

EVALUATION OF XYLANASE PRODUCTION FROM FILAMENTOUS FUNGI WITH DIFFERENT LIFESTYLES

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Xylanase plays an important role in the food, feed, and pulp/paper industry. Filamentous fungi have been considered as useful producers of this enzyme from an industrial point of view, due to the fact that they excrete xylanases into the medium. In this study, four fungal species belonging to different genera, i.e. *Aspergillus*, *Cochliobolus*, *Pyrenophora*, and *Penicillium* were isolated from different sources and compared for their ability to produce xylanase in submerged culture. The fungal species showed enzyme activity as determined by dinitrosalicylic acid (DNS) method. It was found that the two saprophytic *Aspergillus* strains, i.e. *A. terreus* (Fss 129) and *A. niger* (SS7) had the highest xylanase activity of 474 and 294 U ml⁻¹ at pH 7 and 8, respectively, in the presence of corn cob hulls after 120 h of incubation. The production of xylanase seemed to be strongly influenced by the interactive effect of initial pH on the fungi. Interestingly, xylanase was better produced by the saprophytic fungi of *Aspergillus* and *Penicillium* than by the plant pathogenic ones of *Cochliobolus* and *Pyrenophora*. This work provides additional information to support future research on fungi with different lifestyles for food industrial production of xylanase.

Keywords: *Aspergillus*, *Cochliobolus*, *Pyrenophora*, *Penicillium*, xylanase, submerged culture

Xylanase (EC3.2.1.8) is an industrially important enzyme that hydrolyzes xylan by breaking the hemicelluloses of the plant cell wall and produces xylooligosaccharides, xylobiose, and xylose (BEG et al., 2001; PAES et al., 2012). This activity has applications in the food and paper-making industries, along with uses in agriculture and for human health. Recently, interest in xylanase has markedly increased due to its wide utilisation in the food industry such as bread making, the production of corn starch, clarification of fruit juice and wine; animal feeds, and alcoholic fermentation (KUMAR et al., 2017; GUIDO et al., 2019). This enzyme is produced by diverse group of organisms, such as bacteria, algae, and fungi (COLLINS et al., 2005). However, although xylanase can be obtained from bacteria and yeasts, the enzymes from fungi meet generally industrial demand, since they are usually excreted extracellularly, facilitating extraction from fermentation media (POLIZELI et al., 2005).

The diversity of filamentous fungi is extremely high in nature, they have been recognised as a target for screening to find out the appropriate source of enzymes with useful and/or novel characteristics (QUINTANILLA et al., 2015). They are considered useful producers of xylanase, due to their capability of producing high levels of extracellular enzymes and their very easy cultivability (SHANKAR et al., 2018). However, improvement of fungal strains for high xylanase production is needed for reducing the cost of the industrial process and also to possess some specialised desirable characteristics.

Genetic engineering, using classical mutation methods and recombinant DNA technology, has been used to increase the expression levels of a large number of microbial

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enzymes (ADRIO & DEMAIN, 2014). However, application of modern techniques to improve the xylanase production does not invalidate the search for wild organisms producing useful enzymes. In addition, due to the need to obtain xylanases with specific processing characteristics, especially in developing countries with low technological capabilities, screening fungal cultures for enzyme production will be suitable. Therefore, screening of naturally occurring fungi may be the best way to obtain new strains and/or xylanases for industrial purposes.

Aspergillus and *Penicillium* fungi have a saprophytic lifestyle in decaying organic and plant materials; this requires an enzymatic activity that is able to degrade plant cell wall polysaccharides (TSANG et al., 2018). On the other hand, the two plant fungi *Cochliobolus sativus* ‘foliar pathogen’ and *Pyrenophora graminea* ‘seed-borne pathogen’ are cereal pathogens that in addition to their role in plant disease, have potential industrial applications; they have been shown to produce cell wall degrading enzymes, including xylanase, during the infection process. These fungi start their lifestyles as biotrophic pathogens degrading plant cell-walls and then switch to necrotrophic growth behaviour (RODRÍGUEZ-DECUADRO et al., 2014).

In this study, the production of xylanase enzyme from a set of fungi, i.e. *Aspergillus*, *Cochliobolus*, *Pyrenophora*, and *Penicillium*, covering different lifestyles was compared under submerged culture to determine their potential as sources of industrial enzymes.

