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Efficiency of Plant Growth-Promoting Rhizobacteria (PGPR) for the Enhancement of Ginger Growth

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Abstract Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides and supplements. Present studies based on isolation and characterization of PGPR from the rhizosphere soil of ginger field and their application for enhancement of ginger growth. Samples were collected from major ginger growing area i.e. Nauni, Kandaghat and Narag, Rajgarh in Solan and Sirmour districts of Himachal Pradesh. A total of 32 isolates were isolated by using modified replica plate method. On the basis of colony morphological similarities seven isolates, designated as KK_1, KK_2, KK_3, KK_4, KK_5, KK_6 and KK_7 were successfully isolated and characterized. Subsequently, to investigate the effects of PGPR isolates on the growth of ginger a pot culture experiment was conducted. Rhizome bacterization with KK_5 isolate not only showed significant increase in plant parameters such as shoot length (47.6% and 38.0%), number of tillers (46.9% and 48.9%), number of leaves (43.1% and 39.7%) and yields (48.3% and 43.5%) over C_1 (uninoculated absolute control) and C_2 (uninoculated fungicide control) treatments, respectively but also increased available N, P and K by 26.5%, 22.9% and 18.8% respectively over initial contents. Under net house studies the bacterial isolate KK_5 has good potential to be used as biofertilizer or biocontrol agent for ginger production in mid hills conditions of Himachal Pradesh.

Keywords Ginger, PGPR, Rhizome, Phosphate solubilization.

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Introduction

Ginger (*Zingiber officinale* Rosc.), member of family Zingiberaceae is a selender perenial tuber crop consumed whole as a delicacy and spice. The characteristic odor and flavor of ginger is due to a mixture of zingerone, shogads, gingerols and volatile oil [1]. India is a leading producer of ginger in the world. It is grown in an area of 1.06 lakh hectares with a production of 3.70 lakh tonnes mainly in the states like Kerala, Meghalaya, Sikkim, Orissa, Arunachal Pradesh, Mizorum, Nagaland, Orissa, Himachal Pradesh together contributes 70% to the country's total production. Ginger can be grown in well drained, medium fertility loam soils with pH value ranges from 6–7 [2]. In Himachal Pradesh, it is grown as rain fed crop at an altitude of 600—1200 m above mean

Table 1. Morphological characteristics of rhizospheric and endophytic bacterial isolates of ginger roots. *Endorhizobacteria.

Isolates	Form	Elevation	Margin	Surface	Gram's reaction	Shape
KK_1	Circular	Raised	Undulate	Smooth	+	Rods
KK_2	Irregular	Flat	Entire	Smooth	+	Rods
KK_3*	Circular	Raised	Entire	Smooth	+	Rods
KK_4*	Circular	Convex	Erosed	Rough	+	Rods
KK_5*	Irregular	Circular	Curled	Smooth	+	Rods
KK_6*	Rhizoida	Umbonate	Lobate	Smooth	+	Rods
KK_7*	Circular	Convex	Entire	Rough	+	Rods

sea level [3]. Ginger growing soils of the state are, in general, deficient in nitrogen and having problems of availability of phosphorus nutrition. Further, low availability of FYM / compost, other chemical fertilizers and problems of diseases like *soft rot* and *fusarium wilt* of ginger have emerged as the major bottle necks for low productivity and quality production of the crop. In the present scenario of organic production of crops the application of PGPR is the effective, ecofriendly, low volume, supplementing source of nutrients not only for sustaining/increasing crop yields but also to sustain soil health [4]. These rhizobacteria are referred to as endophytes and in order to invade roots they must first be rhizosphere competent. PGPR's are also known to produce plant growth promoting compounds including phytohormones ; auxins, cytokinins and gibberellins as well as siderophores [5]. Since there is no commercial biofertilizers PGPR formulations for the ginger crop, therefore, there is an urgent need to develop an effective inoculum of plant growth promoting rhizobacteria (PGPR) which can solubilize phosphorus, fix atmospheric nitrogen and also act as anti-fungal agent to protect the crop from soil borne pathogens [6]. Present study was focused to isolate and characterize the PGPRs and later these were evaluated for their establishment and growth of ginger both under nursery and field conditions.

Materials and Methods

The rhizospheric soil and roots of ginger (*Zingiber officinale* Rosc.) plants were collected from Solan and Sirmour districts of Himachal Pradesh. On the basis of morphological, cultural and biochemical characteristics and as per the criteria of Bergey's Manual of Systematic Bacteriology [7], the selected isolate was identified.

