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# INHIBITION OF PHYTOPATHOGENIC FUNGI BY CHITINASE PRODUCING *RHIZOBIUM* ISOLATES OBTAINED FROM ROOT NODULES OF *MACROTYLOMA UNIFLORUM* (LAM.) VERDE.

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**Abstract**

Isolates of *Rhizobium* designated as HGR-6 and HGR-25 were isolated from root nodules of *Macrotyloma uniflorum* (Lam.) Verdc. and tested for their ability to inhibit *in vitro* growth of *Aspergillus niger, Fusarium solani, Fusarium oxysporium, Botrytus cinera* and *Rhizoctonia solani* sp. on agar plate assay. Fungal cultures in liquid Czapek-Dox medium were also inoculated with either rhizobial culture or clrarified culture filtrate and growth inhibition was visually examined.

Species of *Rizobium* were isolated from fresh nodules of horse gram, *Macrotyloma uniflorum*, by standard method on yeast extract manitol agar (YEMA) medium (Vincent 1970) containing 0.0025% Congo red dye. Root nodulating ability of these isolates was determined by nodulation test (Weller and Cook 1983). The isolates, HGR-1 (Horse gram Rhizobia) to HGR-32, were Gram-negative, non-spore forming rods with the size of 2 - 2.3 µm long and 0.5 - 1 µm width. The size of the colonies was 7 - 8 mm in diameter after 72 hrs on YEMA medium at room temperature. The optimum pH and temperatures were 7.0 - 7.5 and 35ºC.

Commercially available chitin powder (40 g) was added with 500 ml hydrochloric acid followed by continuous stirring at 4$^{o}$C. The hydrolyzed chitin in the beaker was washed with distilled water and was brought to the pH 6 - 7. The precipitate was collected by filtration and stored at 4$^{o}$C. This was used at 5% concentration (w/v) as the sole carbon source. Freshly grown rhizobia were spot inoculated on chitin agar plates and incubated at 30$^{o}$C for 7 days. Plates were then observed for zone of hydrolysis around the colony. Among the 32 rhizobial isolates, 18 exhibited chitinase activity on chitin agar plates. The clear zone formed by the growth of bacterial strains was visually examined. The chitinase activity in the culture supernatant was estimated following the method as described by Vyas and Deshpande (1989). Two rhizobial strains HGR-6 GQ483458 and HGR-25 GQ483460 were selected for further studies.

Effect of rhizobial culture and culture filtrate was studied on growth of the fungal isolates. Rhizobia grown in 10 ml of chitin broth for 48 hrs, was added to the 90 ml of Czapek-Dox medium. The medium was inoculated with 5-day-old fungal mycelia of *Rhizoctonia solani* (MTCC 4634), *Aspergillus niger* (MTCC 872), *Botrytus cinerea* (MTCC 359), *Fusarium solani* (MTCC 6773) and *Fusarium oxysporium* (MTCC 3075). A control flask was also maintained for each fungus without *Rhizobium* culture. In the medium inoculated with the strain HGR-6, maximum inhibition of growth was observed in *F. solani* followed by *B. cinerea*, *A. niger* and *F. oxysporium* whereas the strain HGR-25 inoculated medium, inhibition was maximum in *F. oxysporium* (Table 1).

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**Table 1. Effect of *Rhizobium* culture and culture filtrate on the growth of some common fungi.**

