Isolation and Identification of Xylanolytic Fungi and Their Enzyme Activity

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ABSTRACT

A total of 51 fungi were isolated from various sources such as groundnut shells, sawdust and the soil samples from two places. The first collection was done under the decomposed leaves from East Yangon University campus and another collection was the heap of rice straw soil from Kamarkalote village, Kyauktan Township at a depth of 6 inches. The xylanolytic fungi were isolated on Potato Dextrose Agar medium from four different sources. 17 strains of potential xylanolytic fungi were isolated from soil samples of EYU campus, 19 strains from soil samples of Kamarkalote village, Kyauktan Township, 6 strains from groundnut shells and 9 strains from sawdust of Kamarkalote village, Kyauktan Township. Those isolated fungi were cultured on Czapek dox Agar medium containing Birch wood xylan as preliminary xylanolytic enzyme production medium by forming the clear zone. Among 51 isolated fungi, 26 fungi showed the positive results of the clear zone as xylanase activity. The crude xylanase enzymes were extracted from *Aspergillus* sp., *Penicillium* sp., *Rhizoctonia* sp., *Trichoderma* sp., *Fusarium* sp., *Botrytis* sp., *Aureobasidium* sp., *Rhizopus* sp., *Cladosporium* sp. and *Gliocladium* sp. according to their macroscopical and microscopical characters.

Key words – xylanolytic fungi, xylanase activity, xylanase enzymes

INTRODUCTION

Nowadays, numerous types of commercial enzymes have been introduced to the market. Xylan is the major hemicellulose constituent of hard wood and soft wood, and is the next most abundant renewable polysaccharide after cellulose. A large number of bacteria and fungi are known to produce xylanases (Kulkarni *et al*., 1999; Subramaniyan and Prema, 2002).

Xylanase activity levels from fungal cultures are typically much higher than those from yeasts or bacteria (Paloheimo *et al*., 2003).

Microbial xylanases are used in the pulp and paper, animal feed, textile and food processing industries, and in the production of several valuable products like xylitol and ethanol (Salles *et al*., 2005).

Filamentous fungi are attracting greater attention than bacteria as potential sources of plant cell wall hydrolyzing enzymes such as xylanases because they secrete high levels of the enzymes into the culture medium. They are non-pathogenic, capable of producing high levels of extra cellular enzymes and can be cultivated very easily. Xylanase is important in the bioconversion of hemicelluloses into their constituent sugars such as xylose (Wong *et al*., 1988).

Xylanases are produced by the pretreatment of pulps prior to bleaching in the pulp and paper industry which is the most important industrial application of xylanases. Bioleaching of pulps using xylanase is one of the suitable applications in the pulp and paper industry to reduce or eliminate the use of chlorine and chlorine dioxide (Singh *et al*., 2013).

In the present study, 51 xylanolytic fungi were isolated from soil samples, groundnut shells and sawdust. Among them, the most prominent 26 isolated fungi showed xylanase enzyme activity.

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MATERIALS AND METHODS

Sample collection

The samples of groundnut shells, sawdust were collected from Kamarkalote village, Kyauktan Township. The soil samples were also collected under the decomposed leaves from East Yangon University Campus and another one was the heap of rice straw soil at a depth of 6 inches from Kamarkalote village, Kyauktan Township. Samples were placed in the clean plastic bags, brought to the laboratory used for further analysis.