Screening of bacterial isolates

The screening of the bacterial isolates for various

Table 2. Screening of selected bacterial isolates for multifarious plant growth promoting activities. *Values ranging from 40–70% (++), ≤ 40% (+), ≥70% (+++), no activity (–), ** Values ranging from 20—40 (++), ≤ 20 (+), ≥ 40 (+++), no activity (–).

Isolates	P-Solubilization	N-free medium	IAA production**	Siderophore production*	HCN production	Antagonistic activities**	Phythium sp.	Fusarium sp.
KK_1	+++	+	+++	+	_	++	++	++
KK_2	++	++	++	+	+	++	–	++
KK_3	+++	+++	++	++	+++	++	++	+++
KK_4	++	+	+	++	+	++	++	–
KK_5	+++	+++	+++	+++	++	+++	+++	+++
KK_6	+++	+++	++	+++	++	+++	+++	+++
KK_7	++	++	++	++	+	++	–	+++

Table 3. Effect of of bacterial inoculums on (Zinger) plant parameter under net house conditions. *C_1–Uninoculated absolute control ; **C_2–Fungicide treated control.

	Plant parameters		
Isolates	Shoot length (cm)	Number of tillers	Number of leaves/ plant
*C_1	54.60	1.00	15.67
**C_2	58.40	2.00	16.67
KK_3	78.40	4.66	21.00
KK_4	70.63	3.00	19.00
KK_5	80.63	5.66	24.33
KK_6	79.57	4.33	22.00
CD 0.05	0.37	0.72	2.05

Table 4. Effect of bacterial inoculum on fruit parameters under net house conditions. *C_1–Uninoculated absolute control ; **C_2–Fungicide treated control.

Isolates	Weight of rhizome/ plant (g)	Yield/ Plant (kg)	% increase in yield/ plant over C_1	% increase in yield/ plant over C_2
*C_1	58.62	0.16	0.00	– 4.25
**C_2	60.63	0.17	4.44	0.00
KK_3	80.67	0.23	45.55	38.33
KK_4	72.50	0.21	29.56	25.33
KK_5	81.37	0.24	48.39	43.48
KK_6	78.37	0.23	42.93	38.40
CD 0.05	0.38	0.01	0.54	0.49

plant growth promoting activities like P-solubilization, siderophore, HCN growth on N-free medium, auxin production and antagonism against *Pythium* spp. and *Fusarium* spp. were performed by adopting the standard methods [8—11].

Evaluation of effective PGPRs isolates on growth and yield of ginger

Four Bacterial isolates i.e. KK_2, KK_3, KK_4, KK_5, KK_6 and KK_7 were selected for net house experiments on the basis of their multifarious plant growth promoting activities.

Preparation of liquid formulation for rhizome treatment

Rhizome of ginger (*Zingiber officinale* Rosc.) was procured from Seed Technology and Production Center, Dr YS Parmar University of Horticulture and Forestry Nauni, Solan (HP). The population density (1.5 OD at 540 nm) that resulted in formation of 10^8 cfu/ml of bacterial isolates were used for preparation of liquid formulation [12].

Preparation of potting mixture

The potting mixture was prepared by mixing sand, soil and farm yard manure (FYM) in a ratio of 1 : 1 : 2. The mixture was then filled in the pots and moistened to one third of its maximum water holding capacity. Healthy rhizome having at least two well differentiated buds dipped in the bacterial cell suspension of 1.5 OD were sown at a uniform depth of 2 cm in each pot. After 2 months rhizome was sprouted.

Physico-chemical properties available nutrients and microbiological status of potting mixture

Potting mixture was analyzed before and after the treatments for important physico-chemical and available nutrient status by adopting their standard protocols [13—16]. Organic carbon, available nitrogen, available phosphorus and available potassium was determined by standard protocols The soil was analyzed for total microbial count by standard pour plate technique. The population was expressed as colony forming units (cfu/g soil).

Plant growth parameters

Plant growth promoting activities in terms number of nodes/leaves, number of tillers, shoot length and rhizome yield per plant comparison to control or untreated plants. The fresh weight of all the plants per plot after removing the aerial part, roots and soil were recorded in g/plant.

Experimental design and statistical analysis

The results under laboratory and glass house condi-

Table 5. Physico-chemical properties, available nutrient content and total bacterial counts of soil mixture used for net house experiment (initial status).

