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Research Article

EFFECT OF PLANT GROWTH PROMOTING *Bacillus* Spp. AND *Pseudomonas* Spp. ON GROWTH, RHIZOSPHERIC POPULATION AND PHOSPHOROUS CONCENTRATION OF ASHWAGANDHA (*Withania somnifera*)

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Abstract- In the present study, *Bacillus* and *Pseudomonas* spp. from the rhizosphere of Ashwagandha were isolated and characterized for various plant growth promoting properties. Out of 30 screened isolates, total six isolates, three belonging to *Bacillus* spp. (WsNB-2, WsNB-6 and WsJB-13) and three belonging to *Pseudomonas* spp. (WsCP-1, WsCP-5 and WsCP-6) were selected and used in the pot experiment to study their effect on growth of Ashwagandha, rhizospheric bacterial population and phosphorus concentration of soil. On the basis of pot trial, *Pseudomonas* spp. (WsCP -1) and *Bacillus spp*. (WsNB -2) were found to be the best in positively influencing both plant and soil.
Keywords- Bacillus, Pseudomonas, Ashwagandha, Biofertilizers, Medicinal plants

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Introduction

Withania somnifera, also known as Ashwagandha or Indian ginseng or winter cherry, is a popular and traditional Indian medicinal herb, belonging to family Solanaceae. It possesses high medicinal value due to its antitumor, antiinflammatory, immunomodulatory, anti-ageing and rejuvenating properties [1]. Ashwagandha has high demand worldwide for its alkaloids and other phytochemicals thus overexploited from natural habitats whereas cultivation is comparatively low, because of which it has reached near extinction [2]. The use of synthetic agrochemicals in the cultivation of medicinal plants is highly delimited due to their detrimental effects on human health and environment. Thus, for meeting the continuously increasing demand of this medicinal plant by the pharmaceutical industries and simultaneously avoiding the use of agrochemicals, it is necessary to multiply these species through the use of bio-based technologies like plant growth promoting rhizobacteria (PGPR). Plant Growth Promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield by a wide variety of mechanisms and also offers an attractive way to replace chemical fertilizers, pesticides, and supplements [3]. A limited number of studies have been undertaken regarding the effect of PGPRs on medicinal plants. Thus, keeping in view the above information, in the present study, an effort was made to isolate indigenous plant growth promoting *Pseudomonas* and *Bacillus* strains from rhizosphere of Ashwagandha and study their effect on plant growth and microbiological properties and phosphorous concentration of soil.

Materials and Methods

Isolation and Screening of the bacterial isolates for multifarious plant growth promoting activities

Rhizospheric soil samples of Ashwagandha (*Withania somnifera*) were collected from various locations of Himachal Pradesh viz. University Forest Product medicinal plant field, University Research Center Neri (Hamirpur), Regional Horticultural Research station Jachh (Nurpur), Krishi Vigyan Kendra Saru (Chamba) and Baidynath medicinal field (Subathu). In total, twenty-seven isolates of *Bacillus* spp. and three isolates of *Pseudomonas* spp. were isolated on nutrient agar and Kings media respectively and were then screened for multifarious plant growth promoting activities such as phosphate solubilizing activity, IAA and biocontrol properties such as Siderophore production, Proteolytic activity, hydrogen cyanide (HCN) production, antifungal activity of bacterial isolates by their respective standard methods [5].

Evaluation of selected strains of *Bacillus* and *Pseudomonas* species for plant growth promoting activity in vivo (pot experiment)

On the basis of screening, total 6 bacterial strains, consisting of 3 isolates of *Bacillus* spp. (WsNB -2, WsNB -6 and WsJB -13) and 3 of *Pseudomonas* spp. (WsCP -1, WsCP -5 and WsCP -6), were further selected to study their influence on the growth of Ashwagandha, rhizosphere total bacterial population, and phosphorus concentration in soil of Ashwagandha in pot experiment. 15 days old rooted cuttings of Ashwagandha were treated with the inoculum density of 8x108 CFU/ml, by dipping in bacterial cultures for 15 minutes and were then planted in the pots. After 15 days of planting, the bacterial inoculum was again applied to the plant roots. After two months of planting, height (cm) of plants, rhizospheric bacterial population and phosphorous content of the soil were recorded [4].

