Original Research Article

Xylanase-producing fungi diversity in the soil of Jeddah, Saudi Arabia

ABSTRACT

Aims: To assess the diversity of xylanase-producing fungi in the soil of Jeddah, Saudi Arabia, as well as the xylanase-producing potential of the isolated fungi.
Methodology: A total of ten soil samples were collected from five different sites in the Jeddah district, Saudi Arabia. The soil samples were subjected to a soil dilution assay to estimate the total fungal counts and density percentage of isolated fungal species on xylan agar medium. Further, the xylanase activity of the isolated fungi was assayed using xylan from birch wood (0.01%) as the substrate.
Results: A total of twelve fungal species (*A. alternata, A. flavus, A. niger, A. terreus, B. cinerea, F. roseum, F. solani, P. chrsogenum, P. italicum, P. canescens, R. microsporus, and R. oryzae*) related to six genera were isolated from the ten soil samples. The population of different fungi and the isolated species varies at different sites. Overall, *A. niger* was isolated with the highest occurrence and population density (37.2%). Moderate occurrence was shown by both *P. chrsogenum* (18.6%) and *R. microsporus* (23.5%). The maximum significant value (P=0.05) of extracellular xylanase enzyme was observed in the culture filtrate of *A. alternata* (0.58 units/ml), whereas the minimum value was detected with *F. roseum* (0.20 units/ml).
Conclusion: The soil in the Jeddah district of Saudi Arabia has a good population of xylanase-producing fungi. The relative density of the isolated fungi varied in different soils. The isolated fungal species were capable of producing xylanase. The isolated fungus, *A. alternata,* should be explored further for its extracellular xylanase production for use in biotechnology applications.

Keywords: soil fungal diversity, extracellular xylanase, xylanase producing fungi, A. alternata

1. INTRODUCTION

In the recent years, there has been a phenomenal increase in the use of enzymes as industrial catalysts. Microbial enzymes in biotechnology have stimulated the investigation of their production with the purpose of selecting promising enzyme producers and increasing their yield [1]. Xylanases are applied in a wide range of industrial processes, such as in the pulp and paper industry, which demands a higher purity degree compared to other industries [2]. Fermentation of agricultural cellulosic wastes such as sugarcane bagasse, rice culms, corn cobs, cotton bushes, barley straw, and beet pulp as substrates for microbial enzyme production shows promise as a process for obtaining low-cost products and also helps solve the problem of waste disposal [3].

Microorganisms in particular have been regarded, including fungi, bacteria, and yeasts, which are widely available, and are capable of producing xylanases [4]. Filamentous fungi

have attracted a lot of attention for their potential industrial application as they produce a wide range of xylanases [5]. Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide β -1,4-xylan into xylose, thus breaking down hemicellulose, which is a major component of the cell wall of plants. Xylanases play an important physiological role in plant tissue, because they are involved in fruit softening, seed germination, and plant defense mechanisms [6]. Xylanase has been extensively studied in various industrial processes, such as the food and feed industries, in textile processes, in pulping and bleaching processes, and in waste treatment processes [7]. The objective of this study was to assess the diversity of xylanase-producing fungi in the soil of Jeddah, Saudi Arabia, as well as the xylanase-producing potential of the isolated fungi.

2. MATERIAL AND METHODS

2.1 Collection of soil sample

A total of ten soil samples were collected from five different sites in the Jeddah district, Saudi Arabia. These sites were near different highways in Jeddah (Figure 1 and Table 2). The samples were taken at a depth of 5–20 cm below the soil surface according to the method described earlier by Johnson *et al.* [8] and Mohammed *et al.* [9]. Soil samples were collected directly into new polythene bags. At least five samples were taken at random from each place and brought together into one composite sample, which was mixed thoroughly. The samples were kept in the car with the air conditioning set to the coldest setting until they were transported to the lab.

