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Morphological metabolic and biochemical characterization of bacterial root endophytes associated with brown sarson (*Brassica rapa* L.)

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Abstract
In the present investigation root samples of brown sarson (*Brassica rapa* L.) were collected from three districts of Kashmir valley viz., Anantnag, Srinagar and Baramulla. The bacterial population per gram of all the root sample was determined and it was observed that one of the site Hugam in the District Anantnag had the highest bacterial population of 4.35×10^5 cfu/g of fresh weight) and site Nowgam in the District Srinagar had the lowest bacterial population of 6.8×10^3 cfu/g of fresh weight). A total of 81 morphologically dissimilar isolates were selected and characterized on Gram's staining, cell and colony morphology basis and it was observed that Gram negative bacteria formed the dominant group. The colony characterization revealed that circular forms dominated, likewise the colonies with entire margins and convex elevation dominated among all the isolates. The metabolic properties of isolated bacterial root endophytes revealed that all the isolates can metabolize glucose and galactose but some isolates did not metabolize maltose and sucrose respectively. All the isolates were screened for indole acetic acid (IAA) production and it was observed that 44 isolates among all produced IAA with the average production of 8.15 µg/mL.

Keywords: Endophyte, *Brassica rapa*, IAA, population

1. Introduction
In nature, many microorganisms play a very domineering role in agriculture by improving the soil health and supporting plant growth through nutrient and growth hormonal supplementation thereby engineer ecofriendly relation between plant and soil. The association of plants with the microorganisms that do not suppress or even stimulate plant development has attracted much attention in recent times because of their possible use in the practice of an environmentally oriented production of agricultural products (Chebotar *et al*., 2015) [3]. Several bacteria, fungi and actinobacteria reside in plant tissue intra- or inter-cellularly or may even remain outside the plant either in phyllosphere (epiphytes) or rhizosphere (rhizobacteria) and support plant growth and soil health. The microbes which reside inside the plant tissues is known as endophyte (Orole and Adejumo, 2011) [23]. The mechanisms of plant growth-promotion known to be employed by bacterial endophytes are similar to the mechanisms used by rhizospheric bacteria, e.g., the acquisition of resources needed for plant growth and modulation of plant growth and development (Santoyo *et al*., 2016) [26]. Bacterial endophytes ubiquitously colonize the internal tissues of plants almost in all the plants and generally promote plant growth (Santoyo *et al.,* 2016) [26]. Presence of bacterial endophytes has been reported from many cultivated and flowering plants including rice, wheat, maize, sugarcane, potato, alfalfa, bean, chickpea, mung bean, clover, cowpea, pea, peanut, soybean, carrot, citrus plants, banana, *Acacia*, *Argyrolobium*, *Conzattia*, Fenugreek, *Hedysarum*, *Kennedia*, *Leucaena*, *Lotus*, *Mimosa*, *Medicago*, *Melilotus*, *Ornithopus*, O*nobrychis*, *Oxytropis*, *Psoralea*, *Scorpiurus*, *Sesbania*, *Tetragonolobus* and *Vicia* (Muresu *et al.,* 2011) [20]. Endophytes have been isolated from almost all plant parts, including fruits, leaves, stems, seeds, nodules and roots (Hung *et al*., 2007) [7]. The population of endophytes inside plants varies from plant to plant and even some endophytic bacteria show tissue specificity. However, mostly the endophytic population in plant tissues ranges between 5.6×10^3 and 6.9×10^5 cfu/g (Vega *et al*., 2005) [32]. Similarly, there is large variation in colony morphology of different isolates from different crops and the differences were observed in colony color, shape and size (Hung *et al*., 2007) [7]. Sgroy *et al*. (2009) [27] reported the presence of 68.9% Gram positive bacteria and 31.1% Gram negative bacteria in the root of *Prosopis strombulifera*. Panchal and Ingle (2011) [24] reported that 91.6% root endophytes were

Gram positive although Vetrivelkalai *et al*. (2010) [34] found Gram negative and Gram positive endophytic bacteria in almost equal proportion in the roots of different crops. Lopez *et al*. (2011) [16] reported all the bacterial root endophytes from cactus were Gram negative except one and these endophytes also show host specificity and even in some cases cultivar specificity. Jonathan *et al*. (2013) [9] reported that there are three main mechanisms that drive root endophyte community structure: (1) soil factors that determine survival, (2) plant factors that determine colonization and compatibility, and (3) microbial factors that determine the ability of the endophyte to survive and compete within the root.