Results

Selection of the bacterial isolates on the basis of multifarious plant growth promoting activities

On the basis of screening data [Table 1 and 2] pertaining to multifarious plant growth promoting activities of total 30 rhizospheric strains, 6 strains *viz*., WsNB -2, WsNB -6, WsJB -13, WsCP -1, WsCP -5 and WsCP -6), were selected for the pot trial on Ashwagandha.

Table-1 *Multifarious plant growth promoting properties of Bacillus spp. and Pseudomonas spp. isolated from rhizosphere of Ashwagandha*

Bacteria	Phosphate solubilization		Siderophore production		Auxins Conc. (µg/ml)	Proteolytic activity (Clear zone)
	Plate assay[1]	Quantitative [2] assay (ppm)	Plate assay[1]	% SU [3]		
Bacillus spp.						
WsCB-2	12.6	0.60	-	21.18	2	18.3
WsCB-4	13.3	0.80	-	-	4	14.3
WsCB-7	18.6	4.10	-	-	3	-
WsCB-8	07.3	0.30	-	-	5	19.3
WsHB-3	22.6	5.20	-	-	5	15.3
WsHB-4	14.3	2.60	-	31.42	2	14.3
WsHB-5	12.3	0.70	-	22.21	4	14.3
WsHB-6	11.3	0.60	-	23.35	4	17.3
WsHB-9	17.3	3.40	-	-	1	18.3
WsHB-10	21.6	6.40	-	17.32	5	-
WsJB-1	14.3	3.90	-	21.34	3	19.0
WsJB-2	15.6	2.80	-	19.65	7	15.3
WsJB-3	17.6	3.70	-	16.23	1	08.3
WsJB-4	16.0	3.40	-	-	11	17.6
WsJB-8	15.3	2.90	-	-	5	17.0
WsJB-11	07.6	0.20	14.3	32.67	2	18.6
WsJB-13	16.6	5.20	15.1	35.45	4	14.0
WsJB- 15	16.3	3.80	-	-	5	19.3
WsNB-2	16.6	5.20	13.3	32.17	7	15.3
WsNB-4	14.0	2.60	-	-	1	-
WsNB-5	12.0	0.60	-	-	3	17.3
WsNB-6	16.0	3.70	15.3	37.49	3	17.3
WsNB-7	13.6	0.30	13.6	30.12	3	17.6
WsSB-2	23.5	7.60	-	17.32	7	W+
WsSB-3	06.6	0.10	-	-	4	W+
WsSB-4	14.0	2.20	14.7	33.56	3	17.6
WsSB-5	17.6	3.90	13.0	28.34	7	15.3
Pseudomonas spp.						
WsCP-1	25.3	7.60	16.3	61.61	19	18.6
WsCP-5	25.3	7.40	17.3	62.08	1	18.3
WsCP-6	35.3	9.20	13.6	55.92	6	-

1 mm diameter of yellow zone / pinkish orange zone produced, 2 ppm of orthophosphate solubilization as calibrated from standard curve of KH2PO4, 3 percent reduction in blue colour as compared to reference

Table-3 *Comparison and effect of Bacillus and Pseudomonas Strains on the growth (height) of Ashwagandha seedlings in pot after 2 months of Plantation*

Treatment inoculum density (conc.) CFU/ml	Plant height						Mean
	Bacillus strain			*Pseudomonas* strain			
	WsNB-2 (T_1)	WsNB-6 (T_2)	WsJB-13 (T_3)	WsCP-1 (T_4)	WsCP-5 (T_5)	WsCP-6 (T_6)	
Control (0)	21.10	21.10	21.10	21.10	21.10	21.10	21.10
$8x10^8$ CFU	27.66	25.70	23.70	26.86	24.63	25.03	25.60
Mean	24.38	23.40	22.40	23.98	22.87	23.07	

$CD_{0.05}$: Treatment = 0.77; Bacteria = 0 .63; Treatment x Bacteria = 1.09

Table -4 *Effect and comparison of Bacillus and Pseudomonas strains on the rhizosphere bacterial population of Ashwagandha seedlings in pot after 2 Months of plantation.*

