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PRODUCTION OF PECTINOLYTIC ENZYMES FROM BACTERIAL ISOLATES ASSOCIATED WITH OLIVE (*OLEA EUROPAEA*) PLANTS

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ABSTRACT

Microbial pectinases are widely used in various industries and represent a major class of industrial enzymes. Their broad applications have attracted significant research interest which include plant fiber processing, pectic waste water treatment, paper pulping, fruit juice extraction, and clarification. This study aimed to isolate endophytic bacteria from olive plants and evaluate their potential for pectinase production using the solid-state fermentation (SSF) method. Samples were collected from olive plants in a home orchard located in Zuru, Kebbi State, Nigeria. Bacterial cultures exhibiting the highest zones of inhibition were selected for enzyme production. Orange peel powder served as the pectin-rich substrate in the submerged production medium. Among the isolates, a single pectinolytic strain demonstrated enzyme activity of 1.387 U/mL. These findings suggest that endophytic bacteria associated with olive plants in Zuru have potential as industrial producers of pectinase enzymes.

Keywords: Olive plants, Endophytic Bacteria, Orange peel powder, Pectinase production, Enzyme activity

INTRODUCTION

Pectinolytic enzymes, commonly known as pectinases, are a group of enzymes that catalyze the breakdown of pectin, a complex heteropolysaccharide abundant in plant cell walls. These enzymes have significant industrial relevance in fruit juice clarification, textile processing, and waste treatment (Kashyap *et al.,* 2022). Among the various sources of pectinases, microbial enzymes, particularly those from bacteria, are highly valued for their stability, cost-effective production, and rapid growth cycles (Haile *et al.,* 2022). The olive tree (*Olea europaea*), a key species in Mediterranean agriculture, supports a diverse microbiome in its rhizosphere and endosphere. These microbial communities, adapted to the tree's often challenging environmental conditions, are rich in functional traits, including the production of hydrolytic enzymes (Sallami *et al.,* 2023). The exploration of such plant-associated bacteria provides a sustainable route for enzyme production, especially as industries seek eco-friendly alternatives to synthetic chemicals. Previous studies have shown that olive mill waste and rhizosphere soils are habitats for bacteria capable of producing industrial enzymes such as pectinases. For example, Bouras *et al.,* (2019) reported that approximately 40% of microbial isolates from olive mill waste exhibited pectinolytic activity. Similarly, recent isolation of *Serratia marcescens* from agro-wastes demonstrated its capacity to produce pectinase useful for juice clarification (Haile *et al.,* 2022), indicating the potential for value addition to olive by-products through microbial biotechnology.

Therefore, the aim of this study was to isolate endophytic bacteria from olive plant and, screen them for their ability to produce pectinase enzymes.

MATERIALS AND METHODS

Collection of Samples

Including leaf, steam bark and root. Samples were collected from *Olea Europeae* (Olive tree) pant. All the organs of the plant are possible niches for the endophytic microorganisms, while sometimes the roots possess the highest density of endophytes (Schulz *et al.,* 2002). Another factor that needs to be considered for plant selection is the health of the plant. The samples were collected from unhealthy plant, because collecting samples from a healthy plant without visible symptoms reduces the chance of isolating pathogens together with the endophytes (Hallmann *et al.,* 1997). The samples were processed within 24hrs from the desired plante (Olive tree). However, the plant material was stored at 4°C in sealed plastic bags to keep the tissue fresh and avoid death of the microorganisms.

Preparation of Media

Sixty three grams 63.0g of Starch Casein Agar (SCA) was weighted using a weighing balance and suspended into 1000ml of distil water in a conical flask, the solution was heated and boiled to dissolve the media completely, the media was sterilized using Autoclaves at 121°C, 15p.s.i. for 15mins.unto the colled medium, 50µg/ml of Cycloheximide was added to restrict the fungal and gram negative bacteria growth. The prepared medium (20mls each) was poured in to fresh sterilized petri dishes covered, then allowed to solidify at room temperature (El-Tarabily and Sivasithamparam, 2006).

Surface Sterilization of the Plant Samples

The surface sterilization was done by three times of rinsing the plant material with sterile water, followed by washing in 70% ethanol for 1min and finally rinsed again with distilled water under a laminar hood. The surface sterilized plant materials were placed on a filter paper to allowed excess water

and sterilants to evaporate in laminar air flow cabinet, then cuts in to smaller fragments and macerated in 10ml sterile distil water using sterile mortar and pestle. A 0.2ml of liquid from the macerated samples was taken and spread-plated on SCA plates,and then incubated in a sterile incubator at 28°C until colonial growth was observed. Thereafter, two (2) distinct colonies ware selected and transferred onto a fresh SCA plates to obtain pure bacterial isolates (Strobel and Daisy, 2003).