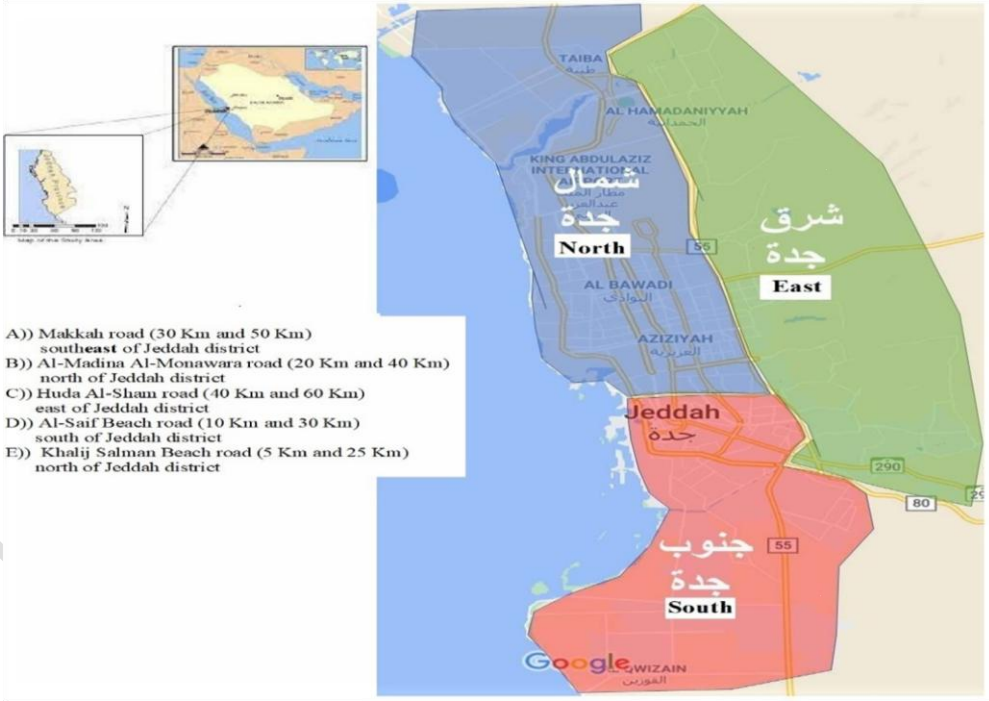

Fig. 1. Jeddah, Saudi Arabia, prepared with the help of Google maps.

Table 1. Location of soil sample collection from Jeddah, Saudi Arabia, for the isolation of xylanase-producing fungi

Site	Sample code	Location
A (Southeast)	JA1	30km from Makkah highway
	JA2	50 km from Makkah highway
B (North	JB1	20 km from Al-Madina highway
	JB2	40km from Al-Madina highway
C (East)	JC1	40 km from Al-Sham highway
	JC2	60 km from Al-Sham highway
D (South)	JD1	10 km from Al-Saif highway
	JD2	30 km from Al-Saif highway
E (North)	JE1	5 km from Salman highway
	JE2	25 km from Salman highway

2.2 Isolation of xylanase-producing fungi from soil samples by dilution plate assay

The dilution plate method was employed for the determination of fungal count in the collected soil sample. Xylan agar medium with streptomycin 30 µg/ml and rose bengal 1:30000 was used for the isolation and identification of the fungal species from the collected soil samples [10, 11]. The soil samples were sieved and air-dried. Ten grams of soil were placed in a graduated cylinder. Water was added to the soil until a total volume of 250 ml was reached. The suspension was serially diluted till the final dilution reached 1:10000. Each Petri-dish received one ml of an aliquot from the previous dilution, followed by 15 ml of sterilized molten xylan medium (just above the solidifying temperature).The dishes were rotated by hand in a broad swirling motion to disperse the soil suspension in the agar. After incubation at 25^{o}C, usually for 5 to 7 days, the resulting colonies were counted. The average number of colonies was multiplied by the dilution factor to obtain the number per gram of the original soil. Three isolation experiments were performed for each treatment, and the resulting fungal species were identified and kept on slants for further studies (Figure 2a & b).

The isolated fungal species were purified by 3 rounds of sub-culturing of a single colony in the culture media. After that, a single colony was aseptically sub-cultured on a slant of xylan medium for the fungal stock culture and stored in the refrigerator at 4 ^{o}C.

2.3 Identification of the isolated fungal species

By microscopic examination, the developed fungal colonies were identified up to the species level by taking the help of various published resources [12-16]. The relative density (RD) of each species was also calculated as a percent of the total count by the following formula:

RD (%) = SS/TS X100; where, SS = the total colony count of a species from a site and TS = the sum of the total colony count of all species from a site.

