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Research Article

A study on Fourier transform infrared (FTIR) spectroscopy for polygalacturonase production by four fungal strains from mangrove soils Krishna district, Andhra Pradesh

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Abstract
Mangrove ecosystems are rich sources of diverse microbial communities, including fungi capable of producing industrially significant enzymes such as polygalacturonase. Identifying potent fungal strains with high enzymatic activity is crucial for biotechnological applications. This study aimed to isolate, identify, and characterize fungal strains from mangrove soils in Gilakaladhindi and Malakayalanka, Krishna District, with a focus on their polygalacturonase production potential. A total of 57 fungal strains were isolated from soil samples using serial dilution and pectin agar plating. The strain exhibiting the highest polygalacturonase activity was selected for further study. Identification of the potent strains was performed through 18S rRNA sequencing, and the sequences were deposited in the NCBI GenBank with accession numbers MK192017, MG271916, KU613360, and KU613362. Fourier Transform Infrared (FT-IR) spectroscopy was conducted to analyze the structural composition of the enzyme, identifying functional groups responsible for its activity. Four fungal strains *Aspergillus nomius, Aspergillus terreus, Penicillium citrinum*, and *Penicillium griseofulvum* were confirmed as potent polygalacturonase producers. Fourier Transform Infrared Spectroscopy (FT-IR) analysis detected hydroxyl, carboxyl, acetyl, and pyruvyl groups, indicating the presence of essential biochemical components. The chemical composition of polygalacturonase consisted primarily of neutral sugars, uronic acids, and proteins. These findings provide valuable insights into fungal polygalacturonase production and its potential applications in various industrial processes.

Keywords: *Aspergillusnomius,* Fourier Transform infrared spectroscopy (FTIR), Mangrove, Polygalacturonase, Uronic acid

INTRODUCTION

Approximately 10% of all enzyme preparations are manufactured using pectinase. The food sector uses pectinolytic enzymes extensively to produce wine and juice (Anuradha *et al.,* 2014). Pectic compounds are complex colloidal acid polysaccharides composed of galacturonic acid residues connected by α (1-4) linkage (Kusuma *et al.,* 2014). The side chains of the pectin molecule are L-rhamnose, arabinose, galactose, and xylose. Galacturonic acid's carboxyl groups are partially esterified by methyl groups and neutralized by sodium, potassium, or ammonium ions. Pectic compounds are characterized as protopectin, pectic acid, pectinic acid, or pectin based on the type of backbone chain changes (Be Miller, 1986).Since microbes are thought to be a rich supply of enzymes, investigating their extracellular enzymatic activity is crucial to producing industrial enzymes from sources including plants, animals, fungi, bacteria, and yeast (Hoa and Hung, 2013). Previous studies have emphasized the significance of microbial enzymes in the manufacture of alcoholic drinks,food,vaccines, pharmaceuticals, and food supplements (Hoondal *et al.,* 2002) (Kumar *et al.,* 2024).

Pectinase enzymes that have the impending utilization in many industrial sectors like textiles, pharmaceutical biotechnology, food, detergents, paper and aquaculture (Mondal *et al.,* 2024). The present work aimed to produce extracellular pectinase enzyme from mangrove soils and determine the enzyme's hydrolytic potency for galacturonic acid and sugars by using FT-IR. This was done in light of the polygalaturonase enzymes industrial importance and its production's inventive and environmentally friendly nature.

MATERIALS AND METHODS

Study area

Soil samples were collected from mangrove habitats in the year 2016 in the villages of Gilakaladindi and Malakayalanka, Krishna District, Andhra Pradesh, India.