Chemically Defined Medium for Enzyme Production

The Following components were used accordingly (i) KH2PO4-3g/L (ii) K2HPO4-6g/L (iii) KCl-2g/L (iv) KNO3-1g/L (v) MgCl2-0.5g/L (vi) NH4Cl20.5g/L (vii) CaCO3-3g/L (viii) FeSO4.7H2O-0.001g/L (ix) Substrate10g/L (Hankin and Anagnostakis, 1975).

Preparation of Seed Culture

During the preparation of seed culture (as starter culture), the colony was collected from subculture plates using a sterile toothpick and agitated in a 100ml conical flask. The inoculated medium was incubated on a shaking incubator at 30°C for 24 hours.

Production Medium

From the seed culture, 50ml of the culture was transferred into 450ml of production medium and placed on a shaking incubator for 48 hours. The negative control contained the 500ml of the production medium and substrate that were not inoculated with the bacterial colony. After 48 hours of fermentation, little amount of fermented culture was poured into glass test tube and centrifuged at 10,000r/pm for 10 minutes. The supernatants contained crude extracellular enzymes.

Pectinase Assay

The crude extracellular enzyme (1ml) was mixed with 1ml of 1% substrate suspended in 0.1M of phosphate buffer at pH 7; the mixtures were incubated in a water bath at 50°C for 10mins. The mixture was allowed to cool at room temperature, and then the solution was boiled in a boiling water bath for 5min. Finally, the absorbance of the solution was measured at 540nm using a spectrophotometer. The enzyme blank and reagent blank also were measured for qualitative analysis of pectinase (Miller, 1959).

RESULTS AND DISCUSSION

Endophytic bacteria were isolated on Starch Casein Agar. The colonies had different morphological appearances such as large white with fluffy spores, small white sporulation powdery colonies producing light brown pigment which was viewed from the base of the plate, yellow colonies pigment diffused through the media (Figure1A). Pure isolates of the endophytic bacteria were obtained by subculture (Figure1B).

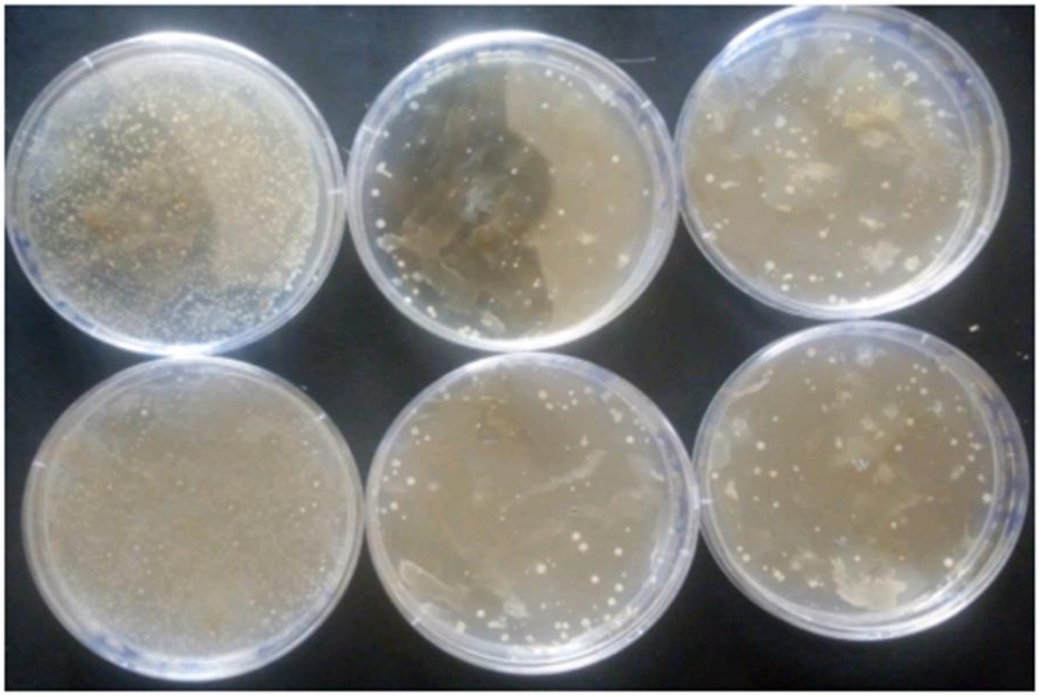

Figure 1A: Varying Cultural Appearances of Endophytic Bacterial Isolated from Olive Tree

