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Research Article

Antibiotic and antibacterial activity of a symbiotic bacterium, *Photorhabdus luminescens*

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ABSTRACT: Antibiotic and antagonistic effects of primary and secondary forms of *Photorhabdus luminescens,* a symbiotic bacterium of an entomopathogenic nematode *Heterorhabditis indica* (RCR) were investigated *in vitro* against plant pathogens and beneficial microorganisms. An inhibition zone assay on potato dextrose agar (PDA) medium revealed that the primary form completely inhibited all plant pathogenic fungi, *viz., Aspergillus flavus, Rhizoctonia solani* and *Fusarium solani* and tested secondary form completely inhibited the growth of *F. solani* and partially inhibited *A. flavus* and failed to inhibit *R. solani.* However, dual culture assay on nutrient agar (NA) in both forms completely inhibited the growth of the bacterial pathogen, *Xanthomonas punicae.* The forms of *P. luminescens* did not inhibit the mycelial growth of entomopathogens *viz., Metarhizium anisopliae, Verticillium lecanii* and *Nomuraea rileyi* but inhibited the growth of bacterial bioagents *Rhizobium* sp. *and Pseudomonas fluorescens.* The present study indicates that *P. luminescens* can be effectively used in the management of these plant pathogens.

KEY WORDS: Antifungal, Antibacterial, entomopathogens, *Heterorhabditis*, *Photorhabdusluminescens,* plant pathogens

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INTRODUCTION

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are used commercially as biological control agents against a range of insect pests (Georgis, 1990). The infective juvenile (IJs) stage of the steinernematids and deterorhabdits are symbiotically associated with the bacteria *Xenorhabdus* and *Photorhabdus*, respectively (Boemare *et al.,* 1993). The IJs enter the insect hemocoel and release the bacteria which kill the insect, establishing conditions for nematode development by providing nutrients (Akhrust and Dunphy, 1993) and also inhibiting the growth of various fungal and bacterial competitors (Chen *et al.,* 1994; Maxwell *et al.,* 1994). The bacterium exists in two forms that differ in their biochemical and physiological properties (Akhrust, 1980; Nagesh *et al*., 2001). The primary form contains para crystalline inclusions and produces antibiotics whereas the secondary form, which occurs after prolonged culture *in vitro*, either lacks or possesses reduced levels of these properties (Boemare *et al.*, 1993). Several metabolites of *P. luminescens* are known to possess antifungal, antibacterial, nematicidal and insecticidal properties (Chen *et al.,* 1994; Bowen *et al.,* 1998; Han and Ehlers, 1999; Hu *et al.,* 1999). Hence, the present study was undertaken to find out the possibility of using *P. luminescens* against important plant pathogenic fungi and bacteria and explore its compatibility with beneficial microorganisms.

MATERIALS AND METHODS

Isolation and purification of P. luminescens from H. indica

Photorhabdus luminescens was isolated from the entomopathogenic nematode, *Heterorhabditis indica* (RCR) as described by Akhurst (1980) and maintained as monoxenic culture. Last instar larvae of the greater wax moth, *Galleria mellonella* (L.) were exposed to the nematodes at the rate of 100 IJs per larva. After 72 hours the dead larvae were surface sterilized by dipping them into 95 per cent ethanol for 2–3 seconds, igniting and plunging into sterilized water. The cadavers were aseptically dissected with sterile forceps and a drop of haemolymph was streaked on to nutrient agar (NA) medium and incubated at 28°C for 24-48 h. The colonies were sub-cultured to obtain the primary form and to obtain the secondary form, the primary form was maintained without subculture for 20 days at 28°C. Based on cultural, morphological and biochemical characterization, the primary and secondary forms were differentiated. The

resulting primary and secondary forms were maintained on NA plates at 28°C for further studies.

