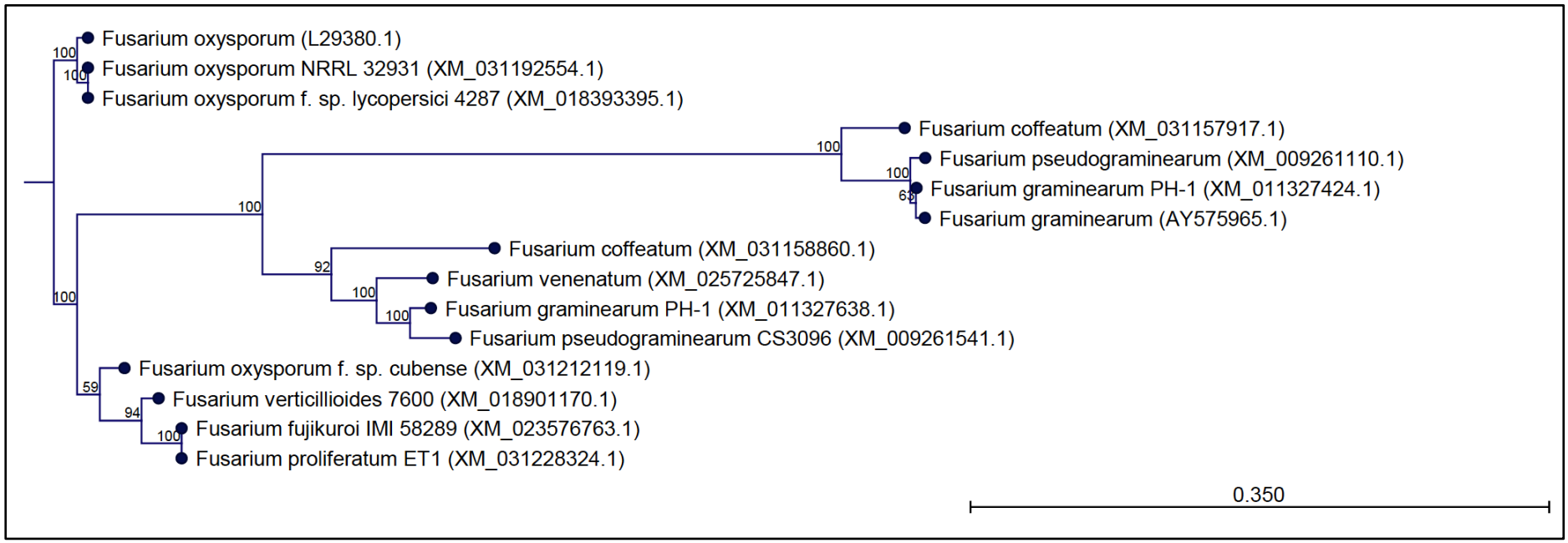
5.3.4. *Phylogenetic analysis of the active genes of the potential fungal enzyme*

The phylogenetic analysis of the XYL2 and XYL5 genes of the xylanases of FO showed that it belonged to different clades with respect to the respective xylanase genes produced by other *Fusarium* sps. (**Figure 5.6 a** and **c**). However, in case of XYL3, FO shared its clade with two closely related *Fusarium oxysporum* species - *F. oxysporum* NRRL 32931 (XM_031192554.1) and *F. oxysporum* (L29380.1) (**Figure 5.6 b**).

