# Productivity of pectinase enzymes by *Aspergillus sp.* isolated from Egyptian soil.

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**ABSTRACT:**

Among ten of *Aspergillus* isolates were detected for pectin hydrolysis isolated from different Governorates in Egypt on pectin screening agar medium (PSA), four isolates of *Aspergillus* were observed with high of pectinase production by clear zone method which were 3.62, 3.70, 3.91, and 4.75mm for these isolates (F4, F5, F9 and F10). The isolate F9 of *Aspergillus* was selected for height pectinase production it was recorded the maximum zone of clearance (4.75mm) as enzyme activity. These isolate has been identified as *Aspergillus flavus* (MH237625.1) according to microscopic characteristics, and molecular biology by ITS. The optimization condition for production of pectinase enzyme such as; pH value, incubation temperature, incubation periods, carbon source, nitrogen source, and mineral salts by isolate F9 of *Aspergillus flavus* (F9 isolate). The productivity of enzymes was assayed in submerged fermentation condition. The maximum pectinase production was observed with pH 7.0 (32.64 U/ml), temperature 25°C (39.09 U/ml), incubation periods 5 days, (38.11 U/ml), maltose (30.79 U/ml), peptone (39.25 U/ml), and $FeSO_4$ (37.41 U/ml) in the production medium.

**Keywords:** Pectinase, *Aspergillus flavus*, Culture condition optimization.

## INTRODUCTION

Pectin or other pectic substances are heterogeneous group of high molecular weight polysaccharides with galacturonic acid residues linked by (1-4) linkages methyl ester (Kashyap, *et al*., 2001). Pectins constituting middle lamella found between the primary cell walls of adjacent young plant cells (Hoondal, *et al*., 2002; Jabeen, *et al*., 2015).

Pectinase is a group of enzymes that is involved in the breakdown of pectin. It also breaks glycosidic linkages by splitting polygalacturonic acid into mono galacturonic acid (Sethi, *et al.,* 2016; Abdullah, *et al.,* 2018). Pectinases have crucial roles in food industries. These enzymes are useful for fruit juice extraction and wine clarification; tea, cocoa, and coffee concentration and fermentation; vegetable oil extraction; preparation of jam and jellies; and pickling (Barman, *et al.,* 2015; Kubra, *et al.,* 2018 ; Sudeep, *et al.,* 2020). Furthermore, these enzymes are used in paper and pulp industries, bleaching of paper, bio-scouring of cotton, retting and degumming of plant fibers, oil extraction, wastewater treatment, poultry feed additives, protoplast fusion technology, and bioenergy production (Oumer, 2017; Kubra, *et al.,* 2018).

Microorganisms are currently the primary source of industrial enzymes: 50% originate from fungi and yeast; 35% from bacteria, while the remaining 15% are either of plant origin (Boopathy and Kulpa 1994). At present, almost all the pectinolytic enzymes used for industrial applications are produced by the fungi, namely *Aspergillus* sp., *Rhizopus stolonifer*, *Alternaria mali*, *Fusarium oxysporum*, *Neurosporacrassa*, *Penicillium italicum* ACIM F-152, and many others. Beulah, *et al.,* (2015). Even though the occurrence of pectinolytic enzymes have been reported in a large number of bacterial and fungal strains, almost all the commercial preparations of pectinases are produced from fungal sources, with *Aspergillus niger* being the most commonly used fungi species for industrial production of pectinolytic enzymes (Oliyad and Dawit, 2018).

Culture conditions such as incubation periods, pH value, incubation temperature, source of carbon, nitrogen and mineral salts etc play an important role in obtaining good pectinase yield. Optimization of the culture conditions is hence, necessary in the selection of the fungal source for industrial exploitation of their extracellular enzymes (Krishnaveni, *et al*., 2012; Kumara, *et al*., 2012).

## MATERIALS AND METHODS

### Sample collection:

Soil samples culture with various crops were collected from different Governorates of Egypt Giza, Gharbia , Kafr El Sheikh , Menoufia and Sharkia were brought to microbiology laboratory from the study.