Study Area

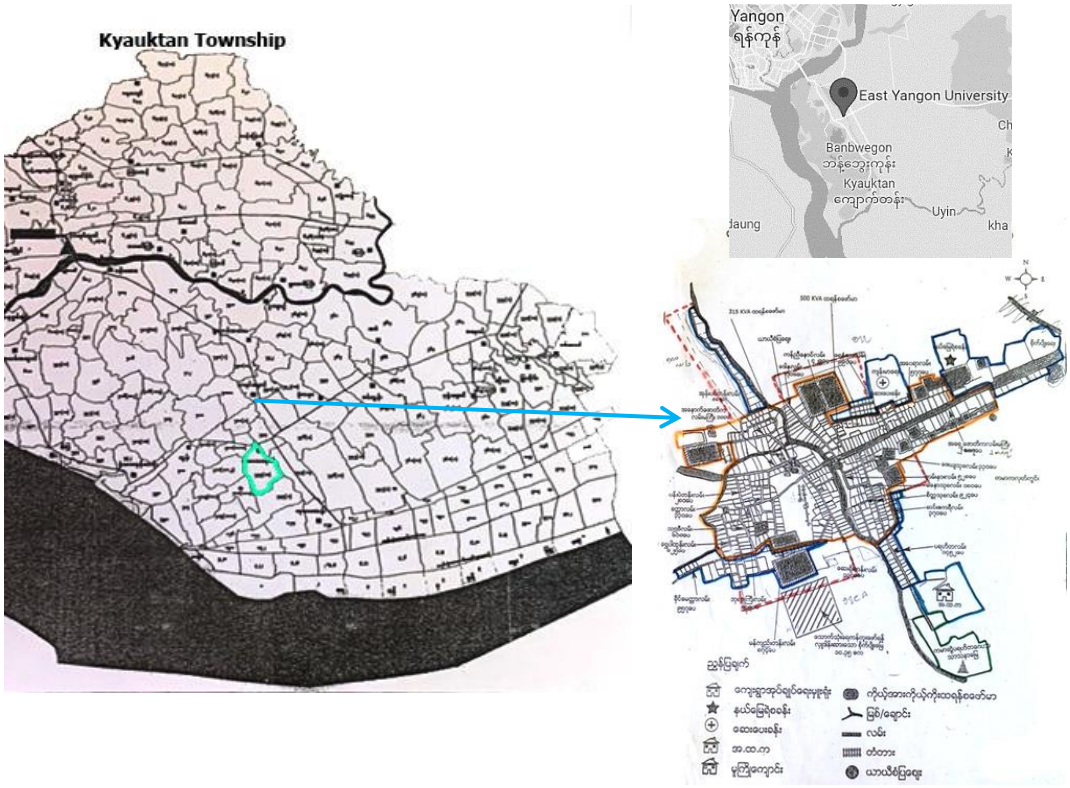


Fig. (1) Location of Kamarkalote village, Kyauktan Township,Yangon Region

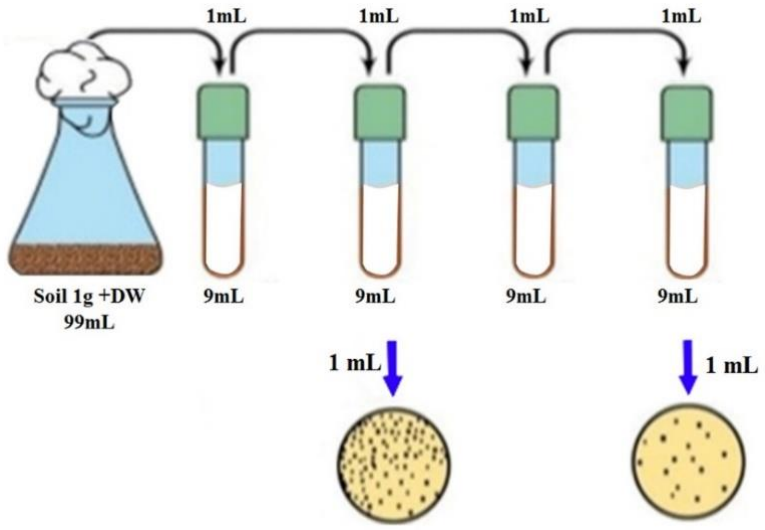


Fig. (2) Isolation procedure for soil samples by the serial dilution method (Pelczar and Chan, 1972)

Isolation of fungal strains

The fungal strains were isolated on Potato Dextrose Agar medium (Dextrose 10 g, Agar 20 g, distilled water 1000 mL, pH 7.0) by Atlas (1993) at room temperature for 7 days. Chloramphenicol was added to the medium after autoclaving.

Preliminary test for enzyme activity

The isolated fungal strains were tested for enzyme activity on Czapek dox agar medium with birch wood xylan (Birch wood xylan 5 g, Peptone 5 g, Yeast extract 5 g, K_2HPO_4 1 g, $MgSO_4$ 0.2 g, Agar 20 g, Distilled water 1000 mL, pH 7.0 at room temperature for 7 days (Nakamura *et al*., 1993).

