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Research Article

ISOLATION AND SCREENING OF INDUSTRIALLY IMPORTANT POLYGALCTURONASE PRODUCING FUNGI FROM THE MANGROVE SOILS OF KRISHNA DISTRICT ANDHRA PRADESH

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Abstract- For the present study, soil samples were collected from mangrove areas of Gilakaladindi and Malakayalanka of Krishna District. Soil samples were serially diluted and plated on pectin agar plates for isolation of Fungi. The isolates were identified as *Penicillium citrinum* RR 101(KU 613360) and *Aspergillus oryzae* RR 103(KU 613361) *Penicillium griseofulvum RR104* (KU613362) and *Aspergillus* sp RR102. Qualitative assay of exo-polygalacturonase of fungal isolates was carried out by growing them on to the pectin agar plates and the zone of hydrolysis around the fungal colonies was measured after flooded with iodine solution. *Aspergillus* sp. RR 102 showed the maximum zone of hydrolysis (14 mm) after 192 h at 37^{0}C. Maximum Polygalacturonase enzyme activity was recorded in *Aspergillus sp* RR 102 (2.40 IU/ml).

Keywords- Polygalacturonase, Pectin Agar Medium (PAM), zone of hydrolysis.

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Introduction

Pectin is a plant polymeric substance with carbohydrate esterified by methanol. In 1930, Pectinases were used commercially for the first time, before which, these enzymes were used at homes. After the knowledge on chemical nature of plant is understood, these enzymes became the leading biological molecules for commercial use [1]. Polygalacturonase enzyme is very crucial for the degradation of pectin commercially. The depolymerisation of pectin is catalysed by pectinases, which are of three types, pectin hydrolases, Pectin lyases and Pectin esterases [2]. There are manifold applications of pectinases, which include fruit juice processing and processing of tea and coffee. Furthermore, it is also used in the textile, food, beverage, oil industry, etc. [3]. Microorganisms are ubiquitous. They play a very important role in all the aspects of our lives, right from food production to the control of diseases. They are inevitable for the sustenance and maintenance of our ecosystem [4].Various reports clearly shows that pectinases are produced by Fungi [5,6] Actinomycetes [7-9] and Yeasts [10] and among them, fungi are potent producers of pectic enzymes[11].

Many important industrial products are now produced from fungi using fermentation technology. A wide range of enzymes, excreted by fungi, play an important role in the breakdown of organic materials and many of these enzymes are now produced commercially. *Aspergillus* is the main source of commercial pectinases [12]. Demand for the highly stable and effective pectinases is increasing day by day as there are various applications in biotechnology and industry. Agro-industrial waste dump yards are the ideal locations for the isolation of pectinase producing fungi. Mangrove forests cover 25% of the coastline of the World. The present study focuses on screening of soils from mangrove areas of Gilakaladindi and Malakayalanka of Krishna District for the fungi which produce pectinase. However there were no reports of pectinolytic fungi from Gilakaladhindi and Malakayalanka of Krishna district (Andhra Pradesh).

Materials and Methods

Isolation of Fungi

Mangrove soil samples were collected from Gilakaldhindi and Malakayalanka of Krishna district Andhra Pradesh at the depth of 20cm after removing approximately 3cm of the top soil. All the samples were collected in the sterile polythene zip lock covers. The soil samples collected from five places in each sites were mixed thoroughly. Serial dilutions are prepared using the soil sample. The Czapek-Dox media plates were inoculated with 1ml of soil suspension at dilution of 10^{-3} and incubation at room temperature for 3days. After incubation the isolated cultures were separated and sub cultured into slants. These pure cultures were screened for the production of enzyme by growing them in pectin agar medium plates (Triplicates were maintained).

Composition of pectin agar medium (g/l, wt/vol)

Citrus Pectin	10.0
Di-ammonium orthophosphate	3.0
K_2HPO_4	2.0
$MgSO_4$	0.1
Agar	20.0
Distilled water	1000
pH	5.5

Screening of pectinolytic fungi

Isolated fungal strains were grown on pectin agar medium and were incubated for seven days at 37^{o}C. Pectin agar medium was amended with 0.1% of ampicillin to restrict bacterial growth. After incubation, utilization of pectin was detected by flooding the culture plates with freshly prepared iodine-potassium iodide solution (iodine-1.0g, potassium-iodine-5.0g in 330ml distilled water). After every 24 hours

of incubation, the plates were observed for zone of inhibition and were measured [13]. Slide culture technique was employed to observe the morphological (macro and micro) characters of the potent fungal strains.

Polygalacturonase assay

Polygalacturonase (PG) activity of the culture filtrate was assayed by the standard method [14].Enzyme activity was determined using pectin as substrate. The reaction mixture containing 1 ml of 1% pectin was prepared in sodium acetate buffer (0.1 M; pH 5.5) and 1ml of crude enzyme was added and was incubated at 40º C in a water bath for 20 min. The reaction was stopped with 3ml of 3, 5-dinitrosalicylic acid (DNS) solution. Then the mixture was boiled for 5 minutes and 1ml of sodium potassium tartarate was added and cooled. The intensity of the colour density was measured at 540 nm using spectrophotometer (Elico). The amount of enzyme was estimated based on the standard graph of reducing sugars as - one unit of PG activity was defined as the amount of enzyme in 1 mL that would liberate reducing sugars equivalent to 1 mg of galacturonic acid, per minute under the specific conditions of reaction.

Results And Discussion

A total of 53 fungal cultures were isolated from mangrove soils. Among them 14 cultures were positive for pectinase production. Four good producers of enzyme were identified by 18S rRNA sequencing (MACROGEN, SOUTH KOREA). These sequences were deposited in gene bank (NCBI). The identified fungi with accession numbers are KU 613360 (*Penicillium citrinum* RR 101), KU 613361 (*Aspergillus oryzae* RR 103), KU 613362 (*Penicillium griseofulvum* RR 104) and *Aspergillus sp* RR102.