### Isolation and purification of pectinolytic fungi:

Pectinolytic fungi were isolated from soil samples by serial dilution method and spread on Potato dextrose agar (PDA) plates. 0.1 ml of sample spread on petri plates from last two dilutions and these plates were incubated at 28°C for 72 hours. After incubation mixed colony were obtained which were purified by streaking on water agar media.

**Screening of pectinolytic fungi:**

Screening was done by the spot inoculation method, The fungal isolates were inoculated on the pectinase selective agar media containing of (Pectin 10 g/l, $(NH_4)_2HPO_4$ 3 g/l, $KH_2PO_4$ 2 g/l, $K_2HPO_4$ 3 g/l, $MgSO_4.7H_2O$ 0.1 g/l Agar 20 g/l). All isolates of fungi were screened by disk (5mm) of growth plate on selective media and incubated at 30 °C for 5 days. The clear zone around the colonies were observed and measured by flooding Iodine solution (iodine 1.0g, potassium iodine 5.0g in 330ml distilled water) on the plates (Satapathy, *et al*., 2019).

**Identification pectinolytic fungal isolate:**

The fungal isolate highly pectinase produced were identified by observing morphological and microscopic characteristics such as color, form, reverse, and margin according to the handbook for the identification of fungi (Alexopoulos, *et al*., 1996).

**Molecular identification of the isolate:**

The fungal isolate of highly pectinase enzymes production was identified by molecular techniques.

**ITS analysis**

***DNA isolation:***

Firstly the DNA was isolated from F9 isolate by CTAB method according to (Sambrook, *et al.,* 1989)

***PCR Reactions:***

The PCR amplification was performed in a total volume of 50 µl, containing 1X reaction buffer, 1.5 mM $MgCl_2$, 1U *Taq* DNA polymerase (Promega), 2.5mM dNTPs, 30 poml of each primer and 30 ng genomic DNA.

| Primer Code | Sequence | Product Size |
|---|---|---|
| *(ITS-1)* F | 5′- TCCGTAGGTGAACCTGCGG -3′ | 600bp |
| *(ITS-4) R* | 5′- TCCTCCGCTTATTGATATGC-3′ | |

***Thermo-cycling PCR program***

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfil 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 30 sec., an annealing step at 45ºC for 30 sec. and an elongation step at 72ºC for 1 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

***Detection of the PCR products:***

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5µg/ml) in 1X TBE buffer at 95 volts. A 100bp DNA ladder was used as a molecular size standard. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

**Purification of PCR products:**

Amplified products for all PCR were purified using EZ-10 spin column DNA clean up mini prep kit. PCR products purification PCR reaction mixture was transferred to 1.5 ml microfuge tube and three volumes was added of binding buffer 1 after that the mixture solution was transferred to the EZ-10 column and let it stand at room temperature for 2 minutes after that centrifuge, 750 µl of wash solution was added to the column and centrifuge at 10000 rpm for two minutes, repeated washing, 10000 rpm was spine for an additional minute to remove any residual wash solution. The column was transferred into a clean 1.5 ml microfuge tube and adds 50 µl of elution buffer, incubated at room temperature for 2 minutes and when store purified DNA at -20 °C.

**ITS sequencing analysis:**

The sequencing of the product PCR was carried through in an automatic sequencer ABI PRISM 3730XL Analyzer using Big Dye TM Terminator Cycle Sequencing Kits following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using Rbcl Forward primer. The fluorescent-labelled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Microgen Company).

**Computational analysis (BLASTn) ITS:**

The sequences were analysed using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST) Sequences were aligned using Align Sequences Nucleotide BLAST.

**Pectinase enzyme productivity by submerged fermentation:**

The fungal isolates were grown in submerged fermentation medium in250ml Erlenmeyer flasks; the flasks were inoculated with 1.0 ml of culture isolates and incubation at 30 °C for5 days at 120 rpm on rotary shaker. After that, the broth culture was centrifuged at 4000 rpm for 10 min. to remove the cells and debris. The supernatant was designated as the crude enzyme which is then used for enzyme assay and characterization (Kaur and Kaur, 2014).