Sl. No.	Parameters	Values
1.	Physico-Chemical Properties	
a.	pH (1 : 2–5)	7.56
b.	Electrical conductivity (dSm^{-1})	0.49
c.	Organic carbon (%)	1.25
2.	Nutritional status [Available nutrients (kg/ha)]	
a.	N	315.6
b.	P	28.3
c.	K	235.2
3.	Total Bacterial Count	
a.	NA (× 105 cfu / soil)	96.8
b.	SEM (× 105 cfu / soil)	110.7

tions on various parameters were subjected to statistical analysis as per method outlined by Gomez and Gomez [17]. The CD at 5% and 1% level was used for testing the significant difference among the treated means. Laboratory experiments were analyzed under completely randomized design (CRD) with three replications. Statistical significance of the data was determined using analysis of variance (ANOVA).

Results and Discussion

Ginger (*Zingiber officinale* Rosc.) occupies considerable value because of its importance in agricultural economy of the country. In Himachal Pradesh, it ranks as one of the most important cash crops particularly in mid-hills. Ginger being heavy feeder and exhaustive crop requires large quantities of inorganic and organic fertilizers. Inorganic or chemical fertilizers besides being costly are also injurious to plants, ground water and environment [18]. Biofertilizers have emerged as promising components of nutrient supply system. It is not only low-cost input but also gives high returns under favorable conditions [19].

In present investigation on the basis of morphological characteristics (form, elevation, margin, surface and shape), Gram's reaction, cell shape and arrangement, phosphate solubilization, IAA production, siderophore production and growth on N-free medium, only seven isolates were selected from total of 32 purified isolates isolated from rhizosphere and roots of ginger (Table 1). Seven isolates namely (KK_1, KK_2, KK_3, KK_4, KK_5, KK_6 and KK_7) exhibited the maximum plant growth promoting attributes viz. phosphate solubilization, siderophore production, growth on N-free medium, auxin production and HCN production. Out of seven isolates, only six isolates were HCN-producers. Six isolates showed antagonism against *Pythium* spp. and only five isolates inhibited the mycelial growth of *Fusarium* spp. under *in vitro* conditions (Table 2). Since the discovery of the PGPR many studies have been made all over the world to evaluate the mechanisms by which these bacteria renders benefits to the plant [20]. The rhizome treatment with different bacterial cultures under net house conditions increased shoot length (47.68%), numbers of leaves/plant (55.12%) and numbers of tillers (183.3%)

Table 6. Effect of bacterial inoculum on physico-chemical characteristic and available nutrient status of soil. *C_1–Uninoculated absolute control ; **C_2–Fungicide treated control.

	Pysico-chemical properties of soil					
Isolates	pH (1 : 2.5)	EC (dSm^{-1})	OC (%)	N (kg/ha)	F (kg/ha)	K (kg/ha)
*C_1	7.68	0.42	0.89	290.3	33.15	251.4
**C_2	7.85	0.39	1.13	305.3	34.12	262.4
KK_3	7.85	0.46	1.24	323.2	37.20	277.5
KK_4	7.75	0.37	1.14	317.5	36.66	267.6
KK_5	7.91	0.44	1.38	330.3	39.73	279.6
KK_6	7.71	0.41	1.02	327.0	38.34	275.6
CD 0.05	NS	NS	NS	0.28	0.34	0.39

Table 7. Rhizosphere and endophytic bacterial population associated with ginger at the end of experiment.

Isolates	Rhizosphere population (× 10^5 cfu soil)		Endophytic bacterial population (×10^1 cfu/g root)	
	[1]NA	[2]SE	[1]NA	[2]SE
*C_1	125.2	160.6	81.20	97.60
**C_2	114.4	155.2	77.60	73.20
KK_3	178.4	196.6	120.6	122.8
KK_4	125.4	190.2	98.20	94.20
KK_5	176.6	197.6	127.2	129.4
KK_6	178.4	192.8	121.8	123.4