Examination of xylanase secretion by congo-red method

Preliminary test for the excretion of xylanolytic enzyme from isolated fungi was performed according to the congo-red method. Three to seven days old culture of isolated soil fungi inoculated in the Czapek dox agar medium and the congo-red reagent (Ethanol 90.0 mL, Congo-red 1.0 g, Distilled water 10.0 mL) was poured onto the surface of agar plate and kept at room temperature for 10 minutes and wash with sodium chloride. The development of a clear zone around the fungal colony was recorded as a positive result of the hydrolysis of xylanase secreted by soil fungi (Sharma *et al*., 1986).

RESULTS

In the present work, all isolated fungi were screened from four different sources and designation of all isolated strains with their enzyme activity as in Table.

Table-1. Location, designation and xylanase activity of isolated fungi

No.	Location of samples	Designated number of isolates	Isolated fungi by Xylanase activity	Total Isolates by Xylanase activity
1	East Yangon University (L)	L-1 to L-10	L-1,L-4,L-6,L-7,L-8	5
1.1	East Yangon University (L)	L-11 to L -17	L-11, L-13,L-16,L-17	4
2	Heap of rice straw soil (K)	K-1 to K- 10	K-1,K-5,K-7,K-9,K-10	5
2.1	Heap of rice straw soil (K)	K-11 to K-19	K-11,K-12,K-14,K-15,K-19	5
3	Groundnut Shells (G)	G -1 to G -6	G-2,G-3	2
4	Sawdust (S)	S-1 to S -9	S-2,S-3,S-6,S-7,S-8	5
	Total	51		26

Macroscopical and microscopical characters of L-17, K-1, K-15, S-2

The mycelium color is dark with a white edge in surface view and a pale green color in reverse view. Conidiophores are upright, simple, terminating in a globose, bearing phialides at the apex or radiating from the entire surface; conidia are 1- celled, globose, often variously colored in mass, in dry basipetal chains. According to these macroscopical and microscopical characteristics, L-17 may be *Aspergillus* sp.

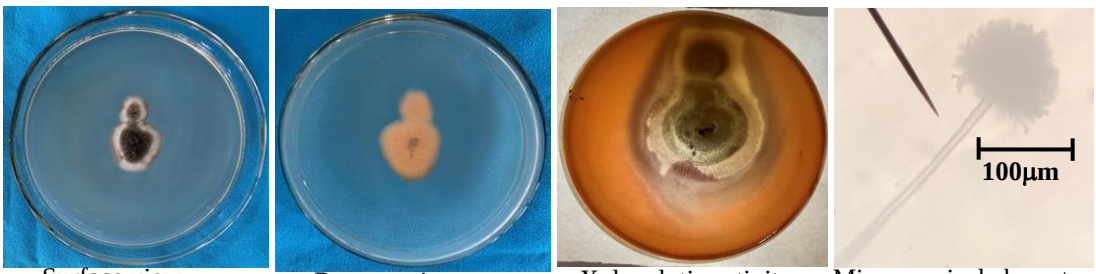


Fig. (3) Xylanolytic activity of L-17 with macroscopical and microscopical characters

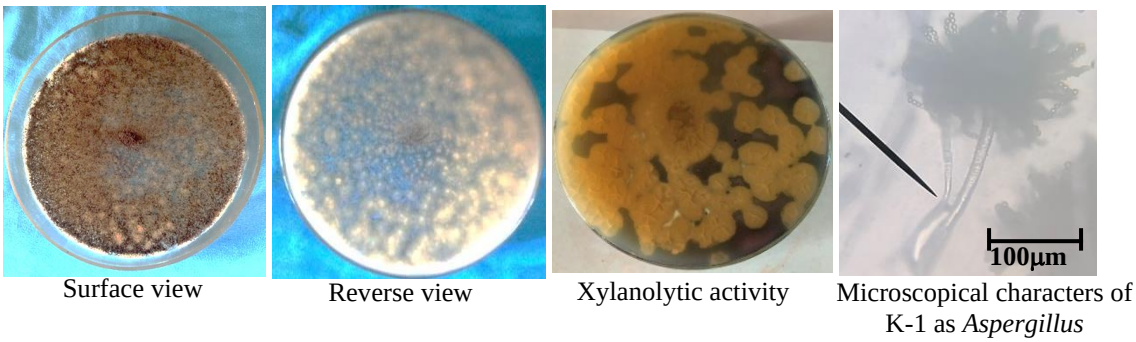


Fig. (4) Xylanolytic activity of K-1 with macroscopical and microscopical characters