**Pectinase enzyme assay:**

The pectinase activity of crude enzyme measured based on the determination of reducing sugars produced as a result of enzymatic hydrolysis of pectin by dinitrosalicylic acid reagent (DNS) according to Miller method (Miller, 1959). The reaction mixture consisted of 0.5ml of 1% pectin in potassium phosphate buffer (pH 7) and 0.5 ml of enzyme extract. The reaction mixture was incubated at 37ºC for 25 min. After incubation 1 ml of DNS was added, mixed and kept on a boiling water bath for 5 min. The tubes were cooled in cold water and by measuring absorbance were recorded at 540 nm (JENWAY-6305 UV-VIS Spectrophotometer). The enzyme and the substrate were run parallel. The standard curve was prepared with of different concentration galacturonic acid (Okonji, *et al*., 2019).

**Total protein estimation of crude enzyme:**

The protein concentration was determined by the Lowry's method, as described by (Lowry, 1951). Standard graph was plotted between concentration of Bovine Serum Albumin (BSA) and optical density. The protein content of crude enzyme sample was calculated from standard graph by comparing the O.D with standard graph (Okonji, *et al*., 2019).

**Optimization of cultural conditions for pectinase productivity:**

Optimization of pectinase produced by the F9 selected isolate studied such as, pH value, incubation temperature, incubation periods, source of carbon, nitrogen and mineral salts.

**Effect of pH value:**

Fermentation medium as prepared in several Erlenmeyer flasks 50ml/250ml, the pH were adjusted as the following (4, 5, 6, 7, 8 and 9), then sterilized, the inoculated broth was incubated for fermentation at 30ºC for 5 days. After that, the enzyme assay was carried out by DNS assay method (Dhital, *et al*., 2013).

**Effect of incubation temperature:**

To study the effect of incubation temperature on pectinase production with selected isolate 50 ml of the fermentation media was prepared and sterilized by autoclaved and inoculated with 1ml of spore dilution these culture were incubated at 20°C, 25°C, 30°C, 35°C, 40°C and 45°C for 5 days at rotary shaker. After that, the enzyme assay was done by usual DNS assay method (Dhital, *et al*., 2013).

**Effect of incubation periods:**

The effect of incubation periods for pectinase production was determined by incubation fermentation medium at different incubation periods viz., 3, 5, 7, 9, 11 and 13days. After that, the enzyme assay was carried out by DNS assay method (Chowdhury, *et al*., 2017).

**Effect of different carbon sources:**

The effect of different carbon sources on pectinase production was studied by adding 1% each of glucose, fructose, sucrose, maltose, lactose, dextrose and galactose in the optimized fermentation medium which were incubated at 30°C for 5 days under shaker conditions. After that, the enzyme assay was carried out by DNS assay method (Kaur and Kaur, 2014).

**Effect of different nitrogen sources:**

Different nitrogen sources ($NH_4Cl$, $NaNO_3$, $KNO_3$, $NH_4NO_3$, yeast extract, peptone and casein) at 0.1% concentration were used in preparation of production medium 50ml/250ml Erlenmeyer flask, and inoculated as mention before. Each flask was incubated at 30ºC 5 days. After that, the enzyme assay was carried out by DNS assay method (Kaur and Kaur, 2014).

**Effect of different mineral salts:**

To study the effect of mineral salts on pectinase activity various mineral salts were used in the media viz., $MgSO_4$, $FeSO_4$, $ZnSO_4$, $MnSO_4$, $CuSO_4$, $CaSO_4$ and $Na_2SO_4$ at 0.1% concentration. After that, the enzyme assay was carried out by DNS assay method.

## RESULTS AND DISCUSSION

### Isolation and purification of pectinolytic fungi:

In the present study, 10 fungal isolates were collected from different soil samples from the local soil in Egypt. The isolation was carried out by serial dilution of soil samples on Potato dextrose agar medium by streak plating isolation techniques were used. Subsequently, the isolates were sub cultured into their respective selective growth media until pure cultures were isolated (Kaur and Kaur, 2014; Okonji, *et al*., 2019).

