Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. **Title of research project:** "Isolation of endophytic and soil actinobacteria and evaluation of their plant growth promoting potential".

2. **Name and Address of the Principal Investigator**: Dr. Rajesh Kumari Manhas Residential: B-3, Guru Nanak Dev University Campus, Amritsar (Punjab).

3. **Name and address of the Institution:** Guru Nanak Dev University, Amritsar-143005.

4. **UGC approval no. and date:** F. No. 43-468/2014(SR), 24 September, 2015

5. **Date of implementation:** 1 July, 2015

6. **Tenure of the project:** 3 years from 01/07/2015 to 30/06/2018

7. **Total grant allocated:** Rs. 8,96,000/-

8. **Total grant received**: Rs. 8,55,000/-

9. **Final expenditure:** Rs. 8,52,025/-

10. **Objectives of the project:**

- Isolation of actinobacteria from surface sterilized tissues (roots, stems and leaves) of healthy plants and their rhizosphere soils.

- Evaluation of these isolates for various direct as well as indirect plant growth promoting activities *viz.* Indole acetic acid production, ACC deaminase activity, phosphate solubilization, rhizosphere competence, siderophore production and antifungal activity against various fungal phytopathogens (*Fusarium oxysporum*, *Alternaria alternata*, *Colletotrichum acutatum*, *Alternaria brassicicola*, *Cladosporium herbarum*, *Rhizoctonia oryzae* f. sp. *sativae*, *Cercospora beticola*) causing diseases in economically important agricultural crops.

- Selection of a potent actinobacterial strain with various plant growth promoting traits and its identification using polyphasic approach based on molecular, morphological, physiological and biochemical characteristics.

- ❖ *In vivo* evaluation of the selected promising strain and its metabolites to further validate its potential as plant growth promoting agent.

- ❖ Purification of antifungal compound/s.

11. **Whether objectives were achieved:** Yes.

12. **Achievements from the project**: Has been able to isolate potent actinobacterial isolates (*Streptomyces* spp.) with plant growth promoting activities, and exhibiting antagonism against various phytopathogens. These could be further developed as bio-fertilizers and biocontrol agents**.**

13. **Summary of the findings**: In the present study, total of 113 different actinobacterial isolates were isolated from different healthy plants (lemon, jamun, candy leaf, radish, turmeric and tulsi) and rhizospheric soil samples (rice, mustard, tea, potato, candy leaf and wheat) collected from Punjab and Himachal Pradesh. In screening for the plant growth promoting activities *viz.* production of indole acetic acid, ACC deaminase, siderophore and ammonia, phosphate solubilisation and rhizosphere competence, 14 (12.38%) isolates showed two or more plant growth promoting activities. All the 14 isolates exhibited antifungal activity against one or more tested phytopathogens. On the basis of broad spectrum activities, the already identified lab isolate (*S. hydrogenans* strain DH16) and two isolates (MR14 and M4) were selected for the further work. Various morphological, biochemical, physiological characteristics showed that the isolates MR14 and M4 belonged to the genus *Streptomyces*. However, the phylogenetic analysis excluded their relatedness with other most closely similar *Streptomyces* spp. Therefore, the isolates could be assigned as new spp. of *Streptomyces* and designated as *Streptomyces* sp. MR14 and *Streptomyces* sp. M4.

    Furthermore, the plant growth promoting potential of *S. hydrogenans* strain DH16 was studied using various *in vitro* and *in vivo* assays. Optimization of indole acetic acid production by strain DH16, using one variable at a time approach, resulted in 2.24 folds (from 36 to 80.06µg/ml) increase in yield of IAA. The strain completely utilized ACC on fourth day of incubation and showed ACC deaminase activity of 363 nanomoles α-ketobutyrate/mg protein/h. The influence of strain DH16 (cells/culture supernatant containing IAA) on growth of pea seedlings was evaluated *in vivo*. Significant enhancement in different agronomic traits of treated pea seedlings *viz.* seed germination, shoot length, root length, fresh and dry weights and lateral roots was observed. Additionally, streptomycete also showed root colonizing ability and its cells were found to be adhered to roots with a cfu count of 7×10^6/cm.