1. Materials and methods

1.1. Fungi

Four different fungal species, from the genera *Aspergillus*, *Cochliobolus*, *Pyrenophora*, and *Penicillium*, were used in this study (Table 1). Two local *Aspergillus* strains, *A. terreus* (Fss 129) and *A. niger* (SS7), isolated from soil by BAKRI and co-workers (2010), were used. The saprophyte *Penicillium canescens* strain F58 from the Institute of Plant Biotechnology, Tbilisi, Georgia was also included in the experiments (ASSAMOI et al. 2008). Two cereal fungal pathogens, *Cochliobolus sativus* (Cs5) and *Pyrenophora graminea* (Pg16) were chosen in this investigation due to their high virulence and ability to produce cell wall degrading enzymes (ARABI et al., 2004) (Table 1).

Table 1. Fungal species used in this study

Fungi	Strain	Origin	Type	Source	Reference
<i>Aspergillus terreus</i>	(Fss 129)	Syria	Saprobe	Soil	BAKRI et al. (2010)
<i>Aspergillus niger</i>	SS7	Syria	Saprobe	Soil	BAKRI et al. (2010)
<i>Penicillium canescens</i>	F58	Georgia	Saprobe	Soil	ASSAMOI et al. (2008)
<i>Cochliobolus sativus</i>	Cs5	Syria	Plant pathogen	Barley-leaves	ARABI & JAWHAR (2003)
<i>Pyrenophora graminea</i>	Pg16	Syria	Plant pathogen	Barley-seeds	ARABI et al. (2004)

1.2. Enzyme production

The fungal strains were screened for xylanase production in Erlenmeyer flasks (250 ml) containing 50 ml of basal culture medium (g l⁻¹): yeast extract 5.0; Na₂HPO₄·2H₂O 10.0; KCl 0.5 and MgSO₄·7H₂O 0.15. Fresh fungal spores were used as inocula and 1 ml spore suspension (containing around 10⁶ spores ml⁻¹) was added to the sterilised medium and incubated at 30 °C for 5 days in a rotary shaker (120 r.p.m). Xylanase production in the basal medium supplemented with the carbon sources (birchwood xylan, wheat straw, wheat bran, or corn cob hulls) was evaluated (Table 2).

Table 2. The optimal substrate (1%) and temperature for xylanase production by fungal species in submerged culture

Fungi	Substrate	T (°C)
<i>Aspergillus terreus</i> (Fss 129)	Corn cob hulls	30
<i>Aspergillus niger</i> (SS7)	Corn cob hulls	43
<i>Penicillium canescens</i> (F58)	Xylan	30
<i>Cochliobolus sativus</i> (Cs5)	Wheat straw	30
<i>Pyrenophora graminea</i> (Pg16)	Wheat bran	30

1.3 Effect of medium pH and incubation temperature

The influence of initial medium pH on xylanase production was assessed by cultivating the strain in the basal media of pH ranging from 3.0 to 9.0. The effect of temperature was studied by performing the fermentation at different temperatures from 25 to 55 °C.

1.4. Enzyme determination

Xylanase activity was determined by the optimised method described by BAILEY and co-workers (1992) using 1% birchwood xylan as substrate. The xylan solution and the enzyme at an appropriate dilution were incubated at 55 °C for 5 min, and the reducing sugars were determined by DNS procedure with xylose as standard (MILLER, 1959). The released xylose was measured spectrophotometrically at 540 nm. Xylanase activity was expressed as 1 µmol xylose per min per millilitre (I U ml⁻¹). Results given are the mean of triplicate experiments.

1.5. Statistical analysis

Data was subjected to analysis of variance using the STAT-ITCF statistical programme (2nd version). Differences in xylanase production among different genera were evaluated for significance by using Newman–Keuls test at 5% probability level.

2. Results and discussion

In the current work, xylanase activity of four fungal species belonging to different genera, i.e. *Aspergillus*, *Cochliobolus*, *Pyrenophora*, and *Penicillium*, was investigated (Table 1). All tested fungi exhibited enzymatic potential, which was highly dependent on the tested species.

However, both saprophytic strains *A. terreus* (Fss 129) and *A. niger* (SS7) showed maximum xylanase production with corn cob hulls as carbon source (474 and 294 U ml⁻¹, respectively) after 120 h of incubation, followed by the other saprophytic strain *Penicillium canescens* F58 (54.01 U ml⁻¹) with xylan as a carbon source (Fig. 1).

This may reflect their lifestyle mechanisms, since these saprophytic fungi are exposed to complex lignocellulosic materials such as corn cob hulls, and their response is complex and leads to the up-regulated transcription of several carbohydrate active enzymes and accessory proteins (BAKRI et al., 2010; CORADETTI et al., 2012; BENZ et al., 2014). Therefore, this biological lifestyle may help genera *Aspergillus* to play different functions in soils, which include either active roles, such as the degradation of dead plant material, or inactive roles, where propagules are present in the soil as a resting stage (ARVANITIS & MYLONAKIS, 2015).

