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ORIGINAL ARTICLE

Evaluation of bacterial consortia isolated from Saffron rhizosphere for its plant growth promotion and disease control Potential

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ABSTRACT
The main objective of the present study was the development of bacterial consortia associated with the rhizosphere of saffron for enhanced plant growth and suppression of corm rot disease. By cultivation dependent approach 165 isolates were obtained out of which only two bacillus strains, Bacillus aryabhattai and Bacillus amyloliquefaciens were selected for bioformulation preparation based upon their in-vitro plant-growth promotion and antifingal activity. The bacillus consortia selected not only effected the growth and number of roots, shoots, and cormlets but also suppressed the severity of corm rot.
***Key words**: Saffron, rhizosphere, bacillus species, corm rot disease, plant growth promotion biological control and bacterial consortia .*

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INTRODUCTION

Crocus sativus L., popularly known as Saffron is the world's costliest spice. It has immense medicinal value and one kg costs around 11,000 US $ [1]. In India, Jammu and Kashmir is the only state with the distinction of saffron cultivation [2]. According to a survey by J&K state agriculture department, the saffron production is adversely effected due to several biotic and abiotic factors due to which saffron cultivation is declining in the state. The major abiotic factors include the less availability of nutrients required by the plants for its proper growth and development. Among the biotic causes, corm rot disease, caused by *Fusarium oxysporum*, is the most destructive [3]. This disease severely damages saffron yields in the saffron growing belts of J&K, India. During corm rot infection, *Fusarium oxypsorum* penetrates the roots and colonizes vascular tissue thereby triggering necrosis and wilting (4). At present, the strategy employed for the control of this disease and for plant growth promotion is the use of chemical fertilizers [4]. The use of these fertilizers is effective but, expensive and due to its overuse it is causing serious environmental problems. Therefore, to protect the yield, development of non-chemical alternative methods is the need of the hour. Biological control and biofertilzer agents are required for sustainable production of saffron and defense against the corm rot disease. Several species of *Pseudomonas* spp. and *Bacillus* spp. have successfully been formulated and now commercially available [5]. Bacillus species based formulations are more stable because of their ease of colonizing rhizosphere, longer shelf-life and spore forming ability [6]. In addition to this they also do not pose any threat to the environment and are biodegradable. Hence the present study aims at the isolation of native bacterial species for the development of bioformulation with biocontrol and plant growth promotion potential.

MATERIAL AND METHODS

Sample collection

Plant samples for the isolation of rhizospheric bacteria were collected in 2017 from Pampore district in Kashmir as described by Luster and co-workers [9]. The collected samples were brought to lab and stored at -20^{0}C until processed.

Pathogenic fungus isolation

Pathogenic fungus, *Fusarium oxysporum* R1, causing corm rot disease isolated from the rooten saffron corms was obtained from the lab repository [7]. The pathogen was grown at 27 for three to four days on Potato dextrose agar (Himedia).

Potential bacterial species isolation

1 gram of soil associated with the roots was dissolved in 5 ml of autoclaved water. The dilution was the spread on different bacterial growth media such as Nutrient broth, Luria bertani and minimal media. The plates were insulated at 37 for overnight and the colonies obtained were purified for pure culture and in-vitro analysis. The isolates were preserved in 70% glycerol.

In-vitro analysis of plant-growth promoting and biocontrol activity

Isolated bacterial species were analyzed in-vitro by agar diffusion plate assay qualitatively as well as quantitatively for biocontrol activity against corm rot causing pathogen and plant growth promoting properties such as phosphate solubilization and siderophore production (Table 1).

Identification of bacterial isolates

The genomic DNA of selected bacterial strains, showing at least one activity, was isolated using protocol given by Pitcher and co-workers [10]. 16S rRNA gene was amplified using universal primers Bac8f (5'-AGAGTTTGATCCTGGCTCAG-3') and Univ529 (5'-ACCGCGGCKGCTGGC-3') [8] following protocol give by Fierer *et al*. [11]. Nucleotide sequences obtained were checked for similarity using nucleotide BLAST and the percentage similarity was also determined (Table 2).

Pot analysis of bioformulation

Bacterial isolates, showing maximum plant growth promotion and biocontrol activity, were selected for bioformulation preparation. Two bacterial isolates showing maximum activities were selected and were analyzed for co-inhibition test. The bioformulation was prepared by mixing the bacterial spores with autoclaved calcium carbonate in 1:2 ratio as described by Gupta and Vakhlu [7]. For in-vivo analysis, pot trials were carried out. The treated corms were dipped in bioformulation and then sown in autoclaved soil, whereas the control corms were dipped in calcium carbonate alone (without biocontrol bacterial consortia) as per the protocol by Kour *et al.* [8]. The data was collected and checked for statistical significance. One-way ANOVA was used for the designing of pot trials.

Table 1: Plant growth promotion and biocontrol activities selected for in-vitro analysis of bacterial strains along with their observations and references.

