Major Research Project Executive Summary

Project Title:

"Studies on fermentative production of allelochemicals by rhizospheric Pseudomonas species and its application for plant growth promotion in viticulture."

By

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Introduction: In India, agricultural customary is a way of life and Grapevine (*Vitis vinifera*) is an agro-industrial cash crop. Grapevine is one of the most important fruit crops of the world containing various most valuable elements essential for life. The crop has a wide adaptability therefore can be grown under varied environments like temperate, sub-tropical and tropical climatic conditions and diverse agro-ecologicalsettings.

Objectives:

1.To Isolate, identify and preserve the fluorescent Pseudomonads rhizospheric soils of grapevine.

2.To study the *in vitro* and *in vivo* bio- control potential of the rhizospheric fluorescent *Pseudomonas* isolates and the allelochemicals produced by them against fungal pathogens of grapevines; *Alternaria alternata*, *Plasmopara viticola*, *Uncinula necator*

and Aspergillus niger

3. To optimize the media and media contents for maximum production of allelochemicals

4.To analyze and identify the products using different physicochemical methods

5.To synthesize the products on lab scale and industrial pilot scale by fermentation

6.To develop commercial products of plant growth promoting substances

7.To conduct poly-house and field trials.

Salient Findings:

1. isolates showing highest zone of inhibition against fungal pathogens were tested for production of different types of allelochemicals such as siderophore, indole acetic acid, HCN, phenazine-1-carboxilic acid, pyocyanin, and chitinase.
2. Selected isolates were identified as species of genus *Pseudomonas* on the basis of cultural, morphological and biochemical characteristics as described in Burgey,s Manual of systematic bacteriology.

3.Strain RSML37 produced 642mg/L of Fe-siderophore in optimized succinate medium in a specially designed 20L fermenter in 48hrs at 30oC. Appearance of parrot green colour indicated production of siderophore in the medium. The absorption maxima at

404nm indicated production of pyoverdin type of siderophore. Purification of siderophore was done by XAD 4 amberlite column chromatography and eluted methanol.

4.The strain RSML35 yielded 641 mg L-1 of phenazine-1-carboxilic acid (PCA). The solvent extraction method using chloroform and benzene in highly acidic conditions were used for extraction of phenazine compounds. A preparatory thin layer chromatography (TLC) on silica gel plates was used to obtained yellow coloured purified PCA. UV – Vis absorption maxima at 252nm accompanied by a broad peak at 365 nm and GC-MS analysis indicated molecular formula as C13H8N2O2 which was confirmed by high resolution mas spectroscopy at m/z 225.0664. These analytical procedures confirmed the presence of phenazine-1-carboxylic acid.

5.The strain RSML24 yielded3 40 mg L-1 of pyocyanin. Appearance of blue green colour in the medium indicated production of pyocyanin. The UV- Vis absorption maxima at279nm and TLC Rf value 0.64 has characterized the product as pyocyanin.

6.The PCA obtained using strain RSML 37 reveled better antifungal potential against commonly occurring fungal pathogens. The phenazine 1 carboxylic acid at 200µg/ml exhibited considerable abiocontrol potential against*plasmopara viticola* as compared to 50µg/ml, 100µg/ml concentration.

7.The sub-merged fermentation process for enhanced chitinase production by RSML06 & RSML09 yielded 21EU/ml & 34 EU/ml respectively of crude extract of chitinase enzyme. The demonstration of antifungal activity of crude chitinase extract against fungal phytopathogens *(Aspergillus niger*, and *Fusarium oxysporum*) reflects the potential of these *Pseudomonas* species to produce chitinase for application as biocontrol agent.

8.The spreying of phenazine 1 carboxylic acid (PCA) on infected grape vein var. Sonaka has succesfully controlled the infection of *Plasmopara viticola* at 200µg/ml concentration in pot trials. However recurrence of the disease need to be tested before coming to concrete conclusion.

9.The highly promising results of crop analysis indicated that iron chelated siderophore application on grape vein (*Vitis vinifera* var. Thompson seedless) resulted in significant increase in shoot length, number of leaves per shoot, petiole iron and chlorophyll content over control.

10. Considerable increase in canopy development was also evident, however, more field trials on various varieties, in various grape growing regions with application of Random Block Designs (RBD) are recommended.