### Screening of pectinolytic fungi:

A total 10 isolates were screened for the pectinolytic activity. Selected fungal isolates were grown on pectin agar plate for screening purpose and pectinolytic activity was observed using plate assay method. The isolated strains showed clear zone around the colonies, after staining with iodine-potassium iodide solution. Among 10 isolates, four isolates showed that the maximum clearance zone of pectinolytic activity (3.62, 3.70, 3.91, and 4.75mm) shown in table 1. Priya and Sashi (2014) reported that the maximum clearance zone of pectinolytic activity in *Aspergillus*, *Penicillium jenseni* and *P. citrinum* of 4.0mm around the colonies. Kamalambigeswari, *et al.,* (2018) who screened three different *Aspergillus niger* strains F-3, F-4, F-P and reported isolate F-4 produced the maximum (9 mm) zone of pectin hydrolysis.

### Pectinase enzyme productivity by submerged fermentation:

The four isolates (F4, F5, F9 and F10) are most efficient at pectinolytic activity with a clear zone were screened for quantitative estimation for pectinase production using selective enrichment approach to draw comparative account. This method was based on the enzymatic hydrolysis of pectin, the galacturonic acid was determined spectrophotometrically. Among 4 isolates F9 shows relatively higher pectinase production than that of other isolates (35.83 U/ml) (Table 2). Therefore F9 was selected finally for further experiments**.** Akbar and Prasuna (2012) found that *Aspergillus oryzae* gave 42 units/ml of pectinase enzymes activity in submerged fermentation medium.

### Identification of the fungal isolate F9 of *Aspergillus* sp.:

Fungal isolate F9 of *Aspergillus* sp. was identified on the basis of morphological and microscopic characteristics. The isolate F9 form colonies was observed circular, color white, margin entire and reverse goldish to red–brown according to (Alexopoulos, *et al*., 1996).

### Molecular identification of the isolate:

Molecular characterization of this strain was done by DNA isolation (CTAB method) according to (Sambrook, *et al*., 1989) and ITS region analysis (Fig. 1). Further, these amplified ITS region sequence of the fungal strain was blasted using online tool. The taxonomical identification was done by the phylogenetic tree construction and the comparison of this fungal strain sequences with other homologous fungal sequences. After morphological, microscopic characteristics, and molecular characterization, the isolate was identified as *Aspergillus* sp. As per the results obtained the strain F9 was identified as *Aspergillus flavus* (MH237625.1).

### Optimization of cultural conditions for pectinase productivity:

Culture conditions such as pH value, incubation temperature, incubation periods, source of carbon, nitrogen and mineral salts are known to influence the synthesis and secretion of extracellular enzymes by microorganisms. Optimization of the culture conditions is hence, necessary in the selection of the fungal source for industrial exploitation of their extracellular enzymes (Krishnaveni, *et al*., 2012; Kumara, *et al*., 2012).

### Effect of pH value:

The pH of the fermentation medium is an important factor in the production of pectinases for it influences the sort and content of those enzymes produced by fungus. As shown in Table 3 the optimum pectinase production by *Aspergillus flavus* (F9 isolate) was observed at pH 7 (32.64 U/ml) and minimum pectinase production was exhibited at pH 4 (25.26 U/ml). The results were more or less similar to those reported by Rohit, *et al.,* (2013). They reported that the enzyme production by *Aspergillus niger* K3 was higher at pH 8. El Garhy, *et al.,* (2020) reported that the pectinase production by *Penicillium chrysogenum* grown on different pH medium showed its highest values at pH 4.0.

### Effect of incubation temperature:

Temperature is directly related to the metabolic activities of the microorganism and it affects the proper growth and product formation by the organism. Table 4 shows the effect of various temperatures on pectinase

production. The maximum pectinase production was obtained at 25°C (39.09 U/ml). Followed by this, 30°C temperature (34.91U/ml) was the second best temperature on pectinase production. On the other hand, the minimum amount of pectinase production was observed at temperature 45°C (27.65 U/ml). Ketipally *et al.,* (2019) reported that at various temperatures between 25°C and 35°C was the most suitable temperature for the growth and production of polygalacturonase by *A. nomius* MR 103.

