Isolation & Screening of Chitinase producing *Rhizobium* strain isolated from the root nodule plants

AkshadaBhadane, Rajendrachoure, SangeetaJangam, Dr. Rupendra Jadhav

Department of Microbiology, The Institute of Science, Mumbai, India.

ABSTRACT -Chitinase is an enzyme which acts on chitin substrate. Chitin are Poly N-acetyl glucosamine, which are naturally found in fungal cell wall. Thus for the degradation of chitin, chitinase are used. Methods to obstruct the fungal diseases need to be found out. However, it has been observed PGPR have some mechanism that can help plants to escape fungal diseases, it could be because of the presence of *Rhizobia* in the root nodules. Thus, in this research, screening of chitinase production from PGPRhad been carried out by using the root nodules of various plants such as Cajanuscajan, Horsegram, and soyabean.

Key words:*Rhizobium* , PGPR, chitinase production, Bromocresol purple, colloidal chitin.

1.INTRODUCTION

Chitin is a biopolymer that is distributed among many soil organisms (i.e., it is a major constituent of the cell walls of fungi and the exoskeleton of invertebrates. Chitin is the second most abundant, natural polymer which is widely distributed as a structural component of crustaceans, insects, and other arthropods, as well as a component of the cell walls of most fungi and some algae.Chitin is the second most major polysaccharide in nature after cellulose, present in cell walls of several fungi, exoskeletons of insects, and crustacean shells. Fungal chitinases play an important role in nutrition, morphogenesis, and developmental process and are known to be produced at various stages during fungus growth. Organisms like fungi, insects & plants have Chitinase and the role of chitinase with respect to other host differ variably in respect to mode of action, the availability of the chitin substrate, optimum conditions, the requirement by the host and the ability to utilize and degrade chitin residues. Some *Rhizobium* isolates may secrete antifungal compounds that acts upon the phytopathogenic fungi. It was noted that enhanced growth rate was obtained because of Rhizobacterial strains; hence *Rhizobium* can be used as bioinoculant (1).

2. MATERIALS AND METHODS

Isolation of *Rhizobium*

Rhizobium was isolated from root nodules of Cajanuscajan, Horsegram&Soyabean. The fresh and plump root nodules of the respective plants were collected. The collected nodules were surface sterilized with 75% ethanol and 0.1% mercuric chloride and washed thoroughly with distilled water. Rhizobium strain was obtained by streaking the crushed root nodules on YEM (yeast extract mannitol, pH 7.0) agar plates containing congo red and incubated at 28°C.. After 2 days of incubation, Rhizobium colonies were obtained. Further streaking, spreading and visual characterization of colony morphology helped in isolation of pure cultures of rhizobium (2).

Solubilisation of chitin to Colloidal chitin

Solubilisation of chitin was done using acidification method. The Shrimp shell chitin flakes were subjected to grinder mixer until fine powder of chitin was obtained. 200 g of ground Shrimpchitin powder was mixed with 1M conc. HCl. The acid was added to the chitin with stirring and the mixture was allowed to stand at 20°C with intermittent stirring until dissolved (1.5-2 hr) and formed a thick paste. The solution

was poured with stirring into a 60L container half-filled with water. A suspension of chitin in water was formed and additional water was added to have the final volume of 50L. The suspension was allowed to stand overnight allowing the chitin to settle and form a concentrated suspension. The mixture was stored for 24 hrs at room temperature. To remove the acidity of colloidal chitin suspension, the paste was washed regularly till the pH of colloidal chitin suspension became neutral (6.0-7.0) .After that, simple filtration was carried out, the thick suspension retained on the filter paper was dried at room temperature for 20 minutes. Then the retained residue (colloidal chitin) was stored and was kept for further use. (3)

Screening of the *Rhizobia* strains

The isolates were grown on CRYEMA at 30ºC for 24-48 hrs. Smooth mucoid, translucent, circular, raisedcolonies were selected. Further, Microscopic examination was carried out, and the isolates were found to be Gram negative short rods.

Primary screening of chitinase on Colloidal Chitin Agar Plates

Primary screening of enzyme production is important for the detection of chitinase. Spot assay was done by inoculating one loopful of a *Rhizobium*isolate cultures from the 4 legumes samples on chitin agar plate .The carbon source for *Rhizobia* was only colloidal chitin. The plates were incubated for 7 days at room temperature.