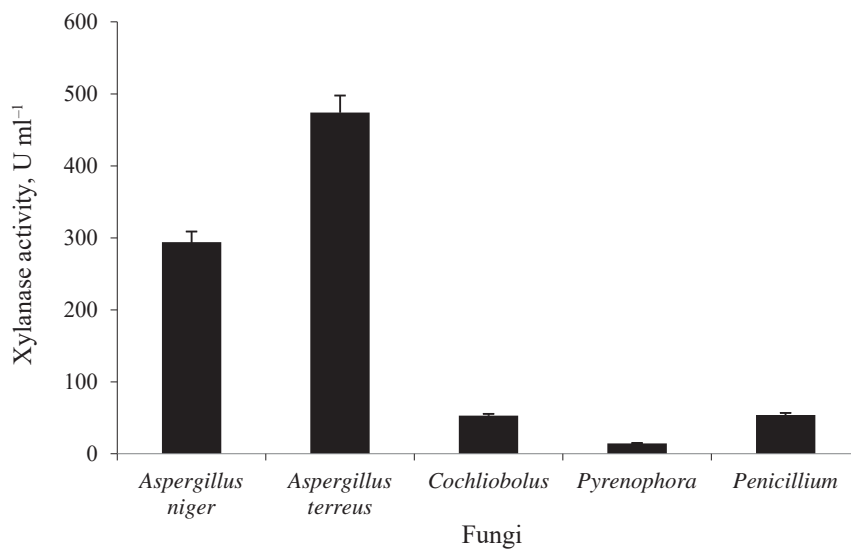


Fig. 1. Xylanase production by four fungal species in submerged culture media in the presence of corn cob hulls for *A. niger* and *A. terreus*, xylan for *Penicillium*, and wheat straw for both *Cochliobolus sativus* and *Pyrenophora graminea*. Error bars display the standard deviation among two biological replicates.

On the other hand, the plant pathogens *Cochliobolus sativus* Cs5 and *Pyrenophora graminea* Pg16 were the lowest producers of xylanase enzyme (52.81 and 14.26 U ml⁻¹, respectively) with wheat straw as a carbon source (Fig. 1). Although these fungi are widely different in their infection behaviour as foliar and seed borne pathogens, however, they started their lifestyles as biotrophs for penetrating plant cell walls and intimate contact with living host plant cell membranes, and then for the necrotrophic stage the fungus can produce a full range of digestive enzymes, and a complete expression of digestive metabolism, so that the fungus can take full advantage of the plant cell as a nutrient source (JOBIC et al., 2007). Interestingly, our data demonstrated that the seed-borne pathogen *Pyrenophora graminea* produced lower amounts of xylanase enzyme than the foliar *Cochliobolus sativus*; this might

be attributed to the foliar infection occurring faster than seed infection, which requires more enzymes for degrading the plant cell wall and consequently spreading the mycelium very fast.

Temperature and pH are important cultural parameters that determine growth rate and have major effect on levels of enzyme production by microorganisms. Fungal xylanases generally exhibit activity within a broad pH range, i.e. pH 3 to pH 8 (SUBRAMANIYAN & PREMA, 2002). The same is true for this study, with all fungal species exhibiting xylanase activities across pH 4–8 (Fig. 2). Our results showed that the optimum pH for xylanase production was between 7 and 8 for the genus *Aspergillus*, whereas, it ranged from 4.5 to 6.5 for the other fungi (Fig. 2). These observations concurred with earlier generalisations that fungal xylanases are more stable at acidic to neutral pH than at basic pH (COLLINS et al., 2005).