Aspergillus sp.RR102 produced maximum zone of hydrolysis with 14 mm diameter followed by *Aspergillus oryzae* RR 103 with 13mm diameter after 168h of incubation [Table-1]. However majority of the previous records indicate that *Aspergillussp*. and *A. oryzae* produced zone of hydrolysis of 3mm diameter only after 72h of incubation [15]. *Penicillium citrinum* RR 101 and *Penicillium griseofulvum* RR 104 produced maximum of 10 mm diameter zone of hydrolysis after 168h of incubation. Similar results were also reported by Banu et al., [16] in *Penicillium crysogenum,* which produced 3mm diameter of zone after 96h of incubation. While *Penicillium citrinum* produced only 4mm diameter of zone after 96hrs of incubation were reported by Priya and Sashi [4].

Table-1 *Measurements of Zone of hydrolysis (mm)*

Name of the strain	Incubation period(h)							
	72	96	120	144	168	192	216	240
Penicillium citrinum RR 101	6	7	8	9	10	10	12	12
Aspergillus sp RR102	8	8	8	8	11	14	14	14
Aspergillus oryzae RR 103	7	7	7	8	8	11	13	13
Penicillium griseofulvum RR104	5	6	8	9	10	10	10	10

Zone of hydrolysis was visible only after 72 hours of incubation. After 72 hours of incubation, *Aspergillus sp* RR102 produced a zone of 8mm, while remaining strains produced zones between 5mm to 7mm. The zone of hydrolysis increased with increase in time of incubation for all the cultures. There was progressive increase in the zone of hydrolysis with incubation period in all the four cultures. In*Aspergillus sp* RR102 the zone of hydrolysis rapidly increased from 8 to 11mm at 168h of incubation and reached peak of 14mm at 192h. However Loera et al.,[17] reported that 72h was the optimum time for maximum polygalacturonase activity by *Aspergillus niger* and 96h for *Penicillium citrinum* was reported by Priya and Seshi [4].

Polygalacturonase activity

Among the four isolates maximum Polygalacturonase enzyme activity was recorded in *Aspergillus sp* RR 102 (2.40 IU/ml) followed by*Penicillium griseofulvum* RR 104 (1.96 IU/ml), *Aspergillus oryzae* RR 103 (1.18 IU/ml) and *Penicillium citrinum* RR 101 (0.52 IU/ml) was showed in [Table-2] [Fig-1].

The pectinase activity in *Aspergillus niger* (PSV23) showed the maximum polygalcturonase activity of 5.411 IU/ml on solid state fermentation in citrus fruit peel was reported by Pramod et al., [20] (2014). Banu et al.,[16] reported that the *Penicillium chrysogenum* exhibited maximum PG production at 35°C (27.07 U/mL) after 5th day of incubation. The culture filtrate was used as crude enzyme extract of isolate *C1 Penicillium sp*. that showed maximum of 8.39 U/ml of pectinase activity after 7th day of incubation was reported by Patil and Chaudhari[18]. However Deshmukh *et al*., [19] reported that *Aspergillus oryzae*produced 224 U/ml of enzyme after 6th day of incubation. *P.citrinum* was the best producer of polygalacturonase enzyme with the maximum value of 129.2U/gdfs, followed by *P. brevi-compactum* that yield 123.2U/gdfs of enzyme activity after 8th day of incubation [20].

Aspergillus sp RR 102 stands out from all other isolates, as it alone produced enzyme on the fourth day. It gradually increased and produced maximum enzyme activity at 144h of incubation. After 192h of incubation, production of enzyme decreased slightly. Production of polygalacturonase was started on the 120h and was maximum on the 168h of incubation. After 168h, the production was decreased.

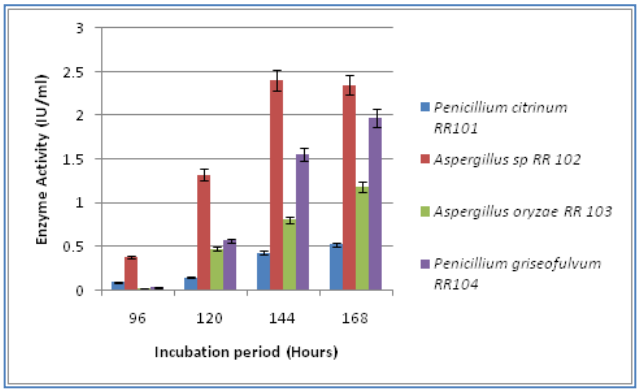

Fig-1 Polygalcturonase (U/ml) produced by different fungal strains at different incubation periods

Table -2 *Polygalacturonase activity by the fungal strains*

Sl. No	Strain Name	Enzyme Units (IU/mL)	Incubation (hours)
1	*Penicillium citrinum* RR 101	0.52	168
2	*Aspergillus* sp RR102	2.40	144
3	*Aspergillus oryzae* RR 103	1.18	168
4	*Penicillium griseofulvum RR104*	1.96	168

Conclusion

Pectinases are industrially important enzymes which are used in several industries. In this study, pectinolytic fungi were isolated from mangrove soil samples. *Aspergillus sp* RR 102 was found to be the potent producer of polygalacturonase. Pectinases are useful for various industrial applications including extraction and clarification of juices, processing of fibers, bleaching of papers, removal of pectic waste and maceration of tea leaves and these selected fungal isolates are promising living organisms that might be useful in producing these valuable biocatalysts for the commercial use in the near future.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Conflict of Interest: None declared

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