Bacillus strains Inoculum density CFU/ml	Rhizosphere bacterial population (10^8 ×CFU/g soil)		Mean	*Pseudomonas* Inoculum density (conc.) CFU/ml	Rhizosphere bacterial population (10^8 ×CFU/g soil))		**Mean**
	Before	After			Before	After	
Control (0)	108.0	148.0	128.0	Control (0)	108.0	148.0	128.0
WsNB-2 (T1)	225.7	277.7	251.7	WsCP-1 (T_4)	231.3	269.7	250.5
WsNB-6 (T2)	230.3	257.0	243.7	WsCP-5 (T_5)	209.7	229.3	219.5
WsJB-13 (T3)	187.0	228.7	207.8	WsCP-6 (T_6)	223.0	260.3	241.7
Mean	187.7	227.8		Mean	193.0	226.8	

$CD_{0.05}$: Treatment = 5.41; Bacteria = 3.83; Treatment x Bacteria = 7.65

$CD_{0.05}$: Treatment = 7.25; Bacteria = 5.13; Treatment x Bacteria = 10.25

Table-5 *Effect and comparison of Bacillus and Pseudomonas strains on phosphorus concentration of Ashwagandha in pot after 2 months of plantation*

Treatment inoculum density (conc.) CFU/ml	Phosphorus concentration (kg/ha)		Mean	Treatment inoculum density (conc.) CFU/ml	Phosphorus concentration (kg/ha)		Mean
	Before	After			Before	After	
Control (0)	429.5	445.2	437.3	Control (0)	431.2	450.3	440.7
WsNB-2 (T1)	436.7	490.9	463.8	WsCP-1 (T_4)	438.7	487.1	462.9
WsNB-6 (T2)	433.5	484.3	458.9	WsCP-5 (T_5)	434.9	479.5	457.2
WsJB-13 (T3)	433.9	476.3	455.1	WsCP-6 (T_6)	433.8	474.9	454.3
Mean	433.4	474.2		Mean	434.6	472.9	

$CD_{0.05}$: Treatment = 8.24; Bacteria = 5.82; Treatment x Bacteria = 11.65
$CD_{0.05}$: Treatment = 9.; 03Bacteria = 6.38; Treatment x Bacteria = 12.76

Table-2 *Antifungal activity, HCN and Ammonia production of Bacillus spp. and Pseudomonas spp. isolated from rhizosphere of Ashwagandha*

Bacteria	Antifungal activity (mm. dia)[1] Indicator test fungi					HCN Production[2]	Ammonia Production[3]
	Alternaria spp.	*Fusarium* spp.	*Pencillium* spp.	*Sclerotium* spp.	*Pythium* spp.		
Bacillus sp.							
WsCB-2	W +	W +	-	13.3	-	-	+++
WsCB-4	W +	-	-	-	10.3	-	-
WsCB-7	15.6	-	-	-	15.6	-	-
WsCB-8	11.6	-	-	17.6	10.6	-	-
WsHB-3	19.3	15.3	-	-	W +	-	-
WsHB-4	-	-	-	W +	-	-	-
WsHB-5	17.3	-	-	W +	W +	-	+
WsHB-6	13.3	-	-	-	-	-	++++
WsHB-9	19.6	16.3	-	-	-	-	-
WsHB-10	-	17.0	-	-	-	-	+++
WsJB-1	W +	-	-	-	-	-	-
WsJB-2	-	-	-	12.6	-	-	-
WsJB-3	15.2	-	-	-	-	-	-
WsJB-4	-	-	-	13.2	-	-	-
WsJB-8	-	-	-	18.0	-	-	-
WsJB-11	10.6	-	-	-	W+	-	++++
WsJB-13	21.3	16.3	-	-	-	-	++++
WsJB- 15	21.6	16.6	-	-	-	-	-
WsNB-2	17.0	14.3	-	13.6	13.6	-	+
WsNB-4	-	15.6	-	16.6	-	-	+
WsNB-5	-	16.3	-	W +	-	-	+
WsNB-6	20.3	17.3	-	W +	15.3	-	++++
WsNB-7	-	W +	-	13.6	-	-	-
WsSB-2	22.3	-	-	16.6	-	-	-
WsSB-3	-	-	-	15.3	-	-	+
WsSB-4	-	W +	-	13.6	-	-	-
WsSB-5	-	14.3	-	13.6	13.9	-	-
Pseudomonas spp.							
WsCP-1	-	+ 17.0	-	16.3	-	+++	+++
WsCP-5	16.3	-	-	-	14.2	+	++++
WsCP-6	21.6	+ 16.6	-	W +	-	+	-

- Indicates no activity; + Indicate activity; W+ Indicates weak activity

1 Antifungal activity expressed in terms (mm diameter) of clear zone produced around the bit at 28 ± 20C after 72h; 2 HCN production on king's media expressed in terms of change of colour of paper strips already dipped in Picric acid from deep yellow to orange brown Ammonia production expressed in terms of change of colour of culture broth from faint yellow to deep brown at 300C for 4days.