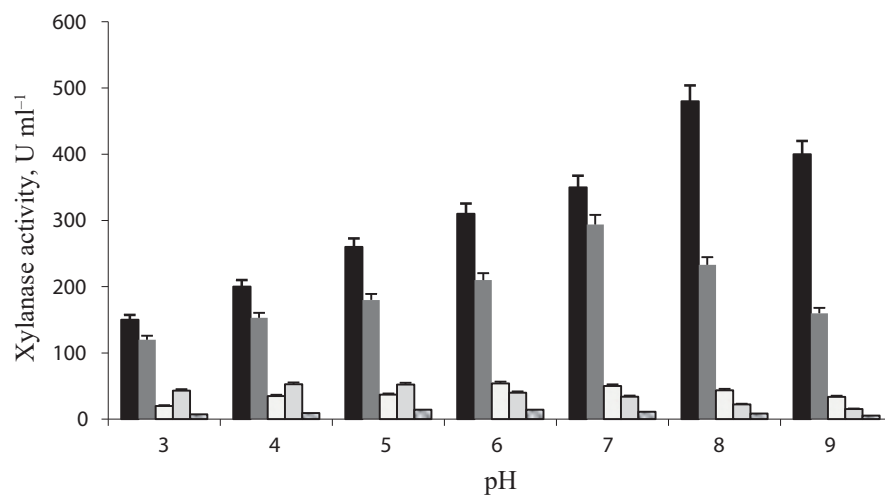


Fig. 2. Effect of different initial pH values on xylanase production by four fungal species
 ■: *A. terreus*; ■: *A. niger*; ■: *P. canescens*; ■: *C. sativus*; ■: *P. graminea*

The fermentation temperature has marked effect on the level of xylanase production, as it plays important role in the metabolic activities of microorganisms (SEYIS & AKSOZ, 2003). In this study, the optimum temperature of 30 °C (except for *A. niger* it was 43 °C) was observed for xylanase production for the fungal species (Fig. 3; Table 2). However, a decrease in xylanase production was observed at 25 °C, and increasing the incubation temperature up to 55 °C significantly reduced enzyme production (Fig. 3). This decline is due to the lower growth rate of fungi at high temperature. The high and low incubation temperatures cause the inhibition of fungal growth that ultimately leads to the decline in enzyme synthesis (LENARTOVICZ et al., 2003). On the other hand, submerged culture was used in this study, which allows control over the degree of aeration, pH and temperature of the medium, as well as control over other environmental factors required for optimum growth of organisms (ESCOBAR et al., 2017).

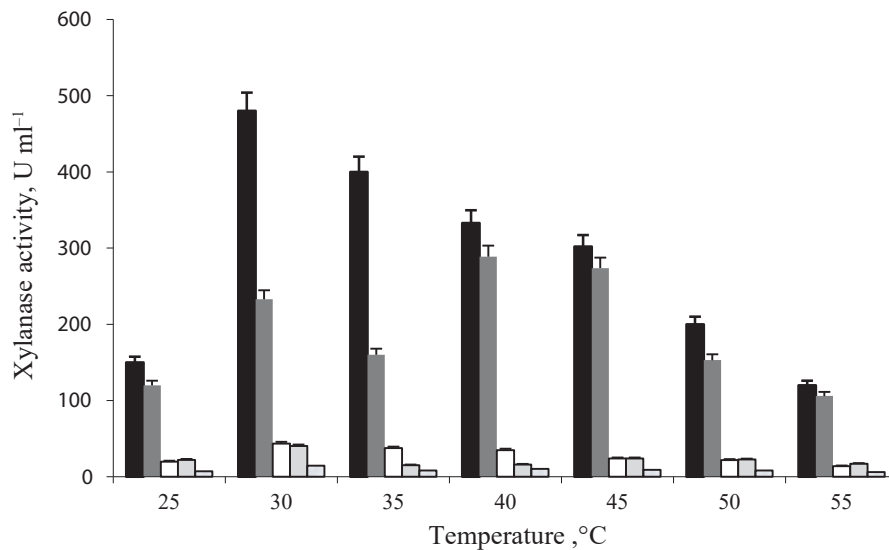


Fig. 3. Effect of temperature on xylanase production by four fungal species
 ■: *A. terreus*; ■: *A. niger*; ■: *P. canescens*; ■: *C. sativus*; ■: *P. graminea*

3. Conclusions

Our study revealed significant differences in xylanase production found among the four different genera, i.e. *Aspergillus*, *Cochliobolus*, *Pyrenophora*, and *Penicillium* in liquid medium. Interestingly, xylanase was produced better by the saprophytic strains of *Aspergillus* and *Penicillium* than by the plant pathogenic ones of *Cochliobolus* and *Pyrenophora*. It was found that the two saprophytic *Aspergillus* strains, *A. terreus* (Fss 129) and *A. niger* (SS7), had the highest xylanase activity of 474 and 294 U ml⁻¹ at pH 7 and 8, respectively. Additionally, data showed that the production of xylanase seemed to be strongly influenced by the interactive effect of initial pH on the fungi. The optimum pH for xylanase production by both isolates was found between 7 and 8 for the genus *Aspergillus*, whereas from 4.5 to 6.5 for others at 30 and 43 °C.

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