2. Materials and Methods

Collection of root samples

A survey was conducted to collect representative root samples of apparently healthy brown sarson (*Brassica rapa* L.) plants from three districts of Kashmir valley *viz.* Anantnag, Srinagar and Baramulla. The samples were randomly collected from two blocks of each selected district. Three villages were chosen per block. In the Anantnag district the villages chosen were Akura, Bona Nambal from block Dachnipora, Hutmara, Panzmulla and Rakh Chandipora from block Khoverpora. In the Srinagar District the villages chosen were Rawalpora, Rangreth, Khunmoah from South Srinagar and Zakura, Gulab Bagh, Ahmad Nagar, Dhara, Tailbal and Batapora from North Srinagar. Three sites were chosen from each village to collect root samples. The sampling was done at peak flowering stage of the crop.

Isolation of root endophytic bacteria

The samples were collected in polythene bags and immediately shifted to laboratory for further studies. The fresh healthy root samples from each site were cut and surface sterilized by 1% (w/v) active chloride (added as a sodium hypochlorite [NaOCl] solution) (Vincent, 1970) [35]. The roots were then crushed in a sterilized petri plate and a loopful of root sap was streaked on TSA plates. Simultaneously, from each batch, uncrushed root sample were kept on TSA medium plates as a control to ensure proper surface sterilization of root samples. The plates were incubated at 28±2 ^{0}C and growth was observed daily for 2-3 days. Well established endophytic bacterial colonies were picked and restreaked on TSA medium for purification. The isolates were maintained on TSA slants at 4 ^{0}C in a refrigerator till further studies. For determining endophytic bacterial population of root samples, fresh root samples (one gram from each sample) were first vigorously washed in distilled water for 5 min, surface sterilized for 5 min in a solution containing 1% (w/v) active chloride (added as a sodium hypochlorite [NaOCl] solution) supplemented with 1 droplet Tween 80 per 100 ml solution, and rinsed three times in sterile distilled water. These roots were then crushed in a sterilized petri plate and population was determined using multiple tube dilution plate technique (Johnson, 1957) [8]. Eight test tubes (25 ml capacity), were taken and 9 ml sterilized distilled water poured into each test tube. One gram of crushed plant sample was transferred into the first test tube containing 9 ml sterilized distilled water. Which gives the dilution of 10^{-1}. One milli liter of the suspension was transferred from 10^{-1} dilution into 2nd test tube in order to get dilution of 10^{-2}. Similarly 1 ml of suspension was serially transferred from dilution 10^{-2} to 10^{-3}, 10^{-3} to 10^{-4}, 10^{-4} to 10^{-5}, 10^{-5} to 10^{-6}, 10^{-6} to 10^{-7} and 10^{-7} to 10^{-8}. From dilution 10^{-8}, 0.1 ml was poured into petri plates containing 20 ml TSA medium. The plates were incubated at 28±2 ^{0}C in a BOD incubator for 2-3 days. The plates were observed daily. Visible bacterial colonies were counted using colony counter and population was calculated by using the formula:

CFU/g plant sample = No. of colonies × 10 × dilution factor

Morphological characterization of isolated endophytic bacteria

The colony morphology was studied on plates after streaking a loopful of isolated colony and colony color, colony size, colony texture and gum production were observed. The bacterial isolates were Gram stained. A smear was prepared from isolated colonies and stained with Gram's stain. Slides were observed under Geytnor microscope at 100X. Cell shape, size, Gram's reaction were observed and these were photographed.

Carbohydrate metabolization

The bacterial isolates were inoculated in phenol-red nutrient broth containing different sugars *viz*. glucose, fructose, maltose, sucrose and lactose separately. The broths were incubated for three days at 25-26 °C. Colour change in the medium from red to yellow was a positive test for carbohydrate metabolization (Bakker and Schipper, 1987) [2].