2.4 Preparation of extracellular crude xylanase enzyme

The fungal stock cultures were transferred to plates containing agar with xylan and incubated for 2–4 days at 25^{o}C. A disc of 1 cm was cut from 2-4 day old cultures and inoculated in conical flasks with 100 ml of liquid medium, which were then incubated at 25^{o}C for 10 days (Figure 2). Culture medium was then filtered using filter paper for separation of

mycelium. The filtrate was treated for the preparation of extracellular xylanase. Solid ammonium sulfate (80%) was mixed with the filtrate. The mixture was cold-incubated (5 ^{o}C) for 48 h before the precipitate was gathered by spinning at 5000 rpm (20 min) using a SIGMA Laboratory Centrifuge. The resulting precipitate was dissolved in buffer containing sodium citrate (pH 7.0) with stirring for 30 min at RT. Excess salt was removed by overnight dialysis at 5^{o}C [17]. In this assay, *A. candidus* was included as a control. The fungus was obtained from the Department of Biology, King Abdul Aziz University, Jeddah. This fungus has been described as a xylanase producer and was isolated from the Jeddah soil [11].

Fig. 2. Isolated fungal species on xylan agar medium (a). Purified fungi on xylan agar medium (b). Fungal species cultured on xylan liquid medium, 10 days of incubation (c).

2.5 Assay of xylanase enzyme

Xylanase activity was assayed using xylan from birch wood (0.01%) as the substrate in 0.05 M sodium citrate buffer (pH 6.2). Specific activity was determined by incubating 3.5 ml of reaction mixture with 1 ml each of enzyme solution and substrate plus 0.5 ml of phenol and 1 ml of sulfuric acid at 50 °C for 10 min. [18]. Measurements were made using a UV-Spectrophotometer (UV-1650 pc, UV-Vis Spectrophotometer, Shimadzu) set to 480 nm [19]. One unit was defined as the amount of enzyme required to produce one µmol of sugar (xylose equivalent) per minute.

2.6 Statistical analysis

There were four replicates for each treatment. The experiment was repeated twice. Data have normal distribution. The data of xylanase activity was statistically analysed using SPSS (version 20). The significance of the data was determined by one-way ANOVA (LSD at 0.05).

3. RESULTS AND DISCUSSION

Xylanase, which breaks down xylans to xylose, has received a lot of attention due to the use of xylose as a source of the sweetener xylitol as well as a number of fermented products [20]. Several filamentous fungi are producers of xylanase and thus have attracted a lot of attention for their potential industrial application. They produce a wide range of xylanases at high titers compared with enzymes derived from bacteria and yeast. Soil is a great source of filamentous fungi. They have been explored for the isolation of potential xylanase producing fungi.

In the present study, ten soil samples collected from five different locations in Jeddah, Saudi Arabia, were subjected to a soil dilution assay to estimate the total fungal counts and density percentage of isolated fungal species on xylan agar medium. Table 2 shows the data of the fungal species isolated from the soil samples collected from two sites in the southeast region

of Jeddah (JA1 and JA2). The data revealed that the total fungal counts in this region were 43 colonies/g of soil, constituting 5 fungal species. *Penicillium italicum* was the most dominant fungal species isolated from the soil of JA2, representing 32.56% of the total fungal count. *Aspergillus terreus* was the second species in dominance, where 11 colonies/g of soil were recovered on xylan medium from JA2 soil, which constituted 25.58% of the total population. The third fungal species in order of density was *A. niger,* isolated from JA2 soil, which constituted 23.26% of the total isolation. *A. flavus* ranked next in the density range that was recovered from the JA1 soil. It constituted 13.95% of the total fungal count. The last and least fungal species in order of density was occupied by *Rhizopus oryzae* isolated from the JA1 soil. It constituted 4.65% of the total isolates.