Isolation of fungi

After excavating about 3 cm of the soil surface, mangrove sediments were taken down to a depth of 20 cm. Samples were taken at a depth of 6 to 10 cm, brought in sterile bags to the lab, and allowed to air dry at room temperature. Each soil sample weighed one gramme. The samples were suspended in 100 millilitres of sterile distilled water, and the mixture wasshaken for thirty minutes before being set aside to allow the suspending particles to settle.Using sterile water, one millilitre of the suspension was serially diluted. Up to 10^{-4} dilutions of serial dilutions were made. Spreaders were used to distribute 0.1 ml soil suspensions at 10^{-4} and 10^{-3} dilutions that were inoculated on Czepak dox Agar plates Bentouhami *et al*. (2024). For 7 days, the plates were incubated at 35 ± 2°C, and the presence of fungal colonies was monitored. After transfer of pure colonies into slants, extracellular polygalacturonase was further examined on pectin agar medium to screen the isolates for potent polygalacturonase producers (Balabanova *et al*. 2018).

Polygalacturonase production

The following ingredients (g/L) were used in the manufacture of the polygalacturonase enzyme: $(NH_4)_2$ SO_4 (1g), K_2HPO_4 (1g), KCl (0.5g), NaCl (5g), $MgSO_4$ (0.5g), $FeSO_4$ (0.01g), and Citrus pectin (5 g). The process took place in a 250 ml conical flask. One millilitre of fungal suspension was used as an inoculant for the flasks, which were then shaken at 2000 rpm for seven days at 30°C. The standard polygalacturonase test was used to measure the polygalacturonase activity in the supernatants Adedayo *et al,.* (2021).

Polygalacturonase assay

The fungal growth's polygalacturonase activity was measured using Miller's technique (1959). Polygalacturonase enzyme activity was measured using pectin as the substrate. A reaction mixture was prepared using 1 ml of 1% pectin in a sodium acetate buffer (0.1 M; pH 5.5). Appropriately diluted crude enzyme was then added, and the mixture was incubated in a water bath at 40ºC for 20 minutes. Three millilitres of Di Nitro Salicylic Acid solution (DNS) were used to halt the reaction. Following a 5-minute boil, 1 ml of sodium potassium tartarate was added and allowed to cool. The colour intensity was measured at 540 nm using UV-visible spectrophotometer. The amount of enzyme needed to release one micromole (1µmol) of galactouronic acid per minute was used to calculate the enzyme activity (U/g). The reaction combination was one ml of 1.2% w/v Pectin in one milliliter of pH 5.0 0.1M citrate-phosphate buffer and one milliliter of culture filterate (raw enzyme solution). The same quantity of substrate and 1 ml of culture filtrates were combined in control experimental tubes, and the combination was heated to a boil for 20 minutes. Subsequently, both the experimental and control tubes were incubated at 35°C for six days. The amount of reducing sugars released into the reaction mixture was measured using the 3,5-dinitrosalicylic acid (DNSA) reagent. Under particular reaction conditions, one unit of Polygalacturonase activity was defined as the quantity of enzyme in 1 mL that would liberate reducing sugars equivalent to 1 mg of galacturonic acid per minute.

Fourier transform infrared (FTIR) data analysis

After the incubation, the culture filtrate was used to analyse polygalacturonase's presence. The filteratewas scanned in at 4 cm^{-1} resolution with 100 scans in the 4000–500 cm-1 spectral range at room temperature. The Fourier transform infrared spectra analysis was made to determine the sugar compositions of the fungal polygalacturonase grown in different agro residues qualitatively and the comparative analysis between the isolated fungal species (Ognyanov *et al*., 2018).To achieve this,theagro industrial residual sugar composition was analysed and compared to the standards of sucrose, fructose, glucose and pectin by using Fourier transform infrared spectra analysis (Kamil *et al.,* 2011). A General observation from these analyses was that the pectin signals overlapped with those of fructose, which produced more dominant peaks, making it difficult to detect pectin in the presence of fructose (Manrique *et al*., 2002).