Estimation of Indole-3-acetic acid (IAA)

Indole-3-acetic acid (IAA) was estimated as per Salkowski's method (Tang and Bonner, 1974) [31]. Selected endophytic bacterial isolates were inoculated in 25 mL of LB broth without supplemented with L-tryptophan. The flasks were incubated at 28±2 ºC in a shaking BOD incubator. After 4 days of incubation, 2 mL culture broth was centrifuged at 7,000 rpm for two minutes and then IAA was determined in culture supernatant by following method:

To 2 ml supernatant, an equal volume of Salkowski's reagent [1 mL of 0.05 M $FeCl_3$ in 50 mL of 35% of perchloric acid ($HClO_4$)] was added. The contents were mixed by shaking and allowed to stand at room temperature for 30 minutes for the development of pink color which was estimated spectrophotometrically at 500 nm. Indole-3-acetic acid was used as a standard (100 mg mL^{-1} IAA in 50% ethanol).

3. Result and Discussion

A total of 22 villages were selected and from three districts *viz*. Anantnag, Srinagar and Baramulla, from each village three random sites were selected for root sampling, roots of all the plant samples collected harbored bacteria capable of growth on the TSA media (Table 4.1). The bacterial population was highest at Hugam in the district Anantnag (4.38×10^5 cfu/g of FW) and lowest at Nowgam in district Srinagar (6.8×10^3 cfu/g of FW). A total of 81 morphologically dissimilar endophytic bacterial isolates were isolated from brown sarson root samples. Amongst the most apparently morphologically similar isolates only one isolate was selected. All the individual colonies of endophytic bacterial isolates were then purified and maintained on TSA slants in triplicates at 4 ^{0}C for further studies. Thirty seven, twenty four and twenty isolates were obtained from district Anantnag, Srinagar and Baramulla, respectively. Nomenclature given to these endophytic bacterial isolates was on the basis of the crop from which these were isolated, as detailed in Table 1. Our results are in agreement with population ranges in previous studies of endophytic bacteria in many crops viz. cotton, sweet corn etc. (Li *et al.,* 2010; McInroy and Kloepper, 1995) [14, 18]. The variation observed in the population is attributed to many factors, as already pointed

out by Mocali *et al.* (2003) [19], the bacterial populations underwent strong fluctuations that were dependent upon the type and physiological status of the host plants, the soil conditions like organic matter, nutrient status, plant source, plant age, tissue type, time of sampling, and environment. Significant variations in the populations of both indigenous and introduced endophytes have been reported (Dawwam *et al*., 2013) [4]. Natural endophyte concentrations can vary between 5.67×10^3 and 6.0×10^5 cfu per g for alfalfa, sweet corn, sugar beet, squash, cotton, and potato, as described by Kobayashi and Palumbo (2000) [12]. Similar results were obtained for endophytic bacteria inoculated by root or seed drenching, with the population levels reaching between 4.03×10^3 and 6.23×10^5 cfu/g of plant tissue for tomato and potato (Kobayashi and Palumbo, 2000) [12]. The levels of colonization by nonpathogenic endophytes tend to be far less than the levels of colonization by pathogenic bacteria; the concentrations of the latter organisms range from 7.08×10^9 to 4.05×10^{14} cfu/g (fresh weight) of tissue in susceptible infected plants (Grimault and Prior, 1994). Mocali *et al.* (2003) [19] attributed increased endophytic population to higher levels of organic matter in the soil and this could be one of the possible reason in our findings because the rural areas apply a very good organic matter to the soil than the urban areas which leads to enhanced microbial population in the soil and ultimately those finding their way into plant tissues had already build a strong population base in the rich organic matter soil and resulted into very good population build up in the tissues may be one of the strong reasons for higher population at site Hugam than the Nowgam. Morphological characterization of endophytic bacterial isolates revealed that the colonies varied from gummy to non-gummy, colony forms varied from circular to irregular, colony margins varied from entire, serrate and lobate, colony elevation varied from flat to raised, convex and umbonate with different colors, 62.96% were gram negative, 58% were circular in form, 60.5% possessed entire margins, 38.3% possessed convex elevation. Among all the isolates 67.9% were rod shaped and 25.9% were cocci in shape (Table 2). Shi *et al*., (2009) isolated 221 bacterial root endophytes from sugar beet, while Panchal and Ingle (2011) [24] isolated 12 isolates from safed musli, similarly Sgroy *et al*. (2009) [27] isolated 29 bacterial endophytes from *Prosopis strombulifera* and Muthukumar *et al*. (2010) [21] isolated 5 from chilli.