The plant growth promoting agents promote the growth of plants either directly by enhancing the production of plant growth hormones (IAA), siderophores, ammonia and ACC deaminase, nitrogen fixation and solublizing inorganic phosphate or indirectly by the production of biocontrol agents for controlling various plant pests like nematodes, insects and fungal phytopathogens. Therefore, the selected strains were further evaluated for biocontrol activities against fungal phytopathogens and root knot nematode *Melidogyne incognita*; and for their effect on plant growth under natural conditions. *In vivo* results demonstrated the efficacy of *S. hydrogenans* strain DH16 cells and culture supernatant to control damping off of radish seedlings (as seed dressing) and black leaf spot of radish (as foliar treatment) caused by *A. brassicicola*, and wilt of tomato plants caused by root knot nematode *M. incognita*. Treatment of *A. brassicicola* infested seeds with culture supernatant or cells significantly improved seed germination (75-80%) and seedling vigour (1167-1538). The visible symptoms of black leaf spot disease were observed in the control plants with 66.81% disease incidence which resulted in retarded growth of root system. On the other side, disease incidence reduced to 6.78 and 1.47% in plants treated with antagonist and its metabolites, respectively. Similarly, soil drenching with culture supernatant and cell suspension of *S. hydrogenans* strain DH16 significantly reduced the galling index and egg masses in nematode infested tomato plants, and therefore, improved plant growth. Additionally, in the absence of pathogen infestation, treatment of plants with culture supernatant and cell suspension, out performed all other treatments, significantly enhanced the root length, shoot length and weight of seedlings as compared to the control plants. These data indicate that *S. hydrogenans* possesses significant nematicidal activity against *M. incognita*, a plant parasitic root knot nematode.

Similarly, the pot experiments also demonstrated the efficacy of *Streptomyces* sp. MR14 cells/supernatant/solvent extract and *Streptomyces* sp. M4 cells/supernatant/solvent extract to control Fusarium wilt in tomato plants caused by a fungal pathogen *F. moniliforme* and a nematode *M. incognita*. Moreover, treatment of plants with culture supernatant and cell suspension in the absence of pathogen infestation significantly enhanced the root and shoot lengths and weight of seedlings as compared to the control plants.

Four antifungal compounds: two from *Streptomyces* sp. M4 and one each from *S. hydrogenans* strain DH16 and *Streptomyces* sp. MR14 were purified. The antifungal compound SH2 purified from *S. hydrogenans* strain DH16 was a new compound identified to be 10-(2,2-dimethyl-cyclohexyl)-6,9-dihydroxy-4,9-dimethyl-dec-2-enoic acid methyl ester with a molecular mass of m/z 377.1 as determined by LC-MS. In *in vitro* and *in vivo* biocontrol assays, the purified compound **SH2** was found to be more potent in comparison to chemical control agents as it caused significant inhibition of pathogen on seeds and resulted in emergence of healthy seedlings. Moreover, in the absence of pathogen challenge, it also promoted the plant growth.

14. **Contribution to the society:** *Streptomyces* spp. isolated during project work might be developed as safe and environment friendly alternatives to harmful chemical fertilizers and chemical agents used to control various plant pathogens.

15. **Whether any Ph.D. enrolled/produced out of the project:** No

16. **No. of publications out of the project:** Three papers published in reputed journals and two are communicated (Appendix I)

PRINCIPAL INVESTIGATOR **REGISTRAR/PRINCIPAL**

Appendix I

PUBLICATIONS

1. Kaur, Talwinder; Amarjeet Kaur; Vishal Sharma and Rajesh Kumari Manhas (2016), 'Purification and Characterization of a New Antifungal Compound 10-(2,2-dimethyl-cyclohexyl)-6,9- dihydroxy-4,9-dimethyl-dec-2-enoic Acid Methyl Ester from *Streptomyces hydrogenans* Strain DH16', Frontiers in Microbiology, 7(1004) , June 29, pp,1-10. DOI: 10.3389/fmicb.2016. 01004. (**Impact Factor: 4.165**).

2. Kaur, Talwinder; Shivam Jasrotia; Puja Ohri and Rajesh Kumari Manhas (2016), 'Evaluation of *in vitro* and *in vivo* nematicidal potential of a multifunctional streptomycete, *Streptomyces hydrogenans* strain DH16 against *Meloidogyne incognita*' Microbiological Research 192 , August, pp, 247–252. (**Impact Factor: 2.723**).

3. **Manhas, Rajesh Kumari** and Talwinder Kaur **(2016) '**Biocontrol Potential of *Streptomyces hydrogenans* Strain DH16 toward *Alternaria brassicicola* to Control Damping Off and Black Leaf Spot of *Raphanus sativus'*, Frontiers in Plant Science, 7 (1869) pp, 1-13, December 16, 2016 DOI: 10.3389/fpls.2016.01869. (**Impact Factor: 4.495**).

4. Rani, Riveka; Talwinder Kaur and Rajesh Kumari Manhas (2018), 'Biocontrol and plant growth promoting potential of phylogenetically new *Streptomyces* sp. MR14 of rhizospheric origin', AMB Express (communicated).

5. Kaur, Talwinder and Rajesh Kumari Manhas (2018), 'Evaluation of ACC deaminase and indole acetic acid production by *Streptomyces hydrogenans* and its effect on growth promotion', (Communicated)

SUMMARY