Table 2. Total fungal colony counts on xylan agar medium and relative density (%) of fungal species isolated from the soil samples collected from site A (JA1 and JA2) Jeddah, Saudi Arabia

Fungal species	Total colony count/ g dry soil JA1	Total colony count/ g dry soil JA2	Relative density (%) (JA1 & JA2)
Aspergillus flavus	6	-	13.95
Aspergillus terreus	11	-	25.58
Aspergillus niger	-	10	23.26
Total Aspergilli	17	10	62.79
Penicillium italicum	-	14	32.56
Rhizopus oryzae	2	-	4.65
Total count	19	24	100.0
Number of fungal species	3	2	

The results of the fungi isolated from the soil collected from the two sites in the northern region of the Jeddah district (JB1 and JB2) are presented in Table 3. A total of 46 colonies/g of soil constituting three fungal species were isolated from site B (Al-Madina Al-Monawara road sites). From the site JB2, 33 colonies/g of soil were isolated, representing three fungal species, while 13 fungal colonies/g of soil were isolated from JB1, constituting two fungal species. According to the data, the most common fungal species (22 colonies/g of soil) was *Aspergillus flavus*, which accounted for 47.83% of all isolates. *Penicillium chrysogenum* was the second fungal species in order of density, at 30.43% of the total population. The least counted fungal species was *Rhizopus microsporus*, isolated only from the soil of JB2. It represents 21.74% of the total fungal isolates.

Table 3. Total fungal colony counts on xylan agar medium and relative density (%) of fungal species isolated from the soil samples collected from site B (JB1 and JB2) Jeddah, Saudi Arabia

Fungal species	Total colony count/ g dry soil JB1	Total colony count/ g dry soil JB2	Relative density (%) (JB1 & JB2)
Aspergillus flavus	8	14	47.83
Penicillium chrysogenum	5	9	30.43
Rhizopus microspores	-	10	21.74
Total count	13	33	100.0
Number of fungal species	2	3	

Table 4 shows the total fungal colony count and the relative densities (%) of the fungi isolated from soil samples collected from two sites (C) of the Huda Al-Sham high way sites, 40 km (JC1) and 60 km (JC2) east of Jeddah district. The data reveals that a total of 115 colonies/g of soil were isolated from soil samples collected from site C. The isolated fungi constitute eight fungal species. The highest number of fungal colonies (83 colonies/g of soil) was isolated from JC2, while the least count (32 colonies/g of soil) was recorded from the soil sample collected from JC1. *Aspergillus flavus* occupied the first position in fungal density (44 colonies/g of soil), constituting 38.26% of the total population. *R. microspores* was the next most isolated fungal species, representing 33.04% of the total isolates. *P. chrysogenum* was the third most isolated species, representing 8.69% of the total soil isolates, where its count was 10 colonies isolated from 60 km of Jeddah district soil (JC2). *P. italicum* came next in order of density, making up 6.09% of the total count. The fifth fungal species in the rank of density was *Alternaria alternata,* which accounted for 5.22% of the total fungal count from all soil samples. *Botrytis cinerea* and *Fusarium roseum* were the next fungal species in rank of density (3.48% of the total population). The last and least dense fungal species in order of density was *Penicillium canescens* (2 colonies/g of soil).

Table 4. Total colony counts on xylan agar medium and relative density (%) of fungal species isolated from the soil samples collected from site C (JC1 and JC2), Jeddah, Saudi Arabia

Fungal species	Total colony count/ g dry soil JC1	Total colony count/ g dry soil JC2	Relative density (%) (JC1 & JC2)
Alternaria alternata	4	2	5.22
Aspergillus flavus	10	34	38.26
Botrytis cinerea	-	4	3.48
Fusarium roseum	2	2	3.48

Penicillium chrysogenum	-	10	8.69
Penicillium italicum	4	3	6.09
Penicillium canescens	2	-	1.74
Total Penicilli	6	13	16.52
Rhizopus microspores	10	28	33.04
Total count	32	83	100.0
Number of fungal species	6	7	

Table 5 includes data on the fungi isolated from the soil samples collected from the two sites (JD1 and JD2) in the south region of Jeddah district. A total of 39 colonies/gram of soil were recorded, representing three fungal species. The data shows that *P. italicum* was the densest fungal species (23 colonies/g of soil), accounting for 58.97% of the total isolates. *A. niger* was the second fungal species in order of density (33.33%) of the total population. The least counted fungal species was *Botryis cinerea*, which was isolated only from JD1, constituting 7.70% of the total fungal isolates.