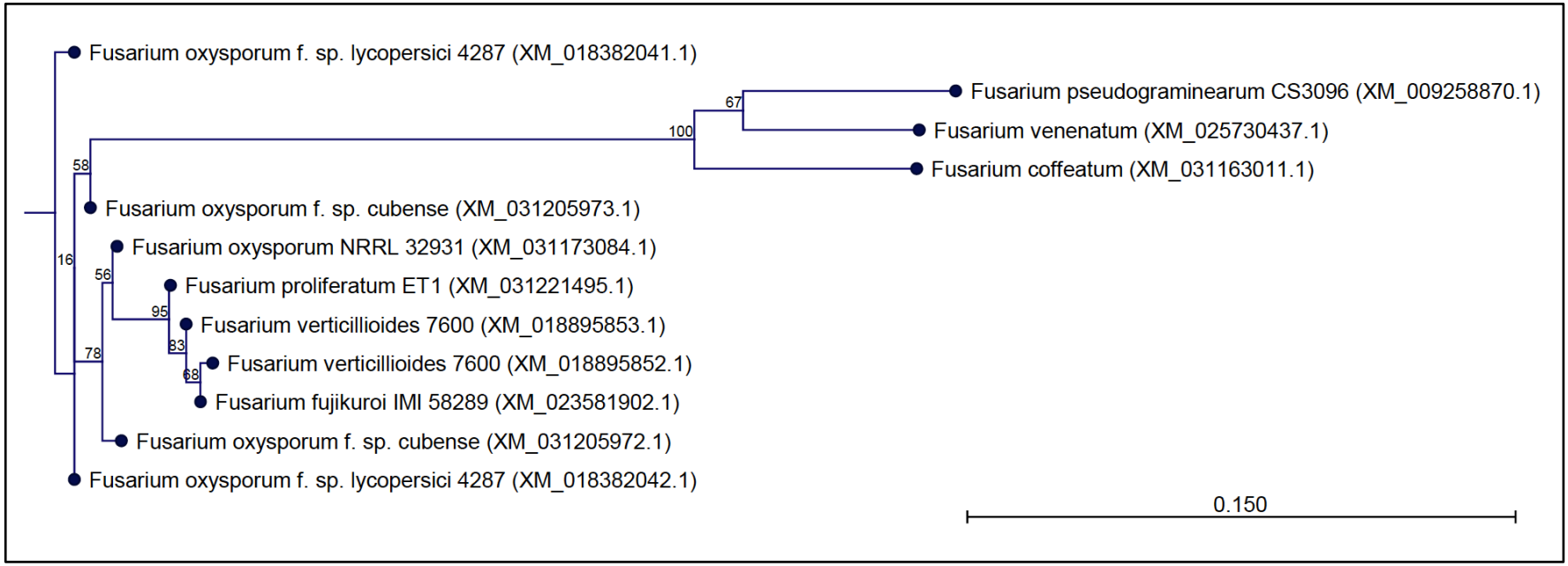
F. coffeatum, F. venenatum, F. pseudograminearum and *F. graminearum* (missing in case of XYL5), belong to an evolved monophyletic group with a significant number of deletions in the amino acid sequences. In comparison to the phyletic-outgroup (involving *F. proliferatum, F. fujikuroi* and *F. verticilloides*) they lie in the evolutionary pathway, of the xylanase genes, from *F. oxysporum* sps. to the initially mentioned monophyletic group.


Fusarium oxysporum f. sp. lycopersici 4287 (XM_018394559.1)
Fusarium commune (KP398856.1)
100
Fusarium coffeatum (XM_031157917.1)
100
Fusarium venenatum (XM_025726046.1)
91
Fusarium pseudograminearum (XM_009261110.1)
77
100
Fusarium graminearum (AY575965.1)
88
Fusarium graminearum PH-1 (XM_011327424.1)
98
Fusarium verticillioides 7600 (XM_018902714.1)
73
Fusarium fujikuroi IMI 58289 (XM_023582248.1)
62
Fusarium proliferatum ET1 (XM_031224066.1)
Fusarium oxysporum f. sp. cubense (XM_031210173.1)
Fusarium oxysporum (XM_031192782.1)
0.300

a. XYL 2


Fusarium oxysporum (L29380.1)
Fusarium oxysporum NRRL 32931 (XM_031192554.1)
Fusarium oxysporum f. sp. lycopersici 4287 (XM_018393395.1)
Fusarium coffeatum (XM_031157917.1)
Fusarium pseudograminearum (XM_009261110.1)
Fusarium graminearum PH-1 (XM_011327424.1)
Fusarium graminearum (AY575965.1)
Fusarium coffeatum (XM_031158860.1)
Fusarium venenatum (XM_025725847.1)
Fusarium graminearum PH-1 (XM_011327638.1)
Fusarium pseudograminearum CS3096 (XM_009261541.1)
Fusarium oxysporum f. sp. cubense (XM_031212119.1)
Fusarium verticillioides 7600 (XM_018901170.1)
Fusarium fujikuroi IMI 58289 (XM_023576763.1)
Fusarium proliferatum ET1 (XM_031228324.1)
0.350

b. XYL3



c. XYL 5

Figure 5.6: The phylogenetic (neighbour-joining) tree of *F. oxysporum* sp. *lycopersici* 4287 with respect to the other closely related *Fusarium* sps. based on the xylanase (a. XYL2; b. XYL3; c. XYL5) genes. The horizontal branches are proportional to the number of the amino acid substitutions per site.

5.4. Discussion

Among several phytopathogenic primary metabolites, xylanases from FO have shown a significant potential in biocontrol of the weed, waterhyacinth. The enzyme has been reported to have shown plant cell wall degradation nature (Gómez-Gómez et al. 2001, 2002), which helps to break the integrity and make the weed susceptible to further secondary infections, that eventually lead to the death and control of the weed. Reports of β-xylosidase degrading minor sugars (Holden and Walton 1992) could also add to breakage of the cell wall moiety of the target host weed, thus enhancing the infection process.