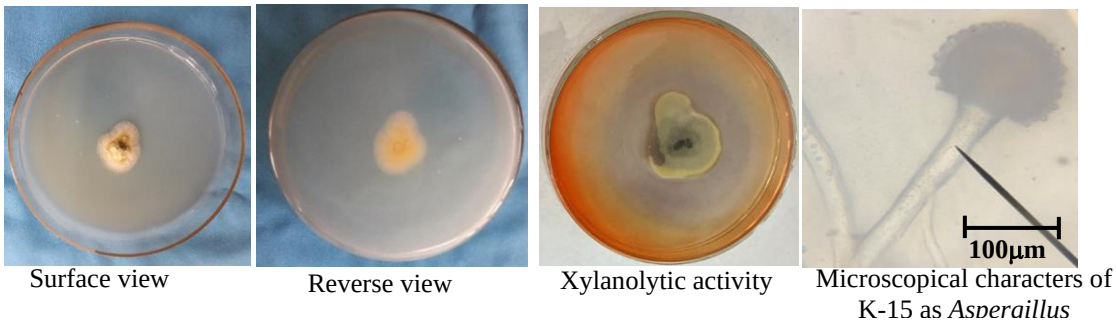


Fig. (5) Xylanolytic activity of K-15 with macroscopical and microscopical characters

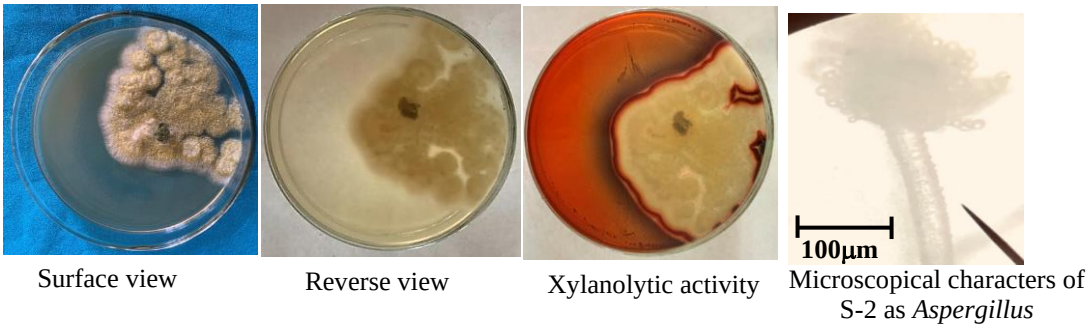
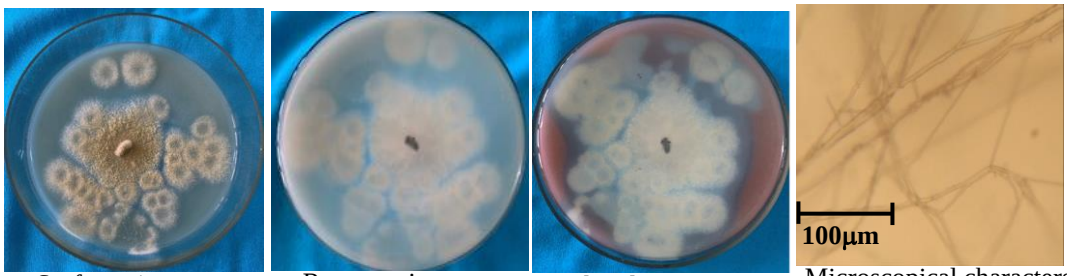


Fig. (6) Xylanolytic activity of S-2 with macroscopical and microscopical characters