RESULTS

Isolation of fungi

Potential four isolates from mangrove soils of Gilakaladhindi and Malakayalanka selected based on the screening and inoculated into pectin broth to know their polygalacturonase enzyme production ability.Several research studies have used similar screen-

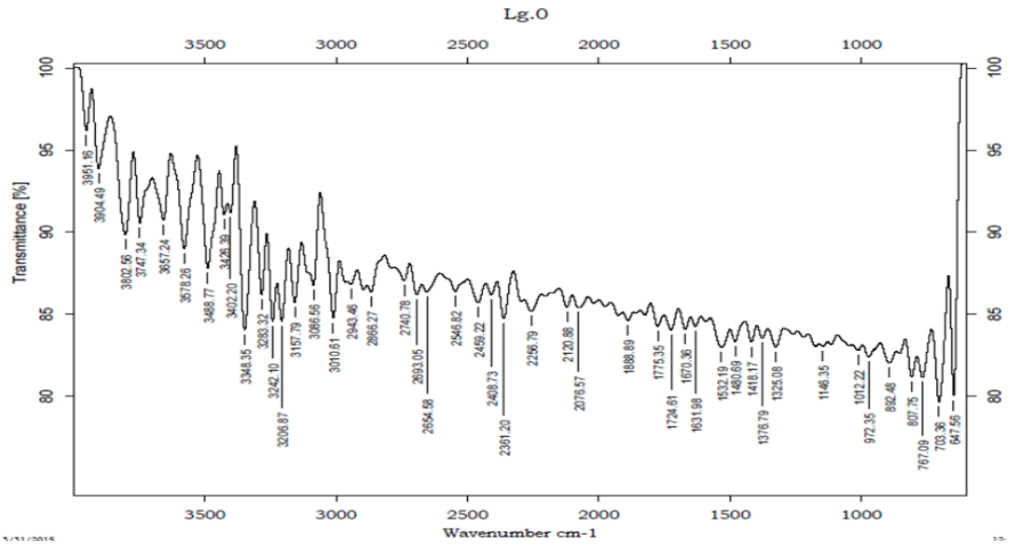

Fig. 1. *Fourier transform infrared (FT-IR) spectroscopy analysis of LG(Penicillium citrinum RM 101)*

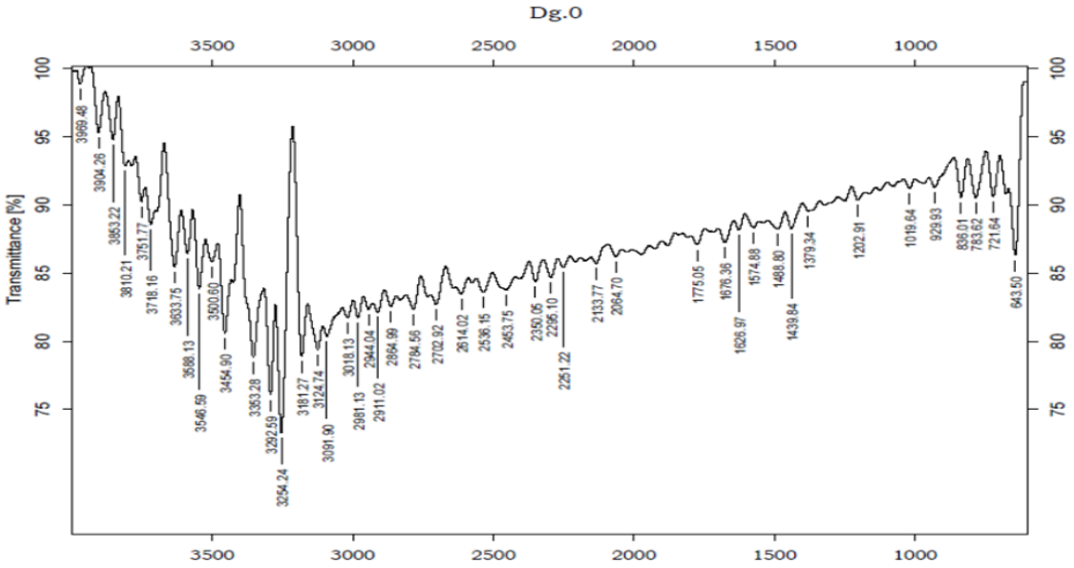

Fig. 2. *Fourier transform infrared (FT-IR) analysis of DG (Penicillium griseofulvumRM104)*

ing techniques to identify fungal species as one of the powerful pectinase producers (Koser *et al.,* 2014).