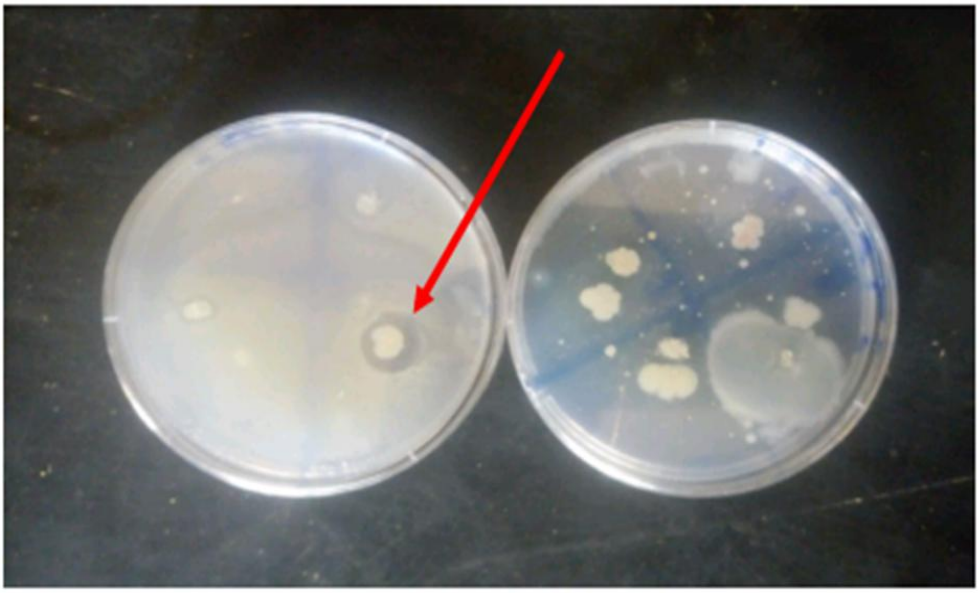

Figure1B: Pure Isolates of Endophytic Bacteria Isolated from Olive Tree

Figure 2 (A): Submerged Fermentation Culture for Enzyme Production. (B) Cell-free Supernatant as Enzyme

Measurement of Enzymes Activity Assay

The crude enzymes as measured spectrophotometerically are presented in table 1.The readings obtained from spectrophotometer were taken multiple times and there means were calculated:

Table.1: Quantitative Analysis of Enzymes Activity Assay Using Spectrometer

S/NO	Enzymes Blank U/M^{-1}	Absorbance (A540nm)
1	1.403	1.387
2	1.514	1.291

Discussion

Endophytic bacteria's were isolated on SCA. Therefore, the processing and isolation technique was carried out in a sterile environment to reduce the number of contaminants that could affect the practical. *Citrus pectin* was used to screen for pectinolytic bacteria, which proved to be a more favourable carbon source for screening the pectinolytic bacteria (Shahriarinour *et al.*, 2025). One screening procedure employed was to select isolates with the highest ability to produce enzymes. (Samira *et al.* (2025) similarly reported pectinase activities in some bacterial isolates. The primary screening was a qualitative test to visualize the hydrolysis of carbon sources on plates. Diameters of clear zones around the colonies were measured, and the ratios of clear zone diameters

to colony diameters were calculated for a comparative assessment of the isolates' activities. Isolates varied in their response to this test. The secondary screening was a more quantitative method used to determine enzyme activity of the isolates. These results suggest that endophytic bacteria may represent a valuable resource for exploring pectin degradation due to their potent activity at high temperatures and their ability to grow under aerobic conditions. Major future goals for pectinase research would be: (A) reducing the cost of pectinase production, and (B) improving the efficacy of pectinases so that smaller quantities are needed. Industrially, using orange peel powder can be highly economical. Dhillon *et al.* (2025) reported the use of citrus peel in semi-solid fermentation for pectinase production. Parameters varied in the current solid-state fermentation of orange peels included incubation time, pH of the basal medium, incubation temperature, and nitrogen source. Production showed optimal growth in the range of 45 to 60°C (Freitas *et al.*, 2025; Rubinder*et al.*, 2025). The source of nitrogen in the growth medium plays a critical role in microbial growth and enzyme production (Mrudula and Anitharaj, 2025).

CONCLUSION

Given the remarkable potential of pectinase enzymes across various industrial sectors, particularly where pectin degradation is crucial it is imperative to continue research focused on isolating and optimizing microbial strains for efficient pectinase production.

The findings of this study highlight the promising role of plant-associated endophytic bacteria, particularly those isolated from olive tree, in producing pectinase enzymes. Furthermore, the use of agro-waste substrates, such as orange peel powder, enhances the economic feasibility and environmental sustainability of enzyme production. These insights underscore the potential of microbial pectinase for wide-ranging applications in biotechnology, textiles, and food processing industries.

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