| Fungi tested | Dry weight (g) of fungal mat without *Rhizobium* sp. | | Dry weight (g) of fungal mat with *Rhizobium* sp. | | | | Dry weight (g) of fungal mat with *Rhizobium* sp. culture filtrate | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | HGR-6 | | HGR-25 | | HGR-6 | | HGR-25 | |
| | Values | Sd | Values | Sd | Values | Sd | Values | Sd | Values | Sd |
| *Rhizoctonia solani* | 0.340 | 0.003 | 0.462 | 0.019 | 0.346 | 0.008 | 0.120 | 0.02 | 0.176 | 0.006 |
| *Aspergillus niger* | 0.600 | 0.047 | 0.404 | 0.002 | 0.160 | 0.02 | 0.570 | 0.004 | 0.364 | 0.003 |
| *Botrytus cinerea* | 0.900 | 0.011 | 0.145 | 0.005 | 0.206 | 0.053 | 0.510 | 0.026 | 0 | 0 |
| *Fusarium oxysporium* | 0.540 | 0.030 | 0.524 | 0.012 | 0.08 | 0.001 | 0.606 | 0.032 | 0.060 | 0.01 |
| *Fusarium solani* | 1.380 | 0.060 | 0.524 | 0.024 | 0.266 | 0.059 | 0.330 | 0.036 | 0.574 | 0.011 |

Ten ml of *Rhizobium* culture, grown in chitin broth was centrifuged at 3,000 rpm and the cell free supernatant was added to the flasks containing 90 ml of Czapek-Dox medium pre-inoculated with fungal mycelia. A control flask was also maintained for each fungus by adding 10 ml of centrifuged chitin broth. In both cases, the inhibition of fungal growth was monitored in terms of dry weight after 9 days. The medium inoculated with the culture filtrate of HGR-6 showed maximum inhibition on the growth of *R. solani* followed by *F. solani*, *B. cinerea* and *A. niger*. The strain HGR-25 showed maximum inhibition on the growth of *F. oxysporium* and least in *F. solani*. The culture filtrate of some wild rhizobia have shown inhibitory effect against some fungi causing root rot disease of faba bean (EI-Batanony *et al.* 2007). *Rhizobium leguminosarum* and heat killed bacterial cell culture filtrate protected lentil plants against infection with the pathogen *F. oxysporium* (Essalmani and Lahlou 2003). Al-Kahal *et al.* (2003) reported that *R. leguminosarum* and *Bradyrhizobium japonicum* controlled faba bean root disease caused by *F. oxysporium*.

Utilization of fungal biomass as a source of chitin by *Rhizobium* sp. was studied by growing several fungi in Czapek-Dox broth for 7 days and the fungal biomass was harvested, washed with distilled water and dried in an oven at 60$^{o}$C overnight. These dried fungal mycelia were then powdered and used at 5 g/l concentration instead of chitin. To observe the ability of *Rhizobium* sp. to utilize dead fungal mycelia, fungal mass of *R. solani*, *A. niger*, *B. cinerea*, *F. solani* and *F. oxysporium* was substituted for chitin. The highest chitinase production was shown by HGR-25 in case of *R. solani*, followed by *F. oxysporium*, *F. solani*, *B. cinerea* and *A. niger*, whereas HGR-6 showed maximum production in *R. solani* and minimum production in *F. oxysporium*. Some rhizobial strains were able to dissolve fungal mycelium at the initial stage (Hossain and Martensson 2008).

It has been reported that rhizobia significantly inhibited the growth of pathogenic fungi such as *Macrophomina phaseolina*, *Rhizoctonia* spp., *Fusarium* sp. and *Pythium* spp. in both leguminous and non-leguminous plants (Hossain and Mohammed 2002). Antagonism against *Verticillium* sp. was observed in 10 rhizobial isolates (Vargas *et al.* 2009). Arfaoui *et al.* (2006) reported that *Rhizobium* isolates significantly reduced wilt incidence of *F. oxyssporium. R. leguminosarum* bv. *viceae* was effective in controlling damping-off of pea infested with *Pythium* sp. (Huang and Erickson 2007). Sridevi and Mallaiah (2008) also reported that *Rhizobium* strains from *Sesbania sesban* were able to produce chitinase and inhibit *F. udum*. Chitinase activity of *Rhizobium* isolates might be advantageous in biocontrol of some common soil fungi including pathogenic species. Thus, the enzyme production has ecological significance in its interaction with soil fungi. The results of this study showed that the chitinase of rhizobia from horse gram may be considered for the biocontrol of plant diseases caused by several phytopathogens like *A. niger*, *F. solani*, *F. oxysporium*, *B. cinerea* and *R. solani*.

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