The bacterial isolates were diverse in their colony characteristics viz. color, texture, secretions, forms, margins, elevations etc. Colony secretions varied from gummy to non-gummy, colony forms varied from circular to irregular, colony margins varied from entire, serrate to lobate, colony elevation varied from flat, raised, convex to umbonate with different colors- light yellow, white, brown, orange, faint white, sharp white, waxy white, deep orange etc. In agreement with our findings bacterial endophytic colonies from sweet potato roots were of similar morphology, round shaped, and color white and pale to bright yellow (Khan *et al*., 2009) [11]. Similarly, there was a large variation in colony morphology of different isolates from soybean, differences were observed in colony—color, shape, and size (Hung *et al*., 2007) [7]. In present study Gram negative bacteria predominated i.e. 51 out of 81 isolates (62.96%), circular forms (58.02%), entire margins (60.49%), convex elevation (38.27%) and rod shape (67.90%) predominated among all the isolates, similar to our findings, Liu *et al*. (2014) [15] reported the existence of thin flat, faint yellow, opaque, round with smooth edge colonies among endophytic bacteria. Gupta *et al*. (2015) [6] reported the similar findings on colony shape, color, margins, elevation and gram staining. Similar observations have been reported in terms of cell shape, colony elevations, color and margins by other workers (Sgroy *et al.,* 2009*;* Pandey *et al*., 2015) [27, 25]. Though, Vetrivelkalai *et al*. (2010) [34] found equal percentage of Gram negative and Gram positive endophytic bacteria from roots of different crops, while Lopez *et al*. (2011) [16] reported all the bacterial root endophytes from cactus to be Gram negative except one. Contrary to our findings of dominance of Gram negative bacterial endophytes Sgroy *et al*. (2009) [27] reported 68.9% Gram positive bacteria and 31.1% Gram negative in the root of *Prosopis strombulifera*. While, Panchal and Ingle (2011) [24] found 91.6% root endophytes to be Gram positive. However, Zinniel *et al.* (2002) [36] reported that among the endophytic bacteria Gram-negative bacteria outnumber the gram positive bacteria in most of the agronomic crops, which supports our findings. Similar findings were shared by Mbai *et al*. (2013) [17].

The metabolic properties of isolated bacterial root endophytes revealed that all the isolates can metabolize glucose and galactose but a total of 16 and 21 isolates among all the isolated 81 bacterial root endophytes did not metabolize maltose and sucrose respectively. In the same way there were 47 isolates among all the isolated bacterial root endophytes which were capable of metabolizing all the tested carbohydrates viz. glucose, galactose, maltose and sucrose (Table 3). Kumar *et al*. (2016) [13] while characterizing the bacterial endophytes associated with *Curcuma longa* L. observed a huge diversity in isolates for metabolizing the various carbon sources, for instance all the isolates metabolized glucose, 50% isolates metabolized maltose and 66.6% metabolized sucrose. Singh *et al*. (2013) while investigating the diversity in metabolization of various carbohydrates by bacterial root endophytes, observed the similar findings. Similar findings were also reported by various researchers (Pandey *et al*., 2015) [25]. The endophytic bacteria grow on a wide variety of carbohydrates depending upon the metabolic pathways they follow and accordingly the expression of these genes leads to the catabolism of a particular or a series of carbohydrates via, tricarboxylic acid cycle, the Entner-Doudoroff, the Embden-Meyerhof-Parnas and the pentose-phosphate pathways (Taghavi *et al.,* 2009) [30]. This could be the possible reason for selective metabolism of different carbohydrates by the isolated endophytic bacteria.

All the 81 bacterial root endophytic isolates were screened for IAA production in Luria Bertani broth without supplementation of L-tryptophan and only 54.3% (44 isolates) produced IAA with the overall average production of 8.15 µg/mL. The highest IAA production (19.54 µg/mL) was observed in isolate SB28 and lowest (2.2 µg/mL) in SB70 (Table 4). All the isolates differed significantly from each other in IAA producing ability, which is in accordance with Verma *et al.* (2012) [33] and Khamna *et al.* (2009) [10] who reported that 56% isolated endophytic bacteria produced IAA, the production varied from 1 to 23 µg/mL. Nimnoi and Pongslip, (2009) [22] reported that IAA synthetic bacteria enhanced root and shoot development of *Raphanus sativus* and *Brassica oleracea* more than five-fold when compared with control. The endophytic actinomycetes present inside root tissues produce IAA that may play an important role in host plant development and growth. Khan and Doty (2009) [11] observed that 33.36% of the isolated bacterial endophytes produce IAA but Mbai *et al*. (2013) [17] reported that only 14% of the bacterial root endophytic isolates produced IAA. Afzal *et al*. (2015) [1] reported that all the endophytic bacterial

isolates produce IAA which ranged from 0.2-5.1 µg/ml in the absence of tryptophan, the precursor of IAA.