Preparation of plant pathogenic and entomopathogenic cultures

Isolates of the fungal pathogens, *Aspergillus flavus* (von Tiegh), *Rhizoctonia solani* (Kuhn) and *Fusarium solani* (Sacc.) and the bacterial pathogen, *Xanthomonas punicae* (Hingorani and Singh) were obtained from the Department of Plant Pathology, College of Agriculture, Raichur. Cultures of three entomopathogenic fungi, *Viz., Metarhizium anisopliae* (Metschnikoff), *Verticillium lecanii* (Zimmermann) and *Nomuraea rileyi* (Farlow) were obtained from the Department of Agricultural Entomology, College of Agriculture, Dharwad, and those of bacterial bioagents, *Rhizobium* sp. and *Pseudomonas fluorescens* (Migula) from Biocontrol laboratory, College of Agriculture, Raichur. All the fungi were cultured on potato dextrose agar (PDA) and bacteria on NA medium. Fungi inoculated Petri dishes were incubated in dark at 28°C for up to 15 days for complete sporulation. Petri dishes containing bacteria were incubated at 28°C for 24-48 hours and stored at 4°C.

Inhibition of fungal growth by P. luminescens *(Inhibition zone assay method)*

A loopful of primary and secondary forms of *P. luminescens* was spread on a line 3 cm away from the edge of the PDA plates (9 cm dia.) separately. Subsequently, the plates were inoculated with one mycelial plug (0.5 cm dia.) of *A. flavus, R. solani, F. solani, M. anisopliae, V. lecanii* and *N. rileyi* separately taken from 15 days old cultures grown on PDA. The plug was placed 3 cm away from the bacterial streak and 3 cm from the wall of the dish. A plate inoculated with the pathogen alone in a similar manner served as control. All the inoculated Petri dishes were incubated at 28°C. Observation on radial growth of test fungi was recorded after 24 hours of incubation for up to 15 days depending on the sporulation period.

Inhibition of bacterial growth by P. luminescens *(Dual culture technique, Ansari et al., 2004)*

A loopful of primary and secondary forms of *P. luminescens* was streaked across one third of the plate separately and incubated for 24 hr after which the test bacteria *X. punicae, Rhizobium* sp. and *P. fluorescens* were streaked perpendicular to the zone of inhibition of antagonistic bacteria. After 24 hours of incubation the inhibition zone was observed.

RESULTS AND DISCUSSION

The primary and secondary forms of *P. luminescens* exhibited varied level of inhibition against the fungal and bacterial cultures tested (Table 1 and 2). The growth of plant pathogenic fungi *A. flavus, R. solani* and *F. solani,* was completely inhibited by the primary form, whereas the secondary form completely inhibited the growth of *F. solani* but partially inhibited *A. flavus* and failed to inhibit *R. solani* (Plate 1). However, both the forms of *P. luminescens* did not inhibit entomopathogens like *M. anisopliae*, *V. lecanii* and *N. rileyi* (Plate 2). Similar from results were obtained by Chen *et al.* (1994) who also reported antimycotic/ antifungal property of *P. luminescens* against *A. niger, F. solani* and *R. solani.* The variation in the level of inhibition by the two forms of *P. luminescens* might be due to quantitative or qualitative differences in the antifungal substances (Hydroxystilbenes and polyketides) produced by the strain. Ghazala M. furgani (2006) reported that the antibiotic production varies with the strain. Put of thirteen different strains of *Xenorhabdus* and *Photorhabdus* tested against *Bacillus cerus* (Frankland and Frankland), Ema strain of *Xenorhabdus* and Arg strain of *Photorhabdus* showed best antibiotic activity with inhibition zones 80-90 mm and 37.5 and 28.5 mm, respectively, followed by KMD15, DSM3370 *X. nematophila* strains and Jun old, IS5 strains of *P. luminescens.* However, the present result on entomopathogens is contradictory to the reports of Ansari *et al.* (2004) who recorded the antagonistic activity of *P. luminescens* to *B. bassiana* and *M. anisopliae*. The difference may be attributed to different strains of the bacterium used.

Table 1. Antibiotic property of *P. luminescens* against plant pathogens

Pathogen	Crop	Activity of forms	
		Primary form	Secondary form
Fusarium solani	Chilli	+	+
Aspergillus flavus	Chilli	+	PI
Rhizactonia solani	Chickpea	+	–
Xanthomonas punicae	Pomegranate	+	+

Note: '–' – Not inhibited, '+' – Inhibited, PI – partially inhibited

Table 2. Antagonism of *Photorhabdus luminescens* against bioagents

Biocontrol agent	Inhibited to	
	Primary form	Secondary form
Metarhizium anisopliae	–	–
Nomuraea rileyi	–	–
Verticillium sp.	–	–
Rhizobium sp.	+	+
Pseudomonas flourescens	+	+