Dhital, *et al.,* (2013) showed that the optimum temperature for maximum enzyme production was found to be at 30°C. At this temperature, 32 U/ml enzymes were produced by *Aspergillus niger* (MG1).

**Effect of incubation periods:**

Incubation period plays an important role in the maximum production of enzymes. The maximum amount of pectinase production was observed with 5 days incubation time (38.11 U/ml). The minimum amount of pectinase production was obtained with 13 days incubation (23.42 U/ml) as shown in (Table 5). Adeleke *et al.,* (2012) concluded that *Penicillium atrovenetum, Aspergillus flavus* and *A. oryzae* produced polygalacturonase optimally on the 5 days which is in accordance with the current findings.

Deshmukh, *et al.,* (2012) reported that *Aspergillus oryzae* produced 224 U/ml of enzyme after 6 days of incubation.

**Effect of different carbon sources:**

Type of the carbon source is one of the most important factors for any fermentation process. Carbon source represents the energy source that will be available for the growth of the microorganism. Among the various carbon source used for pectinase production, maltose was found to be the best carbon sources, showing maximum enzyme activity of 30.79 U/ml (Table 6). This was followed by glucose, sucrose, lactose, fructose, dextrose and galactose. Similar results are observed by Rajmane and Korekar (2012) in *Aspergillus* sp. Abdullah, *et al.,* (2018) reported that xylose proved to be best for optimal production of pectinase by *Aspergillus niger*.

**Effect of different nitrogen sources:**

Nitrogen is the basic element of the cellular components. Table 7 shows the effect of nitrogen sources on the production of pectinase. The maximum pectinase production of 39.25 U/ml was obtained with peptone. This was followed by ammonium nitrate, potassium nitrate, ammonium chloride, yeast extract, casein and sodium nitrate. These results are similar to (Akhter, *et al*., 2011) the findings of peptone were found to support maximal production of pectinase (113.68 U/gm) by *Aspergillus niger*. Rasheedha, *et al.,* (2010) reported that ammonium sulphate has enhanced the production of *P. chrysogenum* pectinase.

**Effect of different mineral salts:**

The mineral salts were considered to be important cofactors for an enzyme to function and hence so analyzed. Among the various mineral salts sources that have been tried, iron sulphate showed maximum enzyme production of 37.41 U/ml followed by magnesium sulphate with 36.05 U/ml and a minimum enzyme production of 23.85 U/ml was observed in zinc sulphate at 5 days as shown in (Table 8). Jagadeesh Babu and Viswanathan (2010) have reported that significant enhancement in the level of pectinase production by *A. foetidus* NCIM 505 was achieved with $Cu^{2+}$ which was found to be deleterious for *A. terreus*.

## CONCLUSION:

In conclusion, Pectinase enzyme was production from the native strain, *Aspergillus flavus* (F9 isolate) that can be used for various industrial applications including extraction and clarification of fruit juices, processing of cotton fabric in textile industries, bleaching of paper, removal of pectic waste waters and maceration of tea leaves. *Aspergillus flavus* gave maximum pectinase production under pH 7.0, 25 °C for 5 days and enhanced growth with maltose, peptone and iron sulphate respectively, in fermentation medium.

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**Table 1:** Screening of pectinolytic fungi.

| Isolates of fungi | Clear zone diameter (mm) | Colony diameter (mm) | Clear zone diameter to colony diameter ratio |
|---|---|---|---|
| F1 | 47 | 18 | 2.61 |
| F2 | 35 | 19 | 1.84 |
| F3 | 41 | 15 | 2.73 |
| F4 | 45 | 20 | 2.25 |
| F5 | 47 | 13 | 3.62 |
| F6 | 37 | 10 | 3.70 |
| F7 | 35 | 18 | 1.94 |
| F8 | 28 | 10 | 2.80 |
| F9 | 57 | 12 | 4.75 |
| F10 | 43 | 11 | 3.91 |