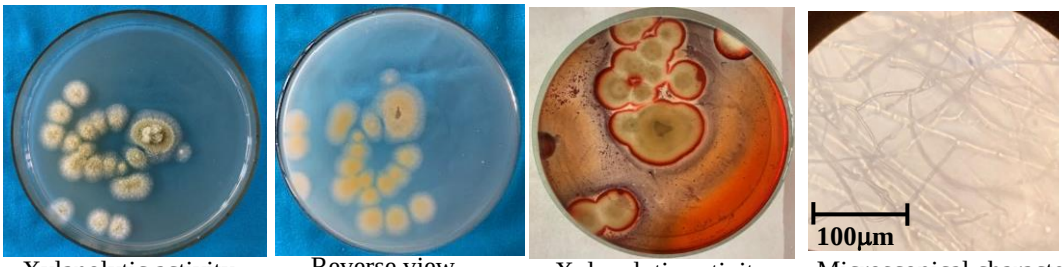


Surface view Reverse view Xylanolytic activity Microscopical characters of K-9 as *Rhizoctonia*

Fig. (7) Xylanolytic activity of K-9 with macroscopical and microscopical characters

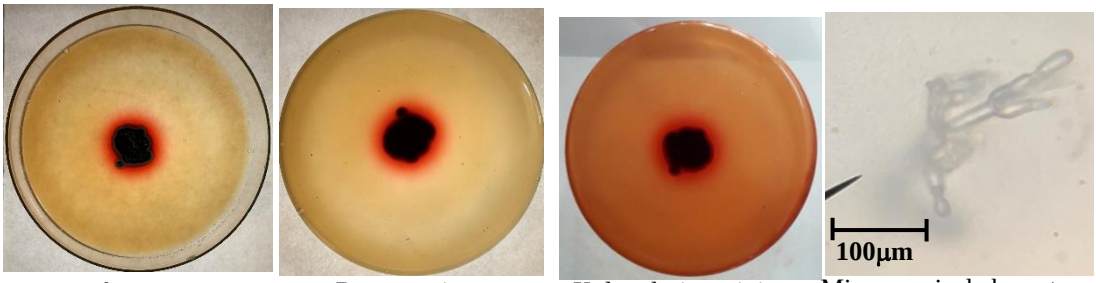
Macroscopical and microscopical characters of K-9, K-14

The mycelium color is pale yellow in surface view and white in reverse view. Asexual fruit bodies and spore lacking, variable inform, frequently small and loosely formed, among and connected by mycelia thread; hyphae with long cell, septa of branch set off from main hypha. According to these macroscopical and microscopical characteristics, K-9 may be *Rhizoctonia* sp.



Xylanolytic activity Reverse view Xylanolytic activity Microscopical characters of K-14 as *Rhizoctonia*

Fig. (8) Xylanolytic activity of K-14 with macroscopical and microscopical characters

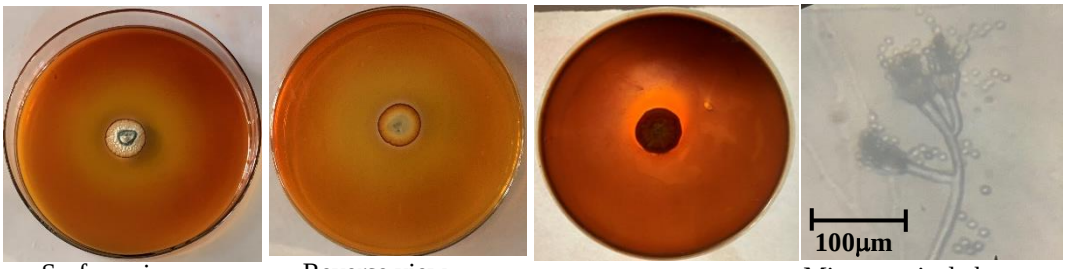


Surface view Reverse view Xylanolytic activity Microscopical characters of L-1 as *Cladosporium*

Fig. (9) Xylanolytic activity of L-1 with macroscopical and microscopical characters

Macroscopical and microscopical characters of L-1

The mycelium color is green with white edge in surface view and red color in reverse view. Conidiophores tall, dark, branched variously near the apex, simple or clustered, conidia dark, 1 or 2 celled, ovoid to cylindrical and irregular, some typically lemon –shaped; often branched acropetalous chain. According to these macroscopical and microscopical characters, L-1 may be *Cladosporium* sp.