Table 5. Total fungal colony counts on xylan agar medium and relative density (%) of fungal species isolated from the soil samples collected from site D (JD1 and JD2), Jeddah, Saudi Arabia

Fungal species	Total colony count/ g dry soil JD1	Total colony count/ g dry soil JD2	Relative density (%) (JD1 & JD2)
Aspergillus niger	5	8	33.33
Penicillium italicm	11	12	58.97
Botrytis cinerea	3	-	7.70
Total count	19	20	
Number of fungal species	3	2	100.0

The results of the fungal population isolated from the soil sample collected from the northern Jeddah district (E) are given in Table 6. The data show that 106 colonies/g of soil were isolated from soil samples from two different sites (JE1 and JE2), representing seven different fungal species. The highest number of fungal colonies (84/g of soil) was isolated from JE2 soil samples, while the least count (22 colonies/g of soil) was recorded in the soil sample collected from JE1. *A. niger* constitutes the highest relative density (33.96%) of the total population. *R. oryzae* was the following fungal species in rank of density, representing 20.75% of the total isolation. Next to it was *P. canescens*, constituting 9.44% of the total count. *P. chrysogenum* had a relative density of 12.26% of the total soil isolates. *A. alternata* came in second in terms of density, accounting for 8.48 percent of the total fungal count. *B. cinerea* and *F. solani* were the next fungal species in rank of density, at 7.55 percent of the total population.

Table 6: Total fungal colony counts on xylan agar medium and relative density (%) of fungal species isolated from the soil samples collected from site E (JE1 and JE2), Jeddah, Saudi Arabia

Fungal species	Total colony count/ g dry soil JE1	Total colony count/ g dry soil JE2	Relative density (%) (JE1 & JE2)
Alternaria alternata	4	5	8.49
Aspergillus niger	8	28	33.96
Botrytis cinerea	-	8	7.55
Fusarium solani	3	5	7.55
Penicillium chrysogenum	-	13	12.26
Penicillium canescens	7	3	9.44
Total Penicilli	7	16	21.70
Rhizopus oryzae	-	22	20.75
Total count	22	84	100.0
Number of fungal species	4	7	

Table 7 represents the cumulative data of all 10 soil samples collected from 5 different sites of the Jeddah district. A total of 204 fungal colonies per g of soil were isolated from all ten soil samples on xylan agar medium, constituting 12 fungal species. *A. niger* was the most isolated fungal species, which constituted 37.2% of the total fungal counts. *R. microsporus* and *P. chrysogenum* were found in moderate numbers, accounting for 23.5% and 18.6% of all fungal isolates, respectively. Five fungal species (*A. alternata, F. roseum, F. solani, P. italicum,* and *R. oryzae*) were isolated with low occurrence, while the rest (*A. niger, A. flavus, A. terreus, B. cinerea*, *P. chrsogenum, P. canescens,* and *R. microsporus*) had rare occurrences.

Table 7. Total fungal counts and numbers of isolated fungal species determined from all ten soil samples collected from 5 sites in Jeddah, Saudi Arabia

Fungal species	Total colony count/ g dry soil	Relative density %	Frequency of occurrence
Alternaria alternata	6.0	3.0	L
Aspergillus flavus	6.0	3.0	R
Aspergillus terreus	11.0	5.3	R
Aspergillus niger	76.0	37.2	H
Total Aspergilli	93.0	45.5	-
Botrytis cinerea	4.0	2.0	R
Fusarium roseum	1.0	0.5	L
Fusarium solani	1.0	0.5	L
Total Fusarium	2.0	1.0	-

Penicillium chrsogenum	38.0	18.6	M
Penicillium italicum	7.0	3.4	L
Penicillium canescens	4 .0	2.0	R
Total Penicilli	49.0	24	-
Rhizopus microsporus	48.0	23.5	M
Rhizopus oryzae	2.0	1.0	L
Total Rhizopus	50.0	24.5	-
Total count	204.0	100.0	-
Total number of species	12.0	-	-

Frequency of occurrence among the ten tested soil samples: High (H): 6-7sites, Moderate (M): 4-5 sites, Low (L): 3-2 sites and Rare (R): 1 site.