**Table 2:** Pectinase enzyme productivity by submerged fermentation.

| Fungal isolates | Enzyme activity (U/ml) | Total protein (mg/ml) | Specific activity (U/mg) |
|---|---|---|---|
| F 4 | 26.56 | 0.855 | 31.08 |
| F5 | 26.56 | 0.855 | 31.08 |
| F9 | 35.83 | 0.569 | 63.01 |
| F10 | 30.41 | 0.781 | 38.94 |

**Table 3:** Effect of pH value.

| pH value | Enzyme activity (U/ml) | Total protein (mg/ml) | Specific activity (U/mg) |
|---|---|---|---|
| PH 4 | 25.26 | 0.89 | 28.38 |
| PH 5 | 27.38 | 0.88 | 31.11 |
| PH 6 | 28.57 | 0.76 | 37.59 |
| PH 7 | 32.64 | 0.54 | 60.44 |
| PH 8 | 31.50 | 0.65 | 48.46 |
| PH 9 | 27.86 | 0.80 | 34.83 |

**Table 4:** Effect of incubation temperature.

| Incubation temperature | Enzyme activity (U/ml) | Total protein (mg/ml) | Specific activity (U/mg) |
|---|---|---|---|
| 20 ºC | 33.29 | 0.51 | 68.45 |
| 25 ºC | 39.09 | 0.44 | 88.84 |
| 30 ºC | 34.91 | 0.57 | 58.40 |
| 35 ºC | 32.04 | 0.58 | 55.24 |
| 40 ºC | 29.38 | 0.67 | 43.85 |
| 45 ºC | 27.65 | 0.84 | 32.92 |

**Table 5:** Effect of incubation periods.

| Incubation periods (day) | Enzyme activity (U/ml) | Total protein (mg/ml) | Specific activity (U/mg) |
|---|---|---|---|
| 3 | 29.49 | 0.61 | 48.34 |
| 5 | 38.11 | 0.43 | 88.63 |
| 7 | 35.35 | 0.53 | 66.70 |
| 9 | 27.65 | 0.77 | 35.91 |
| 11 | 26.13 | 0.81 | 32.26 |
| 13 | 23.42 | 0.93 | 25.18 |

**Table 6:** Effect of different carbon sources.

| Carbon sources (1%) | Enzyme activity (U/ml) | Total protein (mg/ml) | Specific activity (U/mg) |
|---|---|---|---|
| Glucose | 20.98 | 0.84 | 24.98 |
| Fructose | 16.53 | 1.10 | 15.03 |
| Sucrose | 20.76 | 0.89 | 23.33 |
| Maltose | 30.79 | 0.82 | 37.55 |
| Lactose | 20.11 | 0.94 | 21.39 |
| Dextrose | 13.66 | 1.12 | 12.20 |
| Galactose | 9.81 | 1.18 | 8.31 |

**Table 7:** Effect of different nitrogen sources.

| Nitrogen sources (0.1%) | Enzyme activity (U/ml) | Total protein (mg/ml) | Specific activity (U/mg) |
|---|---|---|---|
| Yeast extract | 32.26 | 0.95 | 33.96 |
| Peptone | 39.25 | 0.34 | 115.44 |
| Casein | 27.00 | 1.07 | 25.23 |
| $NH_4Cl$ | 36.21 | 0.60 | 60.35 |
| $NaNO_3$ | 26.18 | 1.18 | 22.19 |
| $KNO_3$ | 37.35 | 0.47 | 79.47 |
| $NH_4NO_3$ | 38.76 | 0.36 | 107.67 |

**Table 8:** Effect of different mineral salts.

| Mineral salts (0.1%) | Enzyme activity (U/ml) | Total protein (mg/ml) | Specific activity (U/mg) |
|---|---|---|---|
| $MgSO_4$ | 36.05 | 0.38 | 94.87 |
| $FeSO_4$ | 37.41 | 0.35 | 105.68 |
| $ZnSO_4$ | 23.85 | 0.57 | 41.92 |
| $MnSO_4$ | 30.25 | 0.44 | 69.22 |
| $CaSO_4$ | 28.57 | 0.47 | 60.27 |
| $CuSO_4$ | 26.89 | 0.51 | 52.42 |
| $Na_2SO_4$ | 28.79 | 0.44 | 65.43 |