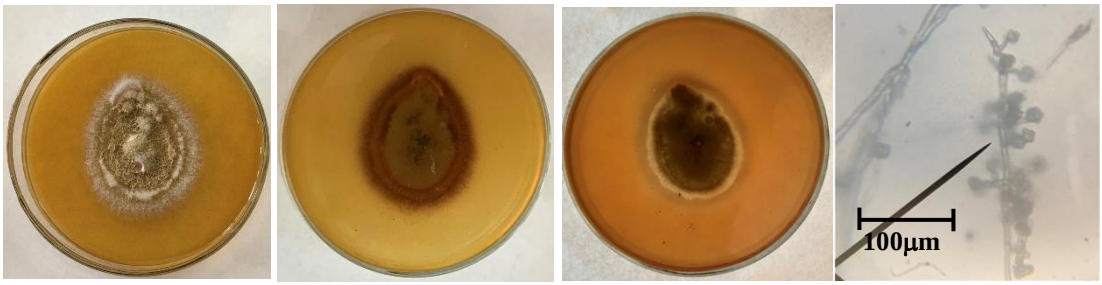


Surface view | Reverse view | Xylanolytic activity | Microscopical characters of L-6 as *Penicillium*

Fig. (10) Xylanolytic activity of L-6 with macroscopical and microscopical characters

Macroscopical and microscopical characters of L-6

The mycelium color is green with white on the surface view and yellow color on reverse view. Conidiospores arising from the mycelium singly, branched near the apex to form a brush- like, conidia- bearing apparatus, conidia brightly colored in mass, 1 –celled, ovoid, produced basipetally. According to these macroscopical and microscopical characters, L-6 may be *Penicillium* sp.

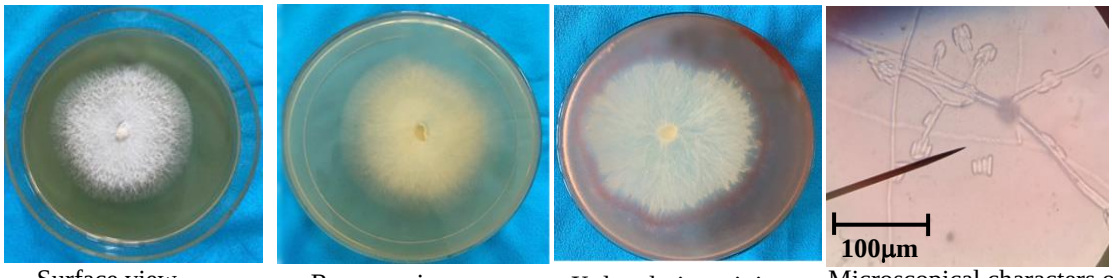


Surface view | Reverse view | Xylanolytic activity | Microscopical characters of L- 8 as *Trichoderma*

Fig. (11) Xylanolytic activity of L-8 with macroscopical and microscopical characters

Macroscopical and microscopical characters of L-8

The mycelium color is olive green in surface view and white in reverse view. Conidiophores hyaline, much branched, not verticillate; phialides in group; conidia hyaline, 1-celled, ovoid, borne in small terminal clusters; usually easily recognized by its rapid growth and green patches of conidia; saprophytic on soil or on wood, very common, some species reported as parasites on other fungi. According to these macroscopical and microscopical characters, L-8 may be *Trichoderma* sp.

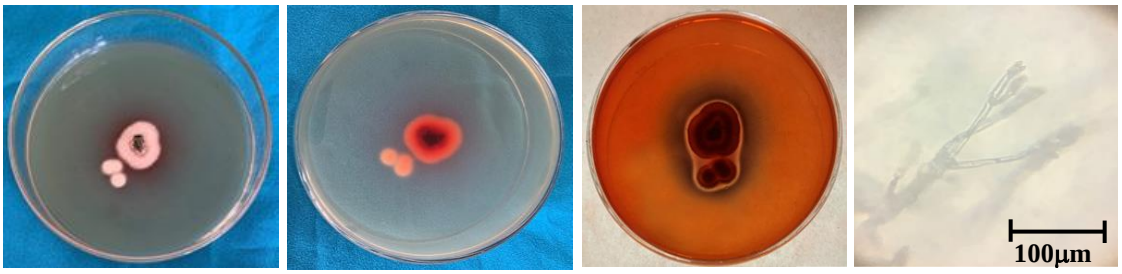


Surface view | Reverse view | Xylanolytic activity | Microscopical characters of L- 16 as *Fusarium*

