MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Studies on pectinolytic fungi isolated from Egyptian soil

Eman M. Shanab, Samia A. Haroun, Ehab A. Metwally, and Gamal M. Abdel-Fattah*

Botany Department, Faculty of Science, Mansoura University

**** Corresponding author*** *E- mail: abdelfattaham@yahoo.com*

Received:16/11/2023
Accepted: 21/12/2023

Abstract: Pectin is a naturally occurring substance that is abundant and has a wide range of uses. It is widely dispersed in different amounts throughout the terrestrial plant's cell walls and middle lamella. The primary constituents of pectin, a heteropolysaccharide, are methanol and galacturonic acid. The pectinase enzyme breaks down pectin to produce several chemicals with industrial uses. A class of enzymes known as pectinases is generated by a variety of organisms, including nematodes, plants, bacteria, fungi, and insects. Pectinases are crucial for numerous industries and are now required commercially for a variety of uses. This study aimed to isolate and screen pectinase-producing fungi from agricultural soils. Sixty fungal isolates belonging to twenty-four species were isolated on culture media supplemented with pectin. All fungal isolates were subjected to morphological identification; the cultural fungal species were identified as *Alternaria alternate, Aspergillus carneus, Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Cunninghamella elegans, Curvularia sp, Penicillium citrinum, Syncephalastrum racemosum,* and *Trichoderma harzianum.* Based on screening of all isolated fungi for qualitative pectinolytic activity, the result showed that *A.carneus* and *A.terreus* had the highest pectinolytic activity when compared with the other isolated fungal species.

Keywords: Pectin, pectinase, fungi, enzymes,citrus, soil

Introduction

Pectin is a complex of polysaccharides; the basic unit is galacturonan (α-D galacturonic acid) .There are two types of pectic substrates. Heterogalacturonan and homogalacturonan were present. Homogalacturonan is a linear polymer containing α (1→4) D galacturonic acid with methyl-and/or acetyl-esterified groups at C-6, O-2, and/or O-3. Additionally, it contains approximately 2-4 percent L-rhamnose units that bind β-(1→2) and β-(1→4) to galacturonate units. Heterogalacturonan is a heteropolysaccharide consisting of 300–1000 chained α (1–4)-linked D-galacturonic acid monomers with a molecular weight ranging from 50,000 to 180,000 Da. It was negatively charged. Although they differ in length and content, their side chains contain simple carbohydrates such as fucose, xylose, arabinan, and galactan. Through their C1 and C2 atoms, they are connected to the center chain [**1**].

The composition of pectin components and the process by which pectolytic enzymes break down pectic materials laid the foundation for the history of pectinases. Subsequently, pectinase synthesis by microorganisms rose to prominence and has remained so for many years. Many microorganisms, including fungi, yeast, and bacteria, are capable of producing pectinases. Plant pathogens use many cell-degrading enzymes to assault target cells, making it easier for pathogens to enter and spread throughout the host tissue [**2**].

Research has revealed that pectinases can be synthesized from a variety of carbon sources and that they are inducible. Many studies on the optimization of microbiological and fermentation parameters as well as various fermentation techniques for the synthesis of pectinases have been published over time [**3**]. Debary (1886) demonstrated the significance of pectinase as the most significant virulence factor in the degradation of pectin found in plant cell walls almost a century ago [**4**]. Subsequently, these enzymes were initially

employed in the home to prepare food, before being applied to the industrial sector in 1930.Pectinases were mostly utilized in fruit juice and wine until the 1960s to aid clarity [**5**].

Currently, pectinases, which represent 25% of the global enzyme market for food and drinks, have drawn worldwide interest as environmentally benign biocatalysts. The diverse class of related enzymes pectinases or pectinolytic enzymes hydrolyze pectic compounds, which are typically found in plants, higher plants, and microbes [**5**].They are crucial to plants because they aid in the expansion of cell walls and the softening of some plant tissues during growth and storage. By encouraging the breakdown and recycling of leftover plant elements, they also contribute to the preservation of ecological equilibrium. Pectinolytic enzymes also play a significant role in the rotting of fruits and vegetables and plant pathogenicity. Three broad categories of pectinolytic enzymes can be separated [**6**] into:

(I) Protopectinases: They produce highly polymerized soluble pectin by breaking down insoluble protopectin.