Test	Observation	Reference
Phosphate solubilization (Pikovskaya's agar)	Clear halos around positive isolates	Paul and Sinha, [12]
Siderophore production (CAS agar)	Orange halos around positive isolates	Pal and Karuna, [13]
Anti-fungal activity	Clear halos around positive isolates	Gupta and Vakhlu, [7]

Table 2: Percentage sequence similarity and 16S rRNA gene based identification of isolated bacillus species

Isolate number	16S rRNA gene identification	% similarity
B3	***Bacillus aryabhattai***	**100**
C2	*Bacillus cereus*	99
C6	*Bacillus megaterium*	100
B9	*Bacillus simplex*	99
D2	*Bacillus thuringiensis*	99
D4	*Bacillus coagulans*	100
C1	***Bacillus amyloliquefaciens***	**100**
D7A	*Bacillus atrophaeus*	99
D3A	*Bacillus mycoides*	100

Table 3: *In-vivo* pot analysis of the bacterial consortia.

Parameters	Control corms	Corms treated with bacterial consortia
Average number of roots	4.71±0.01	8.79±0.02
Average length of roots	3.22±0.02	7.28±0.01
Average number of shoots	1.23±0.03	4.49±0.01
Average length of shoots	5.76±0.02	8.97±0.02
Number of daughter corms	2.35±0.02	4.34±0.01
Flower number	4.76±0.01	5.43±0.01
Disease incidence	7.89±0.02	3.89±0.01

Values are <0.05 level significance

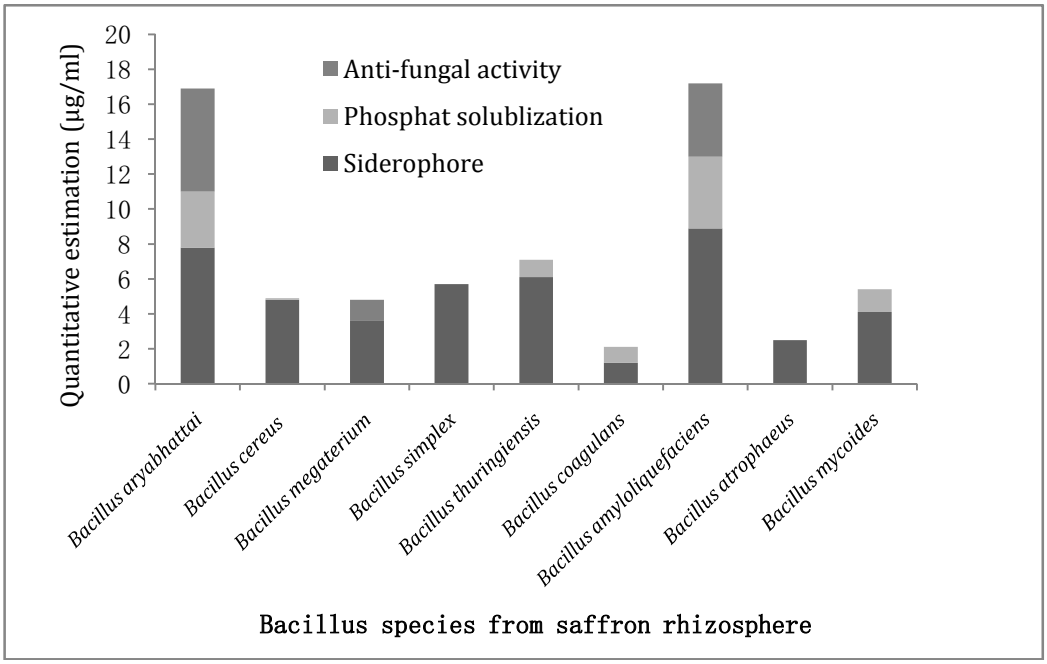

Fig. 1. Amount of plant growth promotion traits shown by selected Bacillus species isolated from the Saffron rhizosphere. Bacillus aryabhattai and Bacillus amyloliquifaciens have the maximum activities and therefore, were selected for the bioformulation preparation.

RESULTS AND DISCUSSION

The bacterial species associated with the roots of saffron plants were isolated and characterized for their plant growth promotion and biocontrol potential. In all, 91 isolates were obtained from all the growth media and these were then initially screened for siderophore production and phosphate solubilization. These are two important activities that have been reported to be important for plant growth promotion and are also associated with the biological control potential of the bacterial isolates. Out of 91 isolates only 8 showed atleast one of the two activities. These 8 isolates were then checked for their biocontrol potential against fungal pathogen and it was seen that only two isolates, *Bacillus aryabhattai* and *Bacillus amyloliquefaciens* had anti-fungal activity (Fig.1). These two isolates were then used for the preparation of Calcium carbonate based bioformulation which was then checked in pots for in-vivo analysis. The pot trial results obtained (Table 3) indicate substantial increase in the root and shoot length and number, corm weight, number daughter corm production and decrease in disease incidence. There disease incidence was reduced to 30% when compared to control samples which could be attributed to the anti-fungal activity of the bioformulation. The number of daughter corms in treated samples was approximately double as compared to control samples. The flower number, though, was not statically relevant. These preliminary results indicate that the bioformulation should be used in the fields for further evaluation and has the potential for commercializing for better grown and disease free saffron.

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CONFLICT OF INTEREST

The authors declare no conflict of interest with the publication of this work.

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