Note: '-' - Not inhibited, '+' – Inhibited

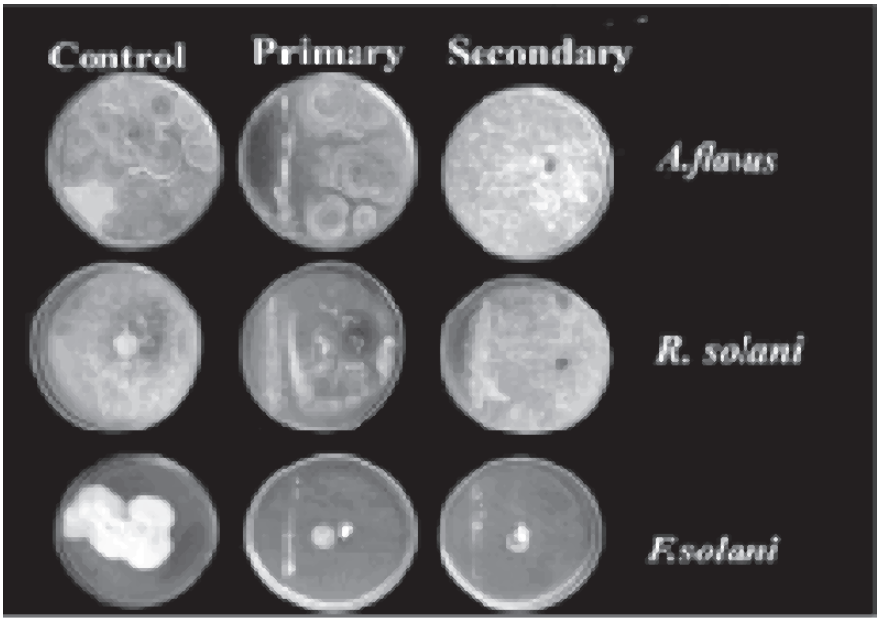

Plate 1. Differential inhibition response of primary form and secondary forms against three plant pathogenic fungi

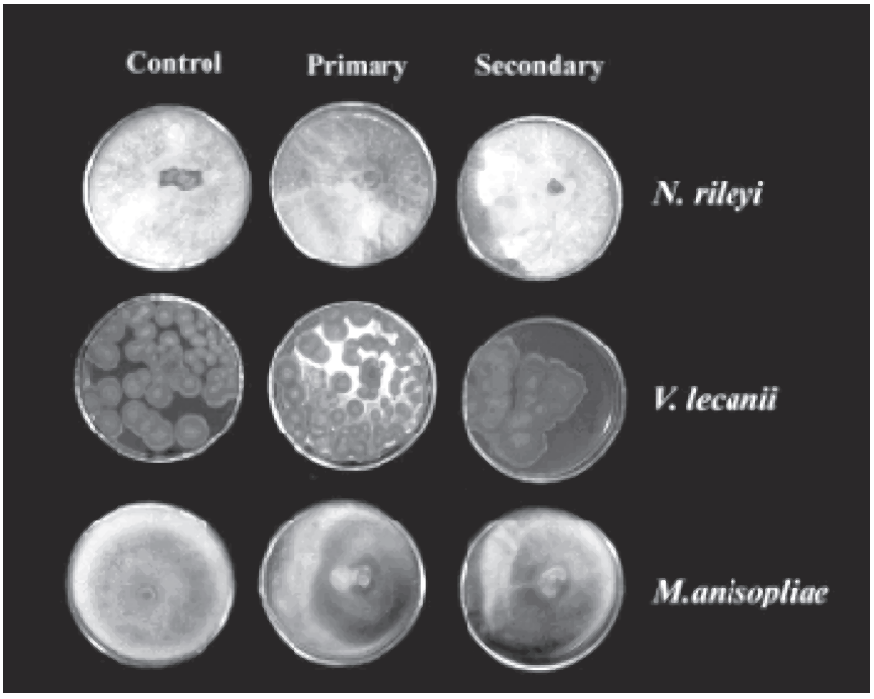

Plate 2. Effect of *P. luminescens* forms on the growth of entomopathogenic fungi

Both the primary and secondary forms completely inhibited the growth of plant pathogenic bacterium, *X. punicae* (Plate 3) and the bacterial bioagents *Rhizobium* sp. and *P.fluorescens* (Plate 4). Since no reports are available with respect to antibacterial activity of *P. luminescens* against *X. punicae, Rhizobium sp.* and *P. fluorescens* the present study forms the first report. The reasons for antagonism against the test bacteria might be due to the production of various antibiotics or antibiosis. Similar opinion was expressed by Jaroz (1991) who observed that the primary form colonies of *P. luminescens* produce an agar diffusible antibiotic compound with broad spectrum of antibacterial activity.