Table 1: Bacterial population obtained from brown sarson (*Brassica rapa* L.) root samples collected from various sites

District	Block/Zone	Village	Bacterial population 10^2×Log10 (cfu/g of fresh weight)	Isolates obtained	
				Number of isolates	Code
Anantnag	Dachnipora	Akura	5.321 to 7.767	9	SB1, SB2, SB3, SB15 SB16, SB51, SB9, SB51, SB9
		Bona Nambal	5.294 to 8.209	6	SB22, SB11, SB23, SB14, SB33, SB27
		Hugam	7.959 to 8.386	4	SB10, SB30, SB32, SB48
	Khoverpora	Hutmara	6.602 to 7.226	9	SB19, SB25, SB58, SB63, SB39, SB40, SB12, SB24, SB73
		Panzmulla	5.287 to 5.839	7	SB26, SB34, SB35, SB17, SB18, SB43, SB41
		Rakh Chandipora	6.082 to 7.226	4	SB13, SB73, SB42, SB44
Baramulla	Tujar	Tujar	5.305 to 6.003	4	SB31, SB6, SB67, SB68
		Bomai	6.110 to 7.001	3	SB49, SB70, SB55
		Brath	5.025 to 6.391	7	SB46, SB47, SB48, SB1, SB6, SB71, SB56
	Baramulla	Juhama	7.323 to 8.004	8	SB72, SB74, SB11, SB17, SB16, SB54, SB26, SB23
		Kanispora	6.093 to 7.776	4	SB80, SB59, SB65, SB79
		Fateh Pora	6.329 to 7.320	4	SB28, SB81, SB75, SB78
Srinagar	South Srinagar	Nowgam	4.227 to 4.329	4	SB38, SB50, SB4, SB5
		Rawalpora	5.045 to 7.331	3	SB20, SB21, SB13
		Rangreth	5.949 to 6.306	5	SB64, SB2, SB25, SB7, SB35
		Khunmoah	6.201 to 7.038	4	SB2, SB54, SB57, SB38
	North Srinagar	Zakura	6.062 to 6.348	4	SB76, SB71, SB35, SB17
		Gulab Bagh	7.204 to 7.842	4	SB29, SB44, SB73, SB38
		Ahmad Nagar	5.330 to 6.116	4	SB66, SB69, SB57, SB18,
		Dhara	5.224 to 5.348	5	SB52, SB53, SB58, SB71, SB44
		Tailbal	5.448 to 6.048	4	SB36, SB60, SB62, SB71
		Batapora	4.258 to 7.305	5	SB37, SB57, SB61, SB45, SB77

Table 2: Morphological characters of bacterial endophytes isolated from roots of brown sarson

Isolate	Colony morphology	Cell morphology	Gram stain	Isolate	Colony morphology	Cell morphology	Gram stain
SB1	Light yellow, entire, circular, convex	Medium rods	+ve	SB21	Brown, lobate, circular, raised	Big rods	-ve
SB2	Yellow, entire, circular, raised	Small oval shaped cells	+ve	SB22	Creamy white, lobate, entire, circular, convex,	Curved rods	+ve
SB3	Brown, entire, circular, umbonate	Minute cocci	-ve	SB23	Orange, entire, circular, convex	Medium rods	+ve
SB4	Faint white, undulate, irregular, flat	Minute cocci	-ve	SB24	Light orange, entire, circular, raised	Cocci	-ve
SB5	White, entire, circular, convex	Small oval shaped cells	+ve	SB25	White, entire, circular, slightly raised	Big rods in pairs	-ve
SB6	Orange, entire, circular, raised	Medium rods	+ve	SB26	Deep orange, entire, circular, convex	Small rods	-ve
SB7	Creamy white, undulate, irregular, flat	Minute cocci	-ve	SB27	Yellow, entire, circular, convex	Long rods	-ve
SB8	Light yellow, entire, irregular, convex	Minute cocci	-ve	SB28	Light creamy white, entire, circular, convex	Long rods	-ve
SB9	Greyish white, serrate, irregular, slightly raised	Small rods	+ve	SB29	Brown, lobate, irregular, raised	Medium rods