Effect of best selected isolates on various parameters of Ashwagandha after two months of plantation

Effect on growth (height) of Ashwagandha

From the data presented in [Table-3], it is evident that WsNB -2 resulted in maximum mean growth (24.38 cm) which was significantly superior from all other strains.

Effect on rhizospheric bacterial population

The effect of all 6 selected strains, on rhizospheric population of Ashwagandha as

presented in [Table-4], depicts that among *Bacillus* strains, WsNB-2 showed maximum increase in rhizosphere soil bacterial population with mean difference of 52 CFU/g soil, whereas, among *Pseudomonas* strains, WsCP -1 resulted in maximum increase in rhizosphere soil bacterial population with mean difference of 38.7 CFU/g soil.

Effect on phosphorus concentration of soil

The influence of 6 *Bacillus* and *Pseudomonas* strains on phosphorus concentration is presented in [Table-5]. WsNB -2 showed maximum increase in phosphorus concentration (436.7-490.9 kg/ha) after 2 months of plantation with mean difference of 54.2 kg/ha, whereas, *Pseudomonas* isolate WsCP -1 resulted in maximum increase in phosphorus concentration (438.7-487.1kg/ha) with mean difference of 48.4kg/ha.

Discussion

The majority of *Bacillus* and *Pseudomonas* isolates screened for the plant growth promoting activities showed positive results for phosphate solubilisation, auxin production, siderophore production, proteolytic activity and HCN production. Similar results have been reported extensively in previous research studies [6], [7], [8]. Under in vivo pot trial, the isolates showed significant increase in the growth in terms of plant height (cm), rhizobial bacterial population and phosphorous concentration which can be credited to the combination of mechanisms contributed by the bioinoculants. The plant growth benefits due to addition of PGPRs has also been reported by Cakmakci, (2005) [9] and Bushra, (2018) [10]. The beneficial effects of inoculation of the PGPRs *i.e*., *B. pumilus* and B. licheniformis on growth of forest plants (alder and pine) and herbaceous plants such as soybean due to production of auxins and gibberellins have also been reported by Gutierrez Manero, *et al*., (1996) [11]. Growth promotion of *Lupinus albus* seedlings by the inoculation of Aureobacterium has also been dedicated to the capacity to auxin and siderophore producing capacity of the strain [12]. In an experiment by Thanuja and Ambika, (2010) [13], bacterial isolates from rhizoplane of Holostemma ada-kodien- an endangered medicinal plant, were screened for their direct growth promoting activities (production of NH3, IAA and phosphate solubilization) and their efficiency was tested on the growth and dry matter accumulation. The results obtained showed overall improvement in the growth terms of shoot length over control. On the basis of present experimental study, *Pseudomonas* spp. WsCP -1 and *Bacillus* spp. WsNB -2 were found to be best for plant treatments to influence the growth of Ashwagandha.

Conclusion

The present study concludes that the plant growth promoting bacteria i.e. *Bacillus* and *Pseudomonas* are active solubilizers of insoluble phosphate and produce several growths promoting substances which are not readily available to the plant, thereby incurring beneficial effects on the Ashwagandha. Thus, the use of plant growth promoting rhizobacteria should be increased in agriculture because it offers an attractive way to replace chemical fertilizers, pesticides, and supplements.

Application of research

Taking into consideration, the negative impact of agrochemicals, the need of an hour is to substitute the chemicals with an environment friendly and sustainable solution. The *Bacillus* and *Pseudomonas* isolated in present study are effective biofertilizers and can be effectively used as biofertilizers in Ashwagandha, to restrain the use of chemical fertilizers.

Research Category: Biofertilizers, Medicinal plants

Abbreviations: CFU: Colony Forming Units

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