(II) Esterases: initiate pectin de-esterification by removing methoxy esters.

(III) Depolymerases: initiate the process of hydrolytically breaking the α- (1, 4)-glycosidic linkages found in the D-galacturonic acid moiety of the pectic materials.

Plants, insects, nematodes, protozoa, fungi, yeasts, and bacteria release pectinolytic enzymes. Although microorganisms and plants are the main sources of pectinase enzyme production, microbial sources such as fungi, yeast, and bacteria have been chosen as the main sources because of their commercial and technical viability [**7**]**.** Currently, the main sources of commercial enzymes are microorganisms: fungi and yeast account for 50% of the origin, bacteria account for 35%, and plants account for the final 15% [**8**]**.**This study aimed to isolate and screen pectinase-producing fungi from agricultural soils.

Materials and methods

Collection of samples

In the current study, two distinct kinds of soil samples yielded fungi that were isolated. and their pectinase activity was investigated. Farmlands at the Nubaria Agricultural Research Station in the Beheira Governorate of Egypt and the Faculty of Agriculture at Mansoura University in the Dakahlia Governorate of Egypt were used to gather clay and sandy soil samples. Using a sterile spatula, Soil specimens were gathered then placed in clean plastic bags then placed in clean plastic bags before being brought to the Microbiology Research Laboratory, Botany Department, Faculty of Science, Mansoura University. for further processing.

Isolation of Fungi

A previously outlined process was followed in the collection of the samples [**9**], with slight modifications. Ten grams of air-dried soil samples were placed in a liter Erlenmeyer conical flask with 100 mL sterile saline solution. The samples were shaken for 15 min, and the soil particles were cemented for 30 min. Next, 10 mL of the supernatant was added to 90 mL of sterile saline solution. Subsequent dilutions were prepared in the same manner after shaking for ten minutes. One milliliter of each dilution was aseptically added to plates of mineral salt pectin agar medium with one percent pectin as the only source of carbon. For every dilution, three plates were created. Developing fungal colonies were selected and purified by subculturing on the same medium after the plates were incubated for eight days at a temperature of 28ºC and a pH of 7.

Identification of selected fungal isolate

Morphological characterization of pectinase-producing fungi

For identification of the isolated fungal colonies were subcultured on the pectin agar medium. Each pure culture was then described and recognized according to its morphological and microscopic features, sporulation, and colony colour. The analysis was completed following the description provided by [**10**. Using a needle and a drop of alcohol on a sterile glass slide, a piece of the sporing surface of the culture was removed. Lactophenol drops were added to stain the fragment, and a coverslip was carefully attached to prevent air bubbles. After that, the preparation was examined with a light microscope. As far as

practicable, the isolated, pure fungus was identified down to the species level. Genera and species of fungi have been identified using the well-accepted keys for the identification of different isolated fungi [**11**]**.**

Screening of Pectinase Producing Fungi

Qualitative assay of pectinase

This experiment was conducted using a pectin agar medium. Pure culture was inoculated and incubated for 72 h at 28°C.An iodine-potassium iodide solution was added after incubation to determine the clearance zone [**12**].

Results and Discussion

Pectinolytic fungi were identified from the citrus soil samples' inoculum. A total of 60 fungal isolates from different dilutions of soil solution belonging to twenty-one genera and twenty-four species were isolated on mineral salt pectin agar medium containing 1% pectin as the sole carbon source for 7 days at 28ºC.Fungal isolates were purified and maintained for further studies. The present results revealed that the highest number of fungal isolates was recorded in the first dilution, whereas the lowest number was recorded in the fifth dilution, as shown in **(Table 1).**

The morphological and cultural traits of the isolates were identified by the current investigation **[10]**, which were identified as *Alternaria alternata, Aspergillus carneus, Aspergillus flavus, Aspergillus terreus, Aspergillus niger, Cunninghamella elegans, Curvularia spp., Penicillium citrinum, Syncephalastrum racemosum,* and *Trichoderma harzianum.*

Primary screening of pectinolytic fungi

Further screening of qualitative pectinolytic activity on citrus pectin plates supplemented with iodine solution, based on the rate of zone clear around the fungal colony, was obtained **(Fig 1 and Table 2)**. Positive isolates were selected based on the clearance zone in the iodine-potassium iodide assay, namely *Aspergillus carneus, Aspergillus terreus, and Curvularia sp. Penicillium citrinum, Alternaria alternata, Aspergillus niger, Aspergillus flavus, Syncephalastrum racemosum, and Cunninghamella elegans* recorded10 mm, 9 mm, 8.8 mm, 8.5 mm, 7 mm, 7 mm, 6 mm, 2 mm, and 1 mm, respectively.