Secondary screening of Chitinase on Bromocresol purple agar plates

Secondary screening (confirmatory screening) was done by adding Bromocresol Purple dye to 1% colloidal chitin agar plates (pH5.2-6.8). All the 4 legumes were spot inoculated on Bromocresol purple agar plates and kept for 3 days and were observed for purple zones. (4)

3.RESULTS

The Rhizobium were isolated from root nodules of legumes on CRYEMA plates (Fig. 1).

Colloidal chitin prepared from Shrimp shells (Fig.2) was used in the screening of Chitinase producing organisms. Growth was observed, indicating that *Rhizobia* species were able to take up chitin as carbon source. During primary screening isolates from Horsegram (local & from Goregaon) and Cajanuscajanshowed no zone of clearance on colloidal chitin agar plate but were only able to utilize chitin as carbon source whereas *Rhizobium* from soyabean showed a zone of hydrolysisafter 7 days of incubation (Fig. 3). From the results, it could be concluded that chitinase of the isolates from Cajanuscajan, Horsegram(local) &Horsegram (Goregaon) could do minimal degradation of the chitin from Shrimp Shells.Fungal chitin and Crustacean chitin have differences in chitin content respectively (5). Chitinase activity against fungal chitin may vary.

For Secondary Screening of chitinase producers,collodial chitin agar plates containingBromocresol purple (pH indicator dye) was used. As shown in fig. 4 purple colour zones were observed around the growth of isolates from Cajanuscajan, Horsegram (local), and Horsegram (HG). And thus it was observed that Rhizobium have ability to produce Chitinase enzyme. Diameter of the zone increased with increase in incubation time (Table 1)

Fig.1 Rhizobium isolated from nodules of soyabean

Fig.2 Colloidal chitin at the bottom during acidification process

Table 1: Day wise zone diameter on Bromocresol purple plates

Isolate	Day 1	Day 2	Day 3
Cajanuscajan	1.2 mm	2.2 mm	4.1 mm
Horsegram	0.7 mm	1.6 mm	2.7 mm
HorsegramGoregaon (HG)	0.8 mm	1.7 mm	3.3 mm

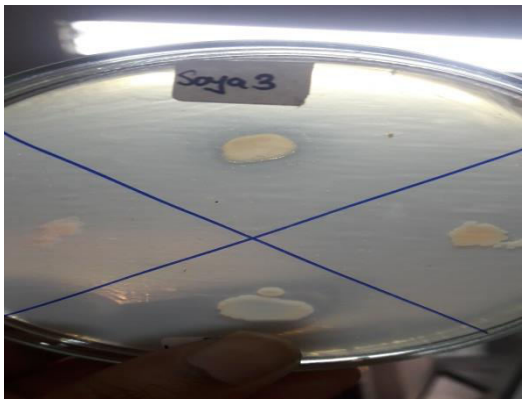

Fig. 3 Zone of hydrolysis shown by Rhizobium isolated from soyabean nodules on colloidal chotin agar plate.

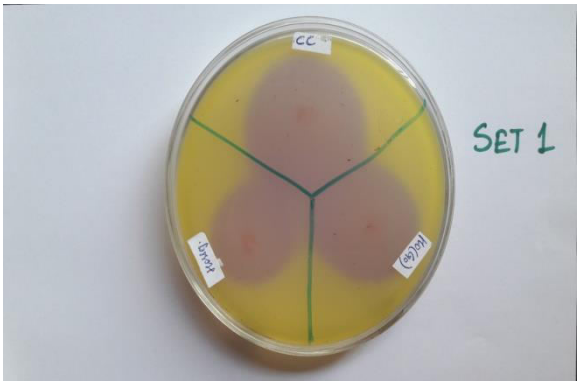

Fig. 4 Zone obtained during secondary screening on bromocresol purple plate.

4.CONCLUSIONS

The present study of all 4 isolates namely Cajanuscajan, Horsegram (local), Horsegram (HG), and soyabean suggests thatRhizobium isolated and screened from root nodules of leguminous plants have ability to produce Chitinase. The potential uses and effects of Chitinase in plant growth promoting activity (as fungal pathogen inhibitor) need to be studied further.

ACKNOWLEDGEMENT

Author is pleased to acknowledge Director ofThe Institute of science for providing the researchsupport and facilities.

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