In the current study, a total of twelve fungal species (*A. alternata, A. flavus, A. niger, A. terreus, B. cinerea, F. roseum, F. solani, P. chrsogenum, P. italicum, P. canescens, R. microsporus, and R. oryzae*) related to six genera were isolated from the ten soil samples. The population of these fungal species varies in different soils. Overall, *A. niger* (37.2%) was isolated with the highest occurrence and population density. Moderate occurrence was shown by both *P. chrsogenum* (18.6%) and *R. microspores* (23.5%). The rest of the fungal species were identified as having low and rare occurrences. It seems the variation in fungal count is probably due to the ability to metabolize the different decomposition products in the soil samples. Thirty-four genera and eighteen fungal species were isolated from 14 soil samples collected from different soils in Saudi Arabia. *Aspergillus* and *Penicillium* contributed the greatest number of species. The most frequent genera were *Botryotrichum* and *Ulocladium,* followed by *Macrophomina, Rhizopus, Fusarium, Alternaria,* and *Cladosporium*. The most common fungal species were *A. fumigatus, A. terreus, A. niger, A. flavus, P. citrinum, P. corylophilum, B. atrogriseum, U. botrytis, M. phaseoli, R. stolnifer, F. moniliforme, A. alternata,* and *C. herbarum* [21]. A total of 12 fungal species were isolated from petroleum-contaminated Saudi Arabian soil. *Aspergillus,* with three species, was predominant, followed by *Trichoderma,* with two species. All isolates of *A. flavus*, *A. niger,* and *T. harzianum* were isolated from all soil samples. *T. harzianum* generally exhibited the highest number of colonies per gram of soil, followed by *A. flavus* and *Chaetomium bostrychodes,* while *Rhizopus* sp. was isolated from only one soil sample [22].

Alternaria alternata, Aspergillus flavus, Cladosporium herbarum, Curvularia lunata, and *Ulocladium chlamydosporium* were isolated from the industrial Al-Jubail city, Saudi Arabia [23]. Microbial contents in soil samples in Al-Khafji town, located in Saudi Arabia, consisted of seven fungal genera belonging to *Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Penicillium,* and *Trichoderma* [24]. Five fungal species (*A. candidus*, *A. niger*, *F. oxysporum*, *P. purpurogenum*, and *U. botrytis*) were collected from soil in Damam city, Saudi Arabia [25]. Recently, from the soil of the Riyadh region (Saudi Arabia), *Aspergillus, Penicillium, Mucor, Fusarium, Alternaria, Trichoderma, Rhizopus*, and *Botrytis* were identified [26]. The results obtained in the present study are consistent with the findings of Al-Sheikh, [27], who stated that the common genera of fungal species isolated from the east, west, and central soil localities in Saudi Arabia were *Alternaria, Aspergillus, Fusarium, Penicillium,* and *Rhizopus.*

The extracellular xylanase enzyme activities of the twelve isolated fungal species were assayed on birch wood xylan incubated at 50°C for 10 minutes (Fig. 3). After a 10-minute

incubation period, the maximum significant value of extracellular xylanase enzyme was observed in the culture filtrate of *A. alternata* (0.58 units/ml), whereas the minimum value was detected with *F. roseum* (0.20 units/ml). The extracellular xylanase activities for the rest of the fungal species can be arranged as follows: *A terreus* (0.54 unit/ml) = *P. chrysogenum* each (0.54 unit/ml) > *B. cinerea* (0.53 unit/ml), while *A. niger* (0.51 unit/ml) = *P. italicum* (each 0.51 unit/ml) > *P. canescens* (0.48 unit/ml) > *R. oryzae* (0.45 unit/ml) > *R. microspores* (0.41 unit/ml) > *A. flavus* (0.39 unit/ml) > *F. solani* (0.35 unit/ml).