Secondary screening of pectinolytic microorganisms based on DNS assay

Isolates with the highest clear zone diameter-to-colony diameter ratio on pectin agar plates were subjected to submerged fermentation using pectin mineral media. Submerged fermentation was performed to select the most potent strains. High pectinase activity was recorded in *Aspergillus carneus and Aspergillus terreus,* as shown in **Fig (2 and 3)** and **Table (2)**. [**13**] screened bacterial strains isolated from soil and vegetable samples by using plate agar and submerged fermentation screening methods

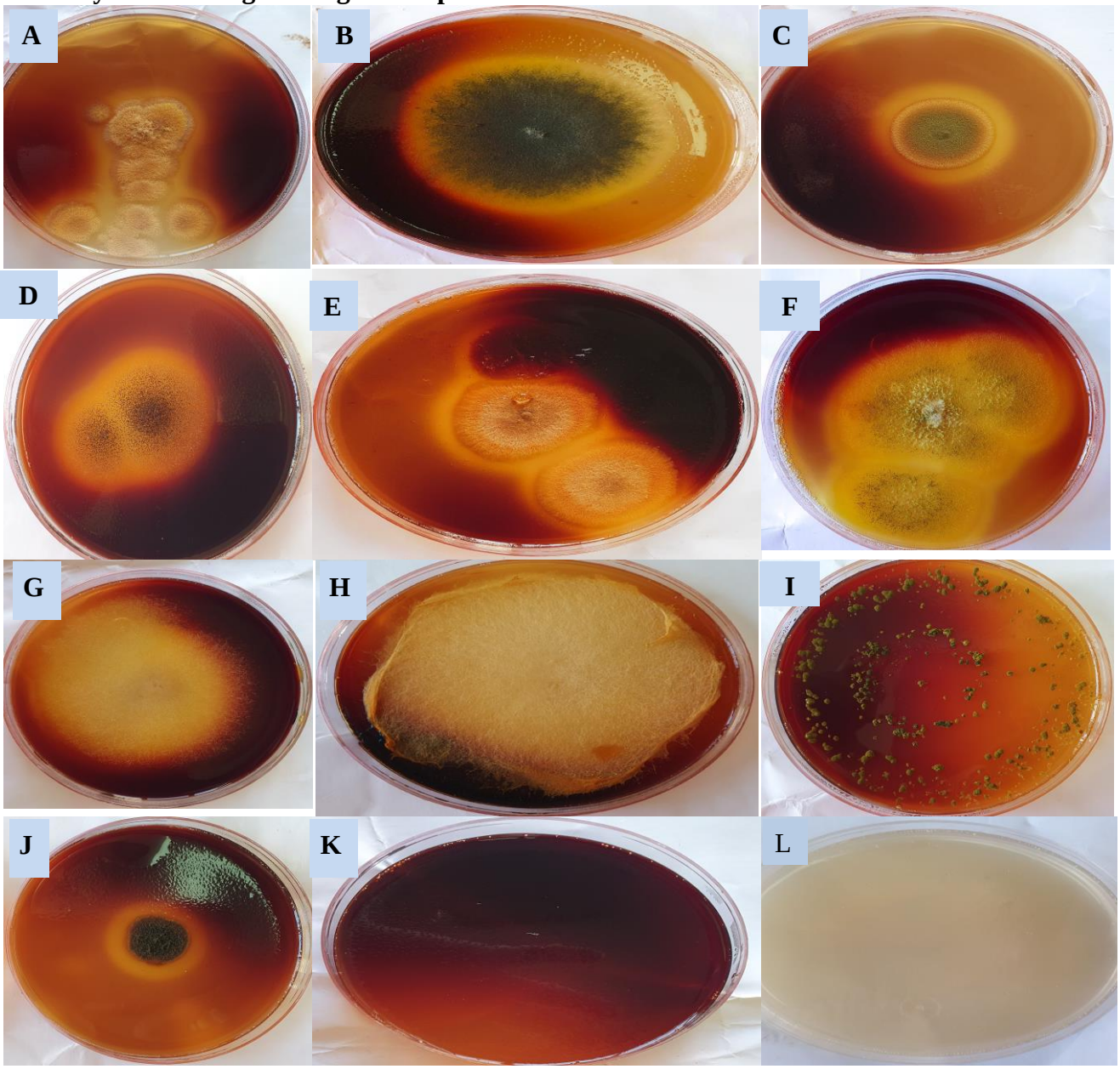
Table (1): Isolation of fungi producing pectinase from different citrus soil samples on a specific medium at 28 °C ± 2.