Isolates	Rhizosphere population (× 10^5 cfu soil)		Endophytic bacterial population (×10^1 cfu/g root)	
	[1]NA	[2]SE	[1]NA	[2]SE
*C_1	125.2	160.6	81.20	97.60
**C_2	114.4	155.2	77.60	73.20
KK_3	178.4	196.6	120.6	122.8
KK_4	125.4	190.2	98.20	94.20
KK_5	176.6	197.6	127.2	129.4
KK_6	178.4	192.8	121.8	123.4

over C_1 (uninoculated absolute control), C_2 (uninoculated fungicide control) (Table 3). The possible reason for enhanced growth and development may be because of better plant stand which is due to direct contribution of PGPR in improving the nutrient status of soil particularly for N, P and Fe. These findings are in conformity with those of Cleyet Marel et al. [21] for vegetable crops particularly tomato and pea. The present results are further supported by significant positive correlation between rhizospheric bacterial population and shoot length (r = 0.87), rhizospheric bacterial population and number of leaves (r = 0.89) and rhizospheric bacterial population and number of tillers (r = 0.86). A significant increase was recorded in rhizome yield 48.39% by the application of KK_5 isolate over C_1 (uninoculated absolute control) and C_2 (uninoculated fungicide control) (Table 4). The present results are supported by significant correlation between rhizospheric bacterial population and weight of rhizome per plant (r = 0.83) and rhizospheric bacterial population and rhizome yield per plant (r = 0.92). These results are further, in agreement with the findings of Sturzand Nowak [18] in potato (Fig. 1). The increased growth by *Bacillus* spp. (KK_5) inoculation may be attributed to the cumulative effects of phosphate solubilization nitrogen fixation and production of plant growth regulators (auxins) and the inhibition of soil borne pathogens particularly *Pythium* spp. and *Fusarium* spp. Further it was interesting to note that no disease symptoms appeared throughout the experimental period bacterial in treated plant as compared to control plants. A significant reduction have also been reported in indigenous bacterial and fungal populations by the application of effective *Bacillus* inoculum in the rhizosphere of potato [22]. The indirect plant growth promoting effects of PGPR (*Bacillus* and *Pseudomonas*) to inhibit deleterious microorganisms have been reported by various researchers.

Availability of nutrients in soils is greatly enhanced through microbial activities which may lead to lowering of pH and release of organic acids to solubilize inorganic complexes present in the soil matrix [23]. The rhizome inoculation with different bacterial isolates not only improved the nutritional content particularly NPK of plants but also increased their uptake significantly over uninoculated controls (C_1 and C_2). The maximum N (105.5 mg/plant), P (84.4 mg/plant), K (149.3 mg/plant) uptake was recorded by application of isolate (KK_5). This increase may be attributed to atmospheric nitrogen fixation, phosphate solubilization in the rhizosphere and increase in root length which might have explored the virgin horizons of soil for absorption of nutrient elements. Phosphate solubilizing bacteria are common in the rhizosphere and the solubilization of P in the rhizosphere by PGPR through organic acid secretion and phosphate production facilitated the conversion of insoluble forms of P to plant available forms and ultimately increased availability to host plant [24]. The data on physicochemical characteristic of soil mixture (initial and at the termination of the experiment) showed that soil was slightly alkaline in reactions pH (7.68–7.91) and EC was in normal range (0.38–0.46 dSm^{-1}). The OC content (0.89–1.38%) was in high range. The application of KK_5 isolate inoculum significantly increased the available NPK content of the soil at the termination of experiment i.e. after 6 months. The increase

was by 27, 19 and 23%, respectively over initial NPK content of the soil. However, there was no significant effect of the inoculation on physic chemical properties (Tables 5 and 6). The maximum endophytic microbial counts on NA and SE medium were (127.2 × 101 cfu/g roots) and (129.4 × 101 cfu/g roots), respectively. However, their respective minimum (77.60× 101 cfu/g roots and 73.20 ×101 cfu/g roots) counts was recorded in C_2 treatment (uninoculated fungicide control) (Table 7).

The KK_5 isolate had showed maximum plant growth promoting attributes and excel all other tried isolates to enhance shoot length, number of leaves, number of tillers and rhizome yield and also increased available nutrients (NPK) content, microbial population (endophytic and rhizosoheric). The KK_5 isolate tentatively identified under *Bacillus* spp. is most predominant colonizer of roots and rhizosphere of ginger plant and has good prospects to be used as biofertilizers and biocontrol agents not only for enhanced growth and rhizome production but also to sustain soil health under mid hill conditions of Himachal Pradesh. Therefore, they are potential candidates for the development of biofertilizer and bioinoculants for crop plants. The world over is changing from inorganic conventional farming towards organic ecofriendly farming methods. This not only requires the isolation of bioinoculants with high potential for use as biofertilizers but also several other factors right from appropriate application procedures to correct marketing practices also being economically cheaper.

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