The species were identified based on the percentage similarities with 18s RNA sequences and identified the species. The identified species were deposited in Gene Bank and accession numbers were generate (Robbertse *et al.,* 2011). The isolated fungi were *Penicillium citrinum* RM 101(LG) (KU613360), *Aspergillus terreus* RR105(MG271916), *Penicillium griseofulvum* RM*104*(DG) (KU613362)and *Aspergillus nomius*rm 103 (MK192017).

Fourier transform infrared (FTIR) analysis

Fungal pectinases were observed to remain stable across a broad pH range, from acidic to alkaline (4.0–8.0). However, their activity and stability may peak at different levels within this pH spectrum. Most of the research reveals that the pectinase peaks were identified in the range between 1000 to 1400cm□¹. The Present investigation reported that all four fungal strains showed the peaks in the above mentioned range. *Penicillium citrinum* RM 101(LG) showed the peaks at 1012.22, 1146.35, 1325.08 and 1376.79 (Fig. 1). All these peaks were within the above mentioned range. In the control (Fig 5) there was no specific peak in this range. This indicated the absence of an enzyme in the control, whereas in the culture filtrates it shows that the enzyme. *Penicillium griseofulvum* RM*104* (DG) showed the peaks at 1019.64, 1202.91 and 1379.34 (Fig. 2). All these peaks were within the range. *Aspergillus terreus* RR105showed peaks at 1097.55, 1143.85, 1210.15, 1251.47, 1317.04 and 1387.58 (Fig. 3). All these peaks were within the range. *Aspergillus nomius*rm 103 showed the peaks at 1091.54, 1194.06 and 1308.49 (Fig. 4). All these peaks were within the range.

Broad peak around 3400 cm^{-1} indicated O-H stretching

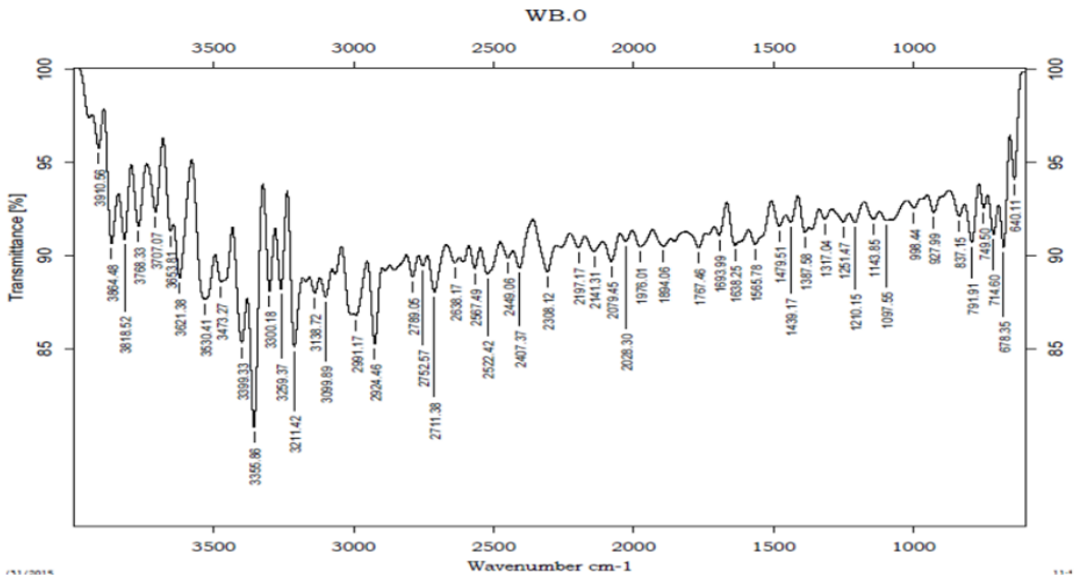

Fig. 3. *Fourier transform infrared (FT-IR)* analysis of WB(*AspergillusterreusRR105)*