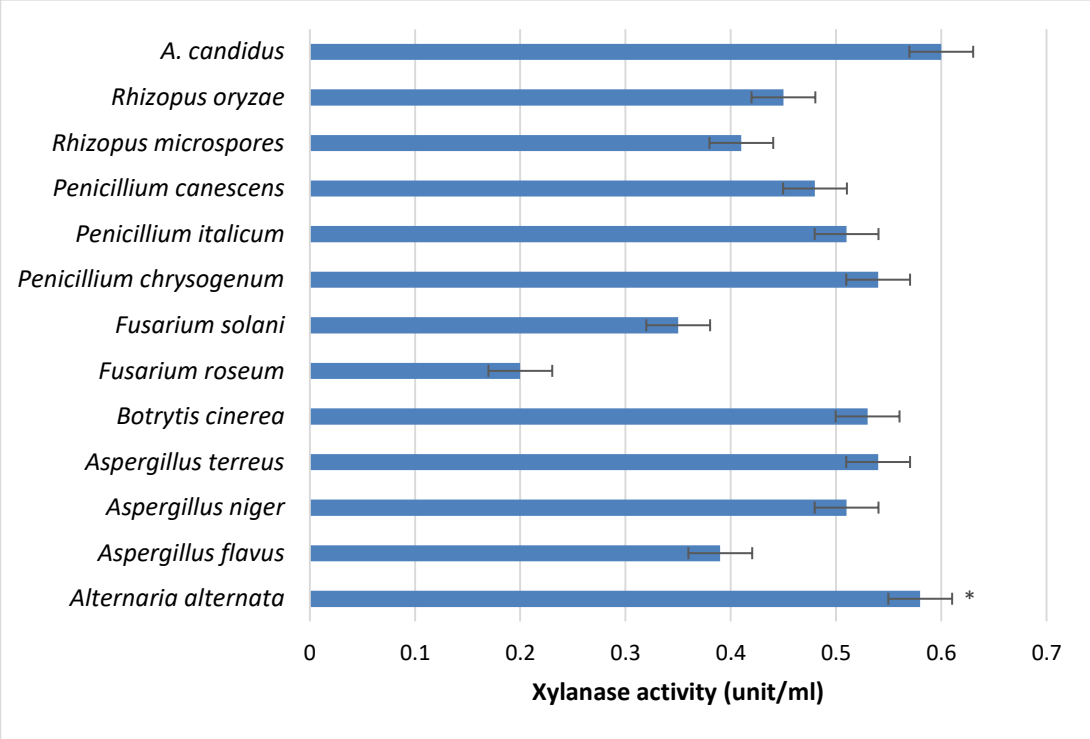

Fig. 3. Assay of extracellular xylanase enzyme activities for the twelve isolated fungal species on birch wood xylan as a substrate, incubated at 50 °C for 10 min.
**Significant from control (A. candidus), P = 0.05, The vertical bars represent ± standard error (n = 4).*

Wipusaree *et al.* [28] isolated 54 fungal species and tested them for the production of xylanase. They found 30 isolates that produced enzymes that degraded when grown on solid xylan agar. *Aspergillus, Trichoderma, and Penicillium* are considered rich sources of enzymes for xylan biodegradation [29].

In a study, a total of twelve filamentous fungal species were isolated from the soil sample, out of which *Aspergillus flavus* and *Trichoderma viride* were selected as the most appreciable xylanase-producers [30]. Different microbes can produce xylanase, including yeast, bacteria, and filamentous fungi. In particular, *Aspergillus, Aureobasidium, Bacillus, Chaetomium, Cryptococcus, Fusarium, Humicola, Penicillium, Phanerochaete, Rhizomucor, Talaromyces, Trichoderma,* are of interest [31]. Another study by Garg *et al*. [32] (2009) showed secretion of high levels of xylanase by filamentous fungi including *Trichoderma, Penicillium*, and *Aspergillus*. Xylanase is produced extracellularly by bacteria, yeast, and filamentous fungi. The filamentous fungi are particularly useful producers of xylanases from an industrial point of view because they excrete larger amounts of xylanolytic enzymes into the medium than yeast or bacteria. The fungal genera *Trichoderma, Aspergillus, Fusarium*, and *Pichia* are considered great producers of xylanases [33]. *A. candidus* xylanase enzyme

activities have been reported to be around 0.60 U/ml [11]. In another similar study, xylanase was isolated from the culture filtrate of *P. janthinellum* [10]. The production of xylanase was 56.31 U/ml from *F. solani* was carried out by Gupta *et al.* [34]. Fungi such as, *T. viride, A. candidus,* has been demonstrated to be an excellent xylanase producer. Furthermore, they have been optimized for increased xylanase production in solid state fermentation utilizing a variety of substrates [11, 35, 36].

4. CONCLUSION

The soil in the Jeddah district of Saudi Arabia is diversely populated with fungi. The soil harbors a good population of xylanase-producing fungi. The relative density of the isolated fungi varied in different soils. A total of twelve different species of xylanase producing fungi were isolated from 10 soil samples collected from 5 sites in Jeddah. The isolated species were capable of producing xylanase and an appreciable amount of crude xylanase was detected. The highest value of extracellular xylanase enzyme was recorded in *A. alternata.* The fungus *A. alternata* can be explored further for its extracellular xylanase production for use in biotechnology applications.

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