Fungal isolates	**Colony number\plate**						**TC%**
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
Alternaria alternata	3	-	1	-	-	-	7
Aspergillus carneus	-	1	-	-	-	-	2
Aspergillus Flavus	2	-	-	-	-	5	12
Aspergillus niger	1	-	-	-	-	2	5
Aspergillus terreus	-	4	2	-	-	-	10
Cunninghamella elegans	2	-	-	-	2	-	7
Curvularia sp	3	-	1	-	1	-	8
Penicillium citrinum	8	-	4	-	2	-	23
Syncephalastrum racemosum	3	2	-	5	-	1	18
Trichoderma harzianum	1	-	-	2	-	2	8
Number of fungal isolates	23	7	8	7	5	10	60
Number of genera	7	2	4	2	3	3	21
Number of species	8	3	4	2	3	4	24

Table (2): Isolated fungal species showed different Zone of Hydrolysis and rates of pectinolytic

FUNGAL ISOLATESNO.		Assay of hydrolysis		Pectinolytic activityU/mL
		Fungal colony	Clear zone (mm) (mm)	
1	***Alternaria alternate***	50	7	624.33
2	***Aspergillus carneus***	19	10	671.95
3	*Aspergillus flavus*	60	6	166.13
4	***Aspergillus niger***	50	7	438.09
5	***Aspergillus terreus***	21	9	634.92
6	***Cunninghamella elegans***	70	1	275.66
7	***Penicillium citrinum***	28	8.5	583.59
8	***Syncephalastrum racemosum***	58	2	495.23
9	***Trichoderma harzianum***	80	0	441.26
10	***Curvularia sp***	30	11	457.14

Activity on Pectin agar using iodine-potassium iodide solution.



Fig(2): Pectinase-producing fungi A) Aspergillus carneus, B) Alternaria alternata, C) Penicillium citrinum, D) Aspergillus niger, E) Aspergillus terreus, F) Aspergillus flavus, G) Syncephalastrum racemosum, H) Cunninghamella elegans**,** I) Trichoderma harzianum, J) Curvularia sp **,** K) iodine sol. Control and L) pure plate before adding iodine sol. Plate showing clear zones on pectin agar plates after adding iodine-potassium iodide solution.