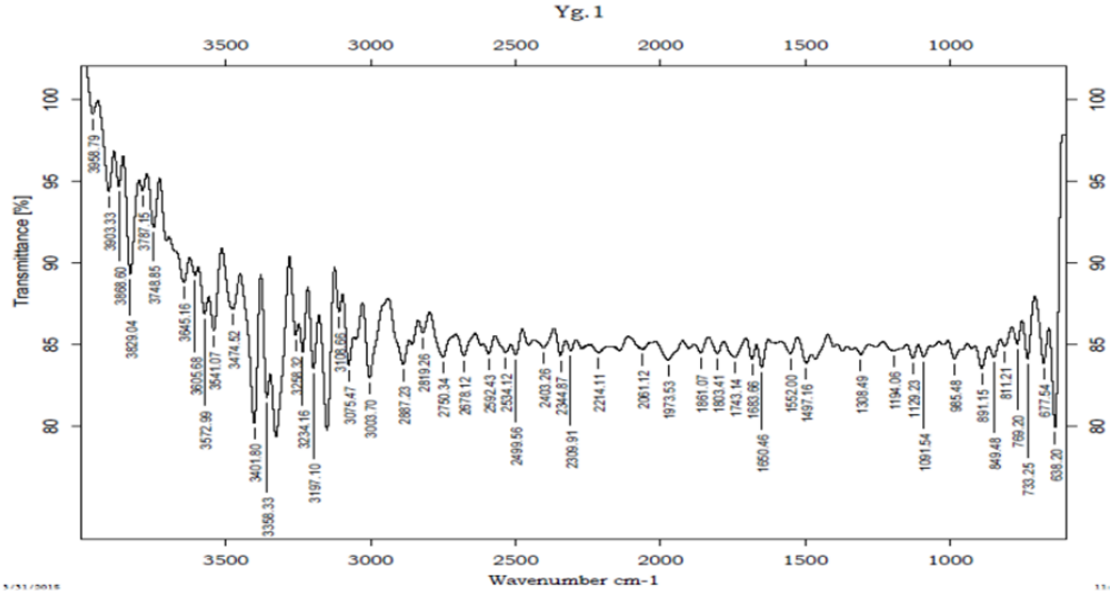

Fig. 4. *Fourier transform infrared (FT-IR)analysis of YG (Aspergillus nomius RM* 103)

vibrations, likely from alcohols or phenols. Peaks from 3200 to 3400 indicated O-H stretching vibration of hydroxyl groups, Amine N-H stretching. Sharp Peak around 2920 cm□¹ suggested C-H stretching from alkanes (CH3, CH2 stretching). Peaks from2350 to 2215 indicated C≡C alkyne stretching. Peak around 1650 cm□¹ Corresponds to C=O stretching (Manriqueand Lajolo, 2002), typical of carbonyl groups (e.g., ketones, aldehydes, esters). Peak from 1025 to 1250 cm□¹ indicated C-O bond, β (1→3) glucan, cell wall polysaccharide. Peak around 1100 cm^{-1}represents C-O stretching, common in alcohols, ethers, and esters.Functional classes of aliphatic amines (C-N stretch) and the sugar group of alkanes (C-H bend) have now been found in pectinase protein. Similarly, the FT-IR spectrum was used to examine vibrations of C-C stretching andfunctional sugar groups C-H. Following the calibration of the peak intensities, sucrose (995 cm-1) and carbohydrate was discovered as glucose (1033 cm-1), as well as galacturonic acid in the range (1200-900 cm-1) and Similar findings were reported by Adina *et al.,*(2010).

