STUDIES ON PRODUCTION AND APPLICATION OF PLANT GROWTH PROMOTING ROOT ENDOPHYTE *PIRIFORMOSPORA INDICA*

by

VINOD KUMAR

Department of Biochemical Engineering & Biotechnology

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on Production and Application of Plant Growth Promoting Root Endophyte *Piriformospora indica*"** being submitted by **Mr. Vinod Kumar** to the Indian Institute of Technology, Delhi for the award of the degree of '**Doctor of Philosophy**', is a record of the bonafide research work carried out by him, which has been prepared under our supervision in conformity with the rules and regulations of the "Indian Institute of Technology, Delhi". The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

Prof. V. S. Bisaria **Dr. Vikram Sahai**

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Abstract

Piriformospora indica, a symbiont root endophytic fungus infests the roots of a broad range of monocotyledonous and dicotyledonous plants. Endophytic root colonization by this fungus confers enhanced growth to the host plant and provides protection against biotic and abiotic stresses. *P. indica* mobilizes the insoluble phosphates and translocates the phosphorus to the host in an energy-dependent process. The fungus is also able to provide systemic protection due to a yet unknown mechanism of induced resistance. The fungus which forms chlamydospores and shows tremendous potential as a biofertiliser and biocontrol agent needs to be mass-produced at a large scale for its successful application as a bio-inoculant to enhance crop productivity.

In this study, the effect of environmental parameters (inoculum size, agitation speed, working volume, initial pH and temperature) and nutritional parameters (various carbon and nitrogen sources) on the growth and sporulation of *Piriformospora indica* in batch bioreactions at shake flask level has been investigated. The composition of Kaefer medium was modified by using statistical central composite design and response surface methodologies. The culture conditions for the cultivation of *P. indica* in 14 L bioreactor have been optimized such that they result in maximum biomass formation during growth phase and in maximum spore density during subsequent sporulation phase. Using glucose deprivation as the strategy for its sporulation, the fungus was made to give rise to the maximum sporulation in a modified Kaefer medium. The value of kinetic parameters, biomass yield ($Y_{X/S}$) and specific growth rate (μ) were 0.79 and 1.15 d^{-1} respectively. An enhancement of 100 % in overall biomass productivity (0.18 g L^{-1} h^{-1}) and reduction of about 70 % in the time required to achieve the maximum spore density (60 h) was achieved in comparison to the original Kaefer medium.

The efficacy of the bio-inoculant formulations containing *P. indica* and fluorescent pseudomonad strains R62 & R81 and their different consortia were tested on *Vigna mungo* and tomato plants under glasshouse and field conditions. For the preparation of these formulations, talcum powder and vermiculite were used as carriers. In both the crops all the treatments resulted in significant increase in growth parameters under glasshouse and field conditions. The results specific to treatments showed consistency in their performance when the conditions were scaled up from glasshouse to field conditions. In case of *Vigna mungo* under glasshouse conditions, a maximum increase of 4.5-fold in dry root weight and 3.9-fold in dry shoot weight with respect to control was obtained when vermiculite-based consortium formulation of *P. indica* and R81 was used. In field studies with *Vigna mungo* using vermiculite as carrier, a maximum enhancement of 3.2-fold in dry root weight, 3.0-fold in dry shoot weight, 8.4-fold in number of nodules and 4.0-fold in number of pods in comparison to control was obtained with the consortium formulation of *P. indica* and R81. The same consortium formulation also caused the highest improvement of 1.9-fold in nitrogen content and 1.7-fold in phosphorus content, while the highest increase of 1.4-fold in potassium content was obtained with *P. indica* alone.

In case of tomato, talcum-based consortium treatment of *P. indica* and R81 resulted in the highest improvement of 8.8-fold in dry root weight and 16.8-fold in dry shoot weight under glasshouse conditions. In field conditions, amongst all the treatments, talcum-based consortium treatment of *P. indica* and R81 resulted in a maximum increment of 2.7-fold in dry root weight, 3.1-fold in dry shoot weight, 3.1-fold in number of branches and 3.9-fold in number of fruits. The same treatment also resulted in maximum increment of 2.8-fold in

nitrogen content, 2.0-fold in phosphorus content and 2.3-fold potassium content. The efficacy shown by the talcum-based bio-inoculant formulation containing *P. indica* and consortium of *P. indica* and R81 against fusarium wilt disease in tomato plant under glasshouse conditions were almost similar. However, R81 alone conferred maximum degree of resistance against fusarium wilt disease.

Agrobacterium rhizogenes - mediated genetically- transformed high yielding hairy root line LYR2i of *L. album* was cultured on Gamborg medium. The maximum dry cell weight (15.4 g/L), podophyllotoxin (4.02 mg/g) and 6-methoxypodophyllotoxin (1.68 mg/g) were obtained after 14 d cultivation. In the literature there are a number of studies where the fungal products such as culture filtrate, spores, mycelium, cell wall extract etc. have been used to enhance the production of secondary metabolites by the plant cell culture. This is the first study where the effects of culture filtrate, cell extract and live cells of *P. indica* have been studied on lignan production by the hairy root culture of *L. album*. The addition of 4 d (log phase) as well as 8 d (decline phase) old, autoclaved and filter-sterilized culture filtrate and cell extract of *P. indica* as well as co-culturing of *P. indica* with hairy root culture of *L. album* resulted in a significant enhancement in podophyllotoxin and 6-methoxypodophyllotoxin content. Podophyllotoxin and 6-methoxypodophyllotoxin content were maximally enhanced by 3.3 times (13.01 mg/g) and 3.8 times (7.23 mg/g) in comparison to control culture when a filter-sterilized culture filtrate at a level of 3 % (v/v) was added to the hairy root culture of *Linum album* on 12^{th} d of growth (exposure time of 48 h) from log and decline phase respectively. The increase in the lignan content coincided with the increase in the PAL activity.

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