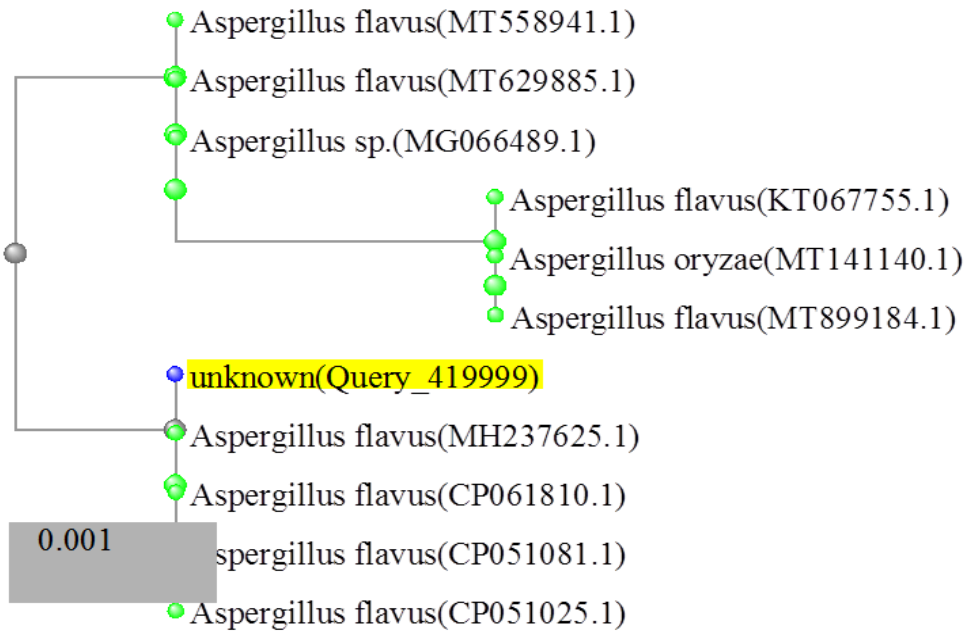


**Figure 1:** Phylogenetic tree for the fungal F9 isolate.

## إنتاجية إنزيمات البكتينيز بواسطة الأسبرجلس المعزولة من التربة المصرية.

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**الملخص العربى:**

يهدف البحث إلى عزل وتوصيف وتعريف أفضل عزلات الإسبرجلس المنتجة لإنزيمات البكتينيز ، حيث تم عزل 10 عزلات من الأسبرجلس من التربة المصرية ، حيث تم اختبار قدرة العزلات على تحليل البكتين على بيئة Pectin Screening Agar ، وأظهرت نتائج تعريف العزلة الأكثر كفاءة في إنتاج إنزيمات البكتينيز عن طريق دراسة الصفات المورفولوجية والميكرسكوبية والبيولوجيا الجزيئية وأظهرت النتائج أنها تنتمى إلى *Aspergillus flavus* (MH237625.1) . وأظهرت دراسة الظروف المثلى لتحديد أفضل وأنسب الظروف لزيادة النشاط الإنزيمي لهذه السلالة من إنزيمات البكتينيز أن أعلى إنتاج لإنزيم البكتينيز من فطر *Aspergillus flavus* (F9 isolate) كانت عند الإس الهيدروجينى 7.0 حيث أعطت ( 32.64 وحدة / مل) ، مع التحضين على درجة حرارة 25 درجة مئوية ( 39.09 وحدة / مل) ، ومن خلال فترة تحضين 5 أيام أعطت (38.11 وحدة / مل) مع استخدام المالتوز كمصدر للكربون أعطت ( 30.79 وحدة / مل) ، وباستخدام الببتون كمصدر للنيتروجين أعطت ( 39.25 وحدة / مل) ، وفى وجود $FeSO_4$ كمصدر للأملاح المعدنية أعطت ( 37.41 وحدة / مل) فى وسط التخمر المغمور.

**الكلمات الاسترشادية:** إنزيمات البكتينيز ، *Aspergillus flavus* ، الظروف المزرعية.