DISCUSSION

The present investigation revealed that pectinase enzyme produced by four different fungal strains exhibited infrared (FTIR) absorbance peaks in the range of 1000 to 1400 cm^{-1}. This spectral region commonly associated with polysaccharide-degrading enzymes likepectinase. This range corresponds to vibrations related to C

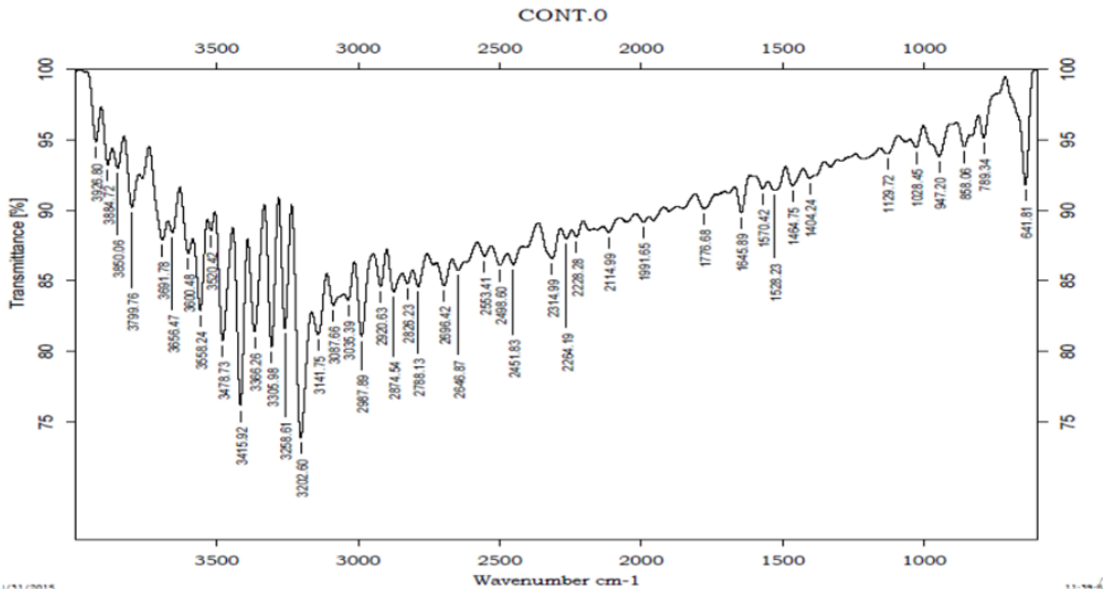

Fig. 5. *Fourier transform infrared (FT-IR) spectroscopy analysis of control contains only medium ingradients without inoculum*

–O, C–C, and C–H bonds in pectin molecules, confirming enzymatic activity involved in pectin degradation (Singh *et al.,* 2018) (Majaliwa *et al.,* 2025).

All tested strains showed peaks in this range, with *Penicillium citrinum RM 101* (LG) producing peaks at 1012.22, 1146.35, 1325.08, and 1376.79 cm^{-1}. These values align closely with those reported by Singh *et al.,* (2024) and Patil and Dayanand (2006), who identified pectinase activity peaks between 1010 and 1380 cm^{-1} in *Penicillium* species. The absence of corresponding peaks in the control confirms that these peaks were enzyme-specific.

Penicillium griseofulvum RM 104 (DG) exhibited three main peaks at 1019.64, 1202.91, and 1379.34 cm^{-1}, which are also in concordance with earlier reports of fungal pectinases showing strong absorbance near 1020 and 1380 cm^{-1} (Zhu *et al.,* 2016) and Strycker *et al.,* (2016).

Aspergillus terreus RR 105 demonstrated the most diverse peak distribution with six peaks (1097.55, 1143.85, 1210.15, 1251.47, 1317.04, and 1387.58 cm^{-1}), indicating possibly higher enzymatic complexity or the presence of multiple pectinolytic isoforms. This is supported by previous findings where *Aspergillus* species displayed a wide FTIR signature due to multi-enzyme production (Bhattacharyya *et al.,* 2021) and (Kashyap *et al.,* 2001).

Aspergillus nomius RM 103 showed peaks at 1091.54, 1194.06, and 1308.49 cm^{-1}, which is consistent with earlier observations of pectinase-related peaks in *Aspergillus niger* and related species (Ahmed *et al.,* 2021) (Bussamara *et al.,* 2010). These findings support the hypothesis that pectinase enzymes produced by *Aspergillus* species generally share similar FTIR absorption profiles, albeit with minor variations depending on strain-specific enzyme composition.