In conclusion, *P. luminescens* was found to possess antimycotic and antibiotic properties against important plant pathogenic fungi and bacteria tested. This provides

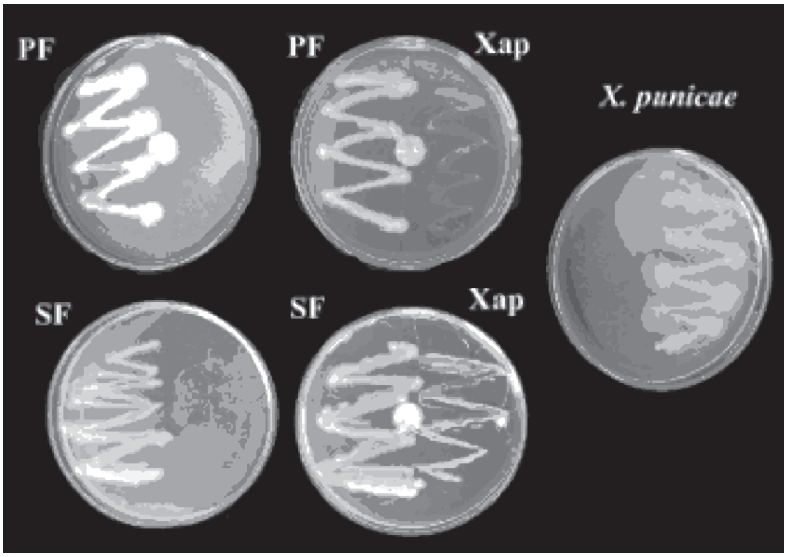

Plate 3. *In vitro* evaluation of *P. luminescens* forms against *X. punicae* by dual culture technique

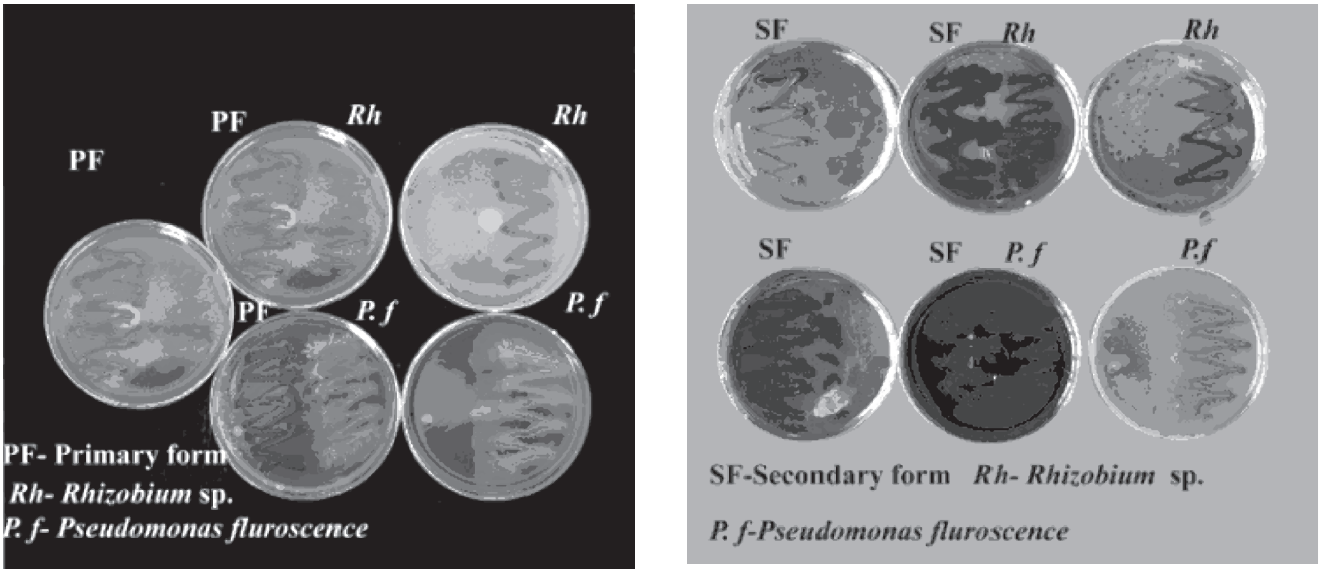

Plate 4. Antagonism of *P. luminescens* primary (a) and secondary (b) form against bacterial bioagents in dual culture