Fig. (12) Xylanolytic activity of L-16 with macroscopical and microscopical characters

Macroscopical and microscopical characters of L-16

The mycelium color is white in surface view and white color in reverse view. Mycelium extensive and cottony in culture, often with some tinge of pink in the mycelium; conidiophores variable, slender and simple, branched irregularly, conidia hyaline, variable, principally of two kinds- macroconidia and microconidia, microconidia 1 –celled, some conidia intermediate, oblong or slightly curve, borne singly. According to these macroscopical and microscopical characters, L-16 may be *Fusarium* sp.

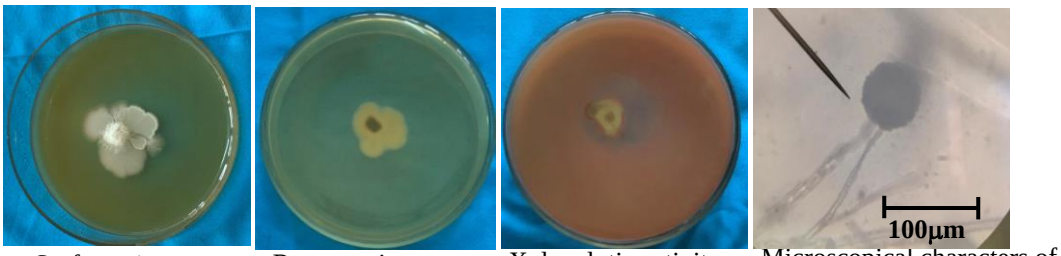


Surface view | Reverse view | Xylanolytic activity | Microscopical characters of K-5 as *Gliocladium*

Fig. (13) Xylanolytic activity of K-5 with macroscopical and microscopical characters

Macroscopical and microscopical characters of K-5

The mycelium color is creamy on the surface view and a white diffusing red pigment color in reverse view. Conidiophores hyaline, the upper portion bearing penicillate branches, forming a compact "brush "as in *Penicillium*; conidia (phialospores) hyaline or brightly colored in mass, 1-celled, produced successively apically and collecting in mucilaginous droplets; saprophytic, common in soil. According to these macroscopical and microscopical characteristics, K-5 may be *Gliocladium* sp.

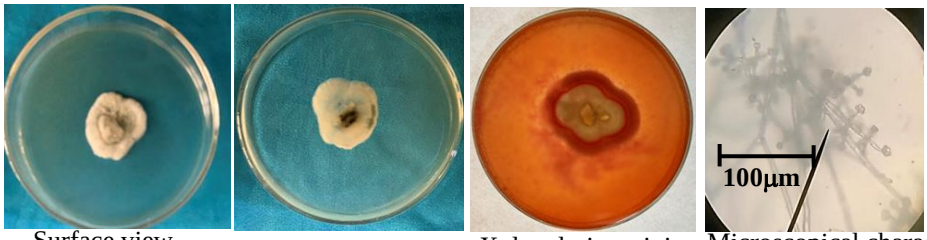


Surface view | Reverse view | Xylanolytic activity | Microscopical characters of G-3 as *Rhizopus*

Fig. (14) Xylanolytic activity of G-3 with macroscopical and microscopical characters

Macroscopical and microscopical characters of G-3

The mycelium color is creamy in surface view and buff color in reverse view. Fungi are characterized by a body of branching mycelia composed of three types of hyphae and usually unbranching sporangiopores. The black sporangia at the tips of the sporangiophores are rounded and produce numerous nonmotile multinucleate spores for asexual reproduction. According to these macroscopical and microscopical characters, G-3 may be *Rhizopus* sp.