Enzymes, like many other metabolites, works best at their optimised condition and for that understanding the nature of these enzymes, becomes absolute necessary, especially when it comes to understanding their mode of action (Anasontzis et al. 2011, Dimarogona et al. 2012, Jorge et al. 2005, Moukouli et al. 2011, Ruiz-Roldan et al. 1999). It is also seen that xylanase when maintained at acidic pH of around 4 (using acetate buffer) have shown more activity than in higher pH conditions. Similar trend of results for xylanase production have been priory reported from *Fusarium* sps. (Saha 2002) or other fungal pathogens (Sardar et al. 2000, Lu et al. 2008)

Microbial xylanases are usually monomeric proteins (Törrönen and Rouvinen 1997), the SDS-PAGE result provided, is considered a good estimation of the purified xylanase molecular weight. The molecular weight of xylanase of FO, after SDS-PAGE was found to be 24 kDa, quite close to other reports from several strains to *F. oxysporum* sps. (Jorge et al. 2005, Christakopoulos et al. 1996a). However, with each level of purification, the enzyme activity units (U) recovered at each step decreases. This usually happens because proteins are either lost in various steps, or they are also denatured during manipulations. On the other hand, the specific activity of the enzyme, which is the ratio of activity units to amount of protein (U/mg), increase during the purification. Several undesired tagged or untagged proteins are purified away, but the preferred protein (who's giving the activity) remains, thus enriching at every step and the ratio of the total activity to total protein increases.

Proteolytic enzymes, like xylanase, hydrolyze the peptide bonds in proteins (Bateman and Basham 1976). Several plant diseases, highlighted particularly by the soft rots;

tissue maceration is a characteristic prominent symptom, which involves the separation of cells from each other within a tissue system. Such effects seen in the form of tissue peeling off were distinctly noticed when the tissue of waterhyacinth was treated with the previously mentioned enzyme, xylanase. The enzymatic basis of tissue maceration has clearly been established in studies dating almost a century back (De Bary 1886, Jones 1909, Brown 1915). This phenomenon was accredited to the digestion of the "inter-cellular cement" or the middle lamella of the plant cell walls (Bateman and Basham 1976). Depletion and/or alteration of the plant cell wall constituents during pathogenesis is the straightest way of showing the involvement of CWDEs in plant disease. As noticed here, enhancement of secondary infections after the damage caused by these primary metabolites have been observed also in trying to understand the biocontrol mechanisms of *Trichoderma koningiopsis* PSU3-2 against post-harvest Anthracnose of Chili Pepper (Ruangwong et al. 2021).

FO strains have shown the presence of three effective xylanase genes, out of four, belonging to the glycoside hydrolases family of the CAZy (Carbohydrate-active enzyme) database with a molecular weight around 22 kDa. They either alone or in combination are required for the xylan growth production by the fungi (Apel-Birkhold and Walton 1996). The presence of redundant enzymes is becoming common in many CWDEs and the significance of redundancy might be due to dissimilarity in their pattern of expression and/or activity. The XYL2, XYL3 and XYL5 needs to be purified to assure the rest. However, the encoding genes have distinct patterns of expression. This pattern of production of cell wall-degrading enzymes during pathogenesis, causing decomposition has been reported for *Fusarium oxysporum* sp. *lycopersici* (Jones et al. 1972), since ages. Similarly, from a study of the cutinases and esterases of *Alternaria brassicicola*, Yao and Köller (1995) have drawn a distinction between enzymes typical of saprophytic growth and those associated with pathogenic growth.

Phylogenetic analysis of the xylanase gene, related to the RNA expression profiling, were done to understand the related taxa and subgroups for the enzyme profiles to evolutionary lineages. Xylanases from *F. oxysporum* was found to be more closely related than the monophyletic clade to which the *F. coffeatum, F. venenatum, F. pseudograminearum* and *F. graminearum* belongs. The degree of catalytic amino acid residues conservation and gene length all tends to favour the existence of an ancestral common enzyme sequence that corresponds to the GH10 and GH11 family

of enzymes, to which the xylanase group of enzymes entails. These evolutionary lineages and relatedness draw a highlight to the relevance of the xylan-degrading enzymes across the *Fusarium* sps.

Though phylogenetic evidence depending on a particular gene is very insufficient to justify taxonomic evolutionary lineages (Rokas et al. 2003), but works on the toxigenic potential and phylogenetic correlation in *Fusarium* sps. have been undertaken lately too (Kristensen et al. 2004), for further understanding of the mode of actions and their potentiality.

5.5. Conclusion

The ability of phytopathogens to produce enzymes that show cell wall degradation properties and can cut down model substrates is one of the initial steps in relation of a particular enzyme to pathogenicity. This approach is of course often helpful in examining the types of enzymes as a pathogen and its capability of producing and in guiding studies relating to infection and disease. But as stated by Hancock (1967), mere demonstration of a degradative enzyme of pathogen origin in infected or diseased tissue does not indicate involvement of that enzyme in pathogenesis or destruction of invaded host tissues. Therefore, prior to understand the nature of the CWDEs, potentiality of these should be tested, in advance for proper understanding of their nature, relatedness and mode of action in the biocontrol potential against environmental threats, like weed infestation.