scope for utilization of *P. luminescens* in the management of these plant pathogens. However, detailed studies on standardization of dosage and formulation of *P. luminescens* are to be carried out. *P. luminescens* was found to be compatible with entomopathogens, which indicates the possibility of using them in combination with entomopathogens. Further studies on the synergistic and antagonistic intentions between the bacteria and entomopathogens need to be carried out. Since *P. luminescens* inhibits the growth of *Pseudomonas* and *Rhizobium* it cannot be combined with these bacterial bioagents.

REFERENCES

Akhurst, R. J. 1980. Morphological and functional dimorphism in *Xenorhabdus* spp. bacteria symbiotically associated with insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *Journal of General Microbiol*ogy, **121**: 303–309.

Akhurst, R. J. and Dunphy, G. B. 1993. Tripartite interactions between symbiotically associated entomopathogenic bacteria *Xenorhabdus* spp. and nematodes (Steinernematidae and Heterorhabditidae) and their insect hosts. In: *Parasites and pathogens of insects*. Academic Press, New York, **2**: 1–23.

Ansari, M. A., Tirry, L. and Moens, M. 2004. Antagonism between entomopathogenic fungi and bacterial symbionts of entomopathogenic nematodes. *BioControl,* **50**: 465–475.

Boemare, N. E., Akhurst, R. J. and Mourant, R. J. 1993. DNA relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathopgenic nematodes and a proposal to transfer *X. luminescens* to a new genus

Photorhabdus gen. nov. *International Journal of Systematic Bacteriology,* **43**: 249–255.

Bowen, D. J., Rocheleau, T. A., Blackburn, M., Andreey, O., Golubeva, E., Bhartia, R. and R. H. ffrench–Constant. 1998. Insecticidal toxins from the bacterium *Photorhabdus luminescens. Science,* **280**: 2129– 2132.

Chen, G., Dunphy, G. B. and Webster, J. M. 1994. Antifungal activity of *Xenorhabdus* spp. and *Photohabdus luminescens*, bacteria associated with the nematodes *Steinernema* spp. and *Heterorhabditis megidis*. *Biological Control*, **4**: 157–162.

Georgis, R. 1990. Formulation and application technology, pp. 173-191. In: (Gaugler, R. and Kaya, H. K. (Eds.), *Entomopathogenic nematodes in biological control*, CRC Press, Boca Raton, FL, USA.

Ghazala, M. F. 2006. *Gnotobiological analysis of Entomopathogenic nematode / bacterium symbiotic complexes.* Ph.D. Thesis, Eeotvos Lorand University, Budapest, Hungary.

Han, R. and Ehlers, R. U. 1999. Trans-specific nematicidal activity of *Photorhabdus luminescens*. *Nematology*, **1**: 687–693.

Hu, K., Li, J. and Webster, J. M. 1999. Nematicidal metabolites produced by *Photorhabdus luminescens* (Entero-bacteriaceae), bacterial symbiont of entomo-pathogenic nematodes. *Nematology,* **1**: 457–469.

Jarosz, J., Blacerzak, M. and Skrzypek, H. 1991. Involvement of larvicidal toxins in pathogenesis of insect parasitism with the rhabditoid nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora. BioControl,* **36**: 361–368.

Maxwell, P. W., Chen, G., Webster, J. M. and Dunphy, G. B. 1994. Stability and activities of antibiotics produced during infection of the insect *Galleria mellonella* by two strains of *Xenorhabdus nematophilus*. *Applied and Environmental Microbiology,* **60**: 715–721.

Nagesh, M., Hussaini, S. S. and Singh, S. P. 2001. Isolation and characterization of symbiotic bacteria from *Heterorhabditis* spp. and *Steinernema carpocapsae. Pest Management in Horticultural Ecosystems,* **8**: 38–42.