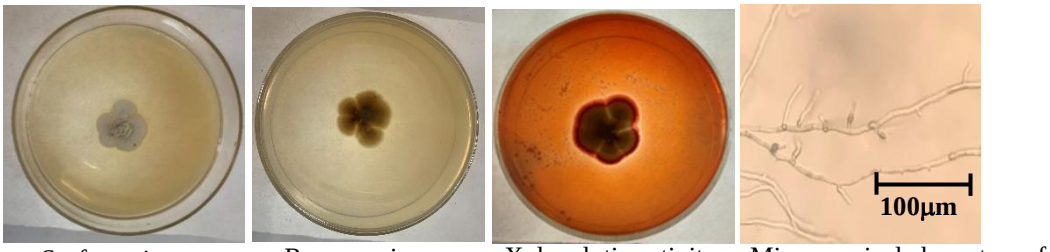


Surface view | Reverse view | Xylanolytic activity | Microscopical characters of S-6 as *Botrytis*

Fig. (15) Xylanolytic activity of S-6 with macroscopical and microscopical characters

Macroscopical and microscopical characters of S-6

The mycelium color is white in surface view and white color in reverse view. Conidiophores tall, slender, determinate, branched irregularly in upper portion, apical cells enlarged or rounded, bearing clusters of conidia; conidia hyaline or gray in mass, ovoid; black irregular sclerotia often present; causing "gray mold " on many plants or saprophytic. According to these macroscopical and microscopical characters, S-6 may be *Botrytis* sp.



Surface view | Reverse view | Xylanolytic activity | Microscopical characters of K-7 as *Aureobasidium*

Fig. (16) Xylanolytic activity of K-7 with macroscopical and microscopical characters

Macroscopical and microscopical characters of K-7

The mycelium color is white in surface view and brown color in reverse view. Mycelium not extensive, hyaline when young, becoming dark with age, black and shingy in old culture, bearing abundant conidia laterally; conidia (blastospores) subhyaline to dark, 1 – celled, ovoid, producing other conidia by budding; saprophytic or weakly parasitic; common in soil. According to these macroscopical and microscopical characters, K-7 may be *Aureobasidium* sp.

DISCUSSION AND CONCLUSION

Addela *et al*., (2015), Godfrey and West, (1996), Kheng and Omar (2005), reported that the fungus is generally considered as a good strain for the production of xylanase enzyme. For these cultures xylanase production was isolated based on the congo- red test, which gave a positive result by forming a reddish orange halo- zone of hydrolysis on Czapek dox agar medium plates containing birch wood xylan. The cultures showing good xylanase activities were stored as pure cultures for further studies.

Only in the past two decades have microbial enzymes been used commercially in many industries such as pulp and paper, the food industry, the textile industry and the feed industry. Xylanase is produced by different microorganisms (fungi and bacteria) and is widely used in pulp and paper industries. In addition, the xylanase enzyme was found to be effective in achieving enhanced sugar extraction from fruit juices, clarification of fruit juices, and substantial dough-raising in baking (Sharma and Sharma, 2017).

The global population is increasing at a rapid rate which results in a high demand for life supporting products. For the production of daily required products such as food, clothes, paper and pharmaceuticals, a larger amount of raw material is consumed (Kumar *et al*., 2017).

In this study, a total 51 xylanolytic fungi were isolated from four different sources of two soil samples, groundnut shells and sawdust. In the present investigation, 26 strains were shown to have a clear zone on Czapek dox agar medium containing Birch wood xylan for estimating preliminary xylanase production.

Among them, 6 strains from soil samples and sawdust were chosen for further investigation of antimicrobial activity according to the best and highest xylanase activity. According to the highest xylanase activity, the soil samples of L-17, K-1, K-15 and sawdust sample of S-2 were identified as *Aspergillus* sp. and then the soil samples of K-9 and K-14 as *Rhizoctonia* sp. and then the soil samples of L-1 as *Cladosporium* sp., L-6 as *Penicillium* sp., L-8 as *Trichoderma* sp., L-16 as *Fusarium* sp., K-5 as *Gliocladium* sp., K-7 as *Aureobasidium* sp. and the sawdust sample of S-6 as *Botrytis* sp. and the groundnut shells sample of G-3 as *Rhizopus* sp. based on their macroscopical and microscopical characters by Barnett (1960) and Dube (1983).

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