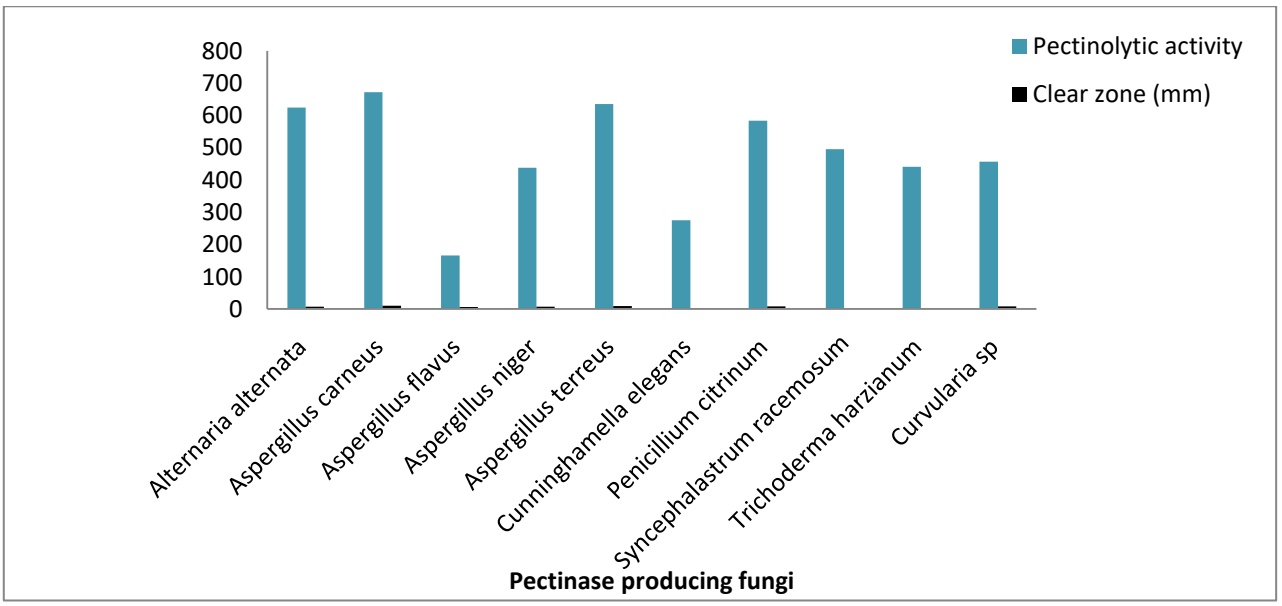


Fig (3): Pectinolytic activity showed in different isolated strains.

4. References

1. Jayani, R.S., Saxena, S. and Gupta, R.(2005): Microbial pectinolytic enzymes. Process Biochemistry. **40(9)**, 2931-2944.
2. Yadav, S., Yadav, P.K., Yadav, D. and Yadav, K.D.S. (2009): Pectin lyase. Process Biochemistry, **44(1)**,1-10.
3. Rao, M.B., Tanksale, A.M., Ghatge, M.S. and Deshpande, V.V. (1998): Molecular and biotechnological aspects of microbial proteases. Microbiology and molecular biology reviews, **62(3)**, pp.597-635.
4. Lang, C. Dörnenburg, H. (2000): Perspectives in the biological function and the technological application of polygalacturonases. Applied Microbiology and Biotechnology. **53(4)**,366-75.
5. Kashyap, DR., Vohra, PK., Chopra, and Tewari, R. (2001): Applications of pectinases in the commercial sector. Bioresource technology. **1**,**77(3)**, 215-27.
6. Jayani, RS., Saxena, and Gupta, R. (2005): Microbial pectinolytic enzymes. Process Biochemistry. **1,40(9)**, 2931-44.
7. Garg, G., Singh, A., Kaur, A., Singh, R., Kaur, J. and Mahajan, R. (2016): Microbial pectinases: an ecofriendly tool of nature for industries. Biotechnology. **6(1)**, 1-3.
8. Anisa, S.K. and Girish, K. (2014): Pectinolytic activity of Rhizopus sp. and Trichoderma viride. *International Journal of Research in Pure and Applied Microbiology*, **4(2)**, 28-31.
9. Siameto, E.N., Okoth, S., Amugune, N.O. and Chege, N.C. (2010): Antagonism of Trichoderma harzianum isolates on soil-borne plant pathogenic fungi from Embu District, Kenya. *Journal of Yeast and Fungal* Research, **1(3)**, 47-54.
10. Abd El-Rahim, W.M., Moawad, H., Hashem, M.M., Gebreil, G.M. and Zakaria, M. (2020): Highly efficient fungal pectinase and laccase producers among isolates from flax retting liquor. Biocatalysis and Agricultural Biotechnology. **25**, 101570.
11. Moubasher, A.H.(1993): Soil fungi in Qatar and other Arab countries. The Centre for Scientific and Applied Research, University of Qatar.
12. Hankin, L., Zucker, M. and Sands, D.C., (1971): Improved solid medium for the detection and enumeration of pectolytic bacteria. Applied Microbiology. **22(2)**, 205-209.
13. Miller, G.L. (1959): Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry. **31(3)**, 426-428.