PRODUCTION OF PECTINASE BY CERTAIN FILAMENTOUS FUNGI AS INFLUENCED BY AGRICULTURAL CONDITIONS AND MICROELEMENTS.

A Thesis Submitted in Partial Fulfillment for a Master of Science Degree in Microbiology

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Summary

The study was conducted in the Faculty of Science Research Botany department laboratory at Fayoum University. It involved collecting agricultural soil samples from various locations in Fayoum Governorate and investigating the production of pectinase, an enzyme with various industrial applications. The study involved screening and identifying pectinase-producing fungi, optimizing the environmental conditions for enzyme production, and evaluating the purified enzyme's properties and potential for textile cleaning.

Firstly: sixty fungal isolates were obtained, and pectinase production was assessed using two methods:

1. Descriptive method: Lugol's Iodine Solution was used to visualize pectin degradation around fungal colonies. 20 out of 60 isolates exhibited positive pectinase production by assessing the contrast in color of Lugol's Iodine Solution test.
2. Quantitative method: Dinitrosalicylic Acid (DNS) assay measured the reduction of DNS detector by D-galacturonic acid, a product of pectin hydrolysis.

The quantitative assessment showed significant enzyme production in 9 isolates. The three best isolates, *A. brasiliensis* showed the highest pectinase production (84.53 ± 1603.67 U/ml), significantly surpassing the other isolates ($P < 0.01$), *A. niveus* and *A. niger* (1311.22±51.40 U/ml, 1264.83 ± 23.94 U/ml), respectively.

Secondly: The optimization of pectinase enzyme production under different environmental conditions, temperature (30-55°C), pH (3.4-7.4), and incubation period (3-11 days) were investigated

The optimal temperature for enzyme production varies depending on the fungal strain. For *A. niveus and A. niger* was 45°C (2840.19 ± 250.223, 3027.859 ± 460.487) U/mL, respectively. For *A. brasiliensis* was 40°C (3033.072684 ± 88.9269100 a) U/ml.

pH 5.4 was the best for *A. niveus and A. niger*, whose enzyme activities are (905.652368 ± 103.4420195), and (1065.169794 ± 64.1197498) U/ml respectively, while for *A. brasiliensis* was 4.4., (1439.983616 ± 91.9157815 [a]) U/ml. Significant differences were recorded at these values ($P = 0.000$). Additionally, the results showed that applying a nutrient medium with a pH of 7.4 led up to a significant decrease in enzyme production in *A. brasiliensis* ($P = 0.000$).

Five days of incubation yielded the highest enzyme production for *A. niveus* (1745.46 U/ml) with statistically significant differences (P = 0.000)., while seven days were optimal for *A. niger* (3040.89 U/ml) and *A. brasiliensis* (2389.27 U/ml) with a significant difference (P < 0.01).

The current study comprehensively analyzes pectinase production by different fungal isolates, highlighting the importance of optimizing environmental factors for enhanced enzyme productivity.

Thirdly: partial purification of polygalacturonase from the three most productive isolates (*A. niveus*, *A. niger*, and *A. brasiliensis*) was achieved using ammonium sulfate precipitation by concentrations(20-60%), chilled acetone, and absolute ethanol, then specific activity was calculated for each purification method which was the highest for absolute ethanol purification in *A. brasiliensis* (3254.42 U/mg) and *A. niger* (1277 U/mg), and for acetone purification in *A. niveus* (943.14 U/mg). The maximum PG activity enrichment was obtained from *A. niger* after ethanol partial purification 14-fold increase.

Electrophoresis analysis of polygalacturonase enzyme revealed one band represented good purity at (30 KDa).

Polygalacturonase was characterized based on the effects of temperature (30-70°C), pH (3-8), and micronutrients (Mn, Zn, Co, Fe) on enzyme stability, activity, and optimum conditions were determined. The enzyme exhibited optimal activity at 60°C and pH 7. Enzyme stability belonging to *A. niger* was maintained within a broad temperature (40-70°C) and pH (5-8) ranges, with activity retention of 90.6-99.9% and 93-99.6%, respectively. Micronutrients have varying inhibition effects on enzyme production. However, there were variations between the elements, iron enhanced pectinase production in *A. niveus* (729.97 ± 73.75 U/ml, P < 0.05), this effect was unique to this species. Zinc promoted the highest enzyme production (1470.22 ± 462.945 U/ml). Iron addition also increased pectinase production in *A. niger*, but the effect was not significantly different from other micronutrients.

The GC-MS analysis identified and quantified the products formed by the enzyme. A cytotoxicity test using this enzyme yielded an IC50 of 151.86 µg.

The purified enzyme was applied for its ability to remove pectin from cotton fabrics. Effectively, it removed pectin from cotton fabrics, demonstrating its potential for bio-scouring (20%).