Collectively, these results reaffirm that FTIR spectroscopy serves as a reliable, non-destructive method for confirming the presence of pectinase activity based on specific absorbance patterns (Koziol *et al.,* 2022) and (Kaur *et al.,* 2014). The variations in the number and intensity of peaks among different strains could reflect differences in enzyme yield, substrate affinity, or the presence of synergistic enzymes.

The FTIR spectra of the fungal culture filtrates revealed several key absorbance peaks indicative of functional groups commonly associated with pectinase enzymes and their associated metabolites. A broad peak around 3400 cm^{-1} was observed in all spectra, suggesting O–H stretching vibrations, likely derived from alcohols or phenolic compounds, as well as N–H stretching indicative of primary and secondary amine groups. These features are typically associated with proteinaceous enzyme components and hydroxyl-rich polysaccharides (Pasieczna-Patkowska *et al.,*2025) and (Manrique and Lajolo, 2002).

A sharp peak at approximately 2920 cm^{-1} was attributed to C–H stretching vibrations from aliphatic hydrocarbons, specifically the methyl ($-CH_3$) and methylene ($-CH_2$) groups. These peaks suggest the presence of alkyl side chains, possibly from fatty acid residues or hydrocarbon chains associated with protein or substrate molecules.

Peaks in the region of 2215–2350 cm^{-1} correspond to C≡C triple bond stretching, which may originate from alkyne groups or unsaturated carbon compounds, though this is less commonly observed in proteins and more likely reflects trace metabolic intermediates.

The peak near 1650 cm^{-1} is indicative of C=O stretching from carbonyl groups, typical of ketones, aldehydes, carboxylic acids, or esters. This is consistent with pectinase structure, where carbonyl groups are part of the active site or the polysaccharide degradation products (Pasieczna-Patkowska *et al.,*2025) and (Manrique and Lajolo, 2002).

Notably, a series of peaks in the 1025–1250 cm^{-1} range were observed across all samples, corresponding to C–O bond stretching, which is characteristic of β-(1→3)-glucan and other cell wall polysaccharides. The prominent 1100 cm^{-1} peak represents C–O stretching vibrations found in alcohols, ethers, and esters, consistent with the hydrolytic action of pectinases on glycosidic bonds in pectic substances.

Additional functional groups such as C–N stretching of aliphatic amines and C–H bending of sugar moieties further support the presence of enzyme-associated biomolecules. The peak at 995 cm^{-1} was associated with sucrose, while a strong band at 1033 cm^{-1} indicated the presence of glucose. Peaks in the 900–1200 cm□¹ range also align with galacturonic acid, a key component of pectin substrates. These findings are in agreement with the work of Adina (2010), who also reported similar FTIR signatures for pectinase-related carbohydrate residues enzyme structures.

Conclusion

The present research successfully isolated four potential polygalacturonase producing two *Aspergillus* species and two *pencillium* species from mangrove soil samples. Among these *Aspergillus* species showed good ability of polygalacturonase production. These potent isolated strains were identified by 18S rRNA sequencing and morphologically characterized. The identified fungi were *Penicillium citrinum* RM 101(LG) (KU613360), *Aspergillus terreus* RR105(MG271916), *Penicillium griseofulvum* RM104 (DG)(KU613362) and *Aspergillus nomius*rm 103 (MK192017). The Fourier transformation infrared spectroscopy technique together with chemometric methods found prime advantage in perceptive chemotypic resemblance between different *Aspergillus* and *Penicillium* strains. Fourier transformation infrared spectroscopy data of enzymes report the functional groups of pectin and the polygalacturonase interactions. The aliphatic amines (C-N stretch) and sugar group of alkanes (C-H bend) functional classes were identified. This conforms the presence of pectinase enzyme especially polygalacturonase based on the FTIR data analysis. Pectinases have vast applications in industries including clarification and extraction of juices, textile, paper making, waste removal and tea leaves maceration. These selected fungal isolates would be vital industrial strains to boost production of polygalacturonase production cost-effective manner and an eco-friendly in near future.

Conflict of interest

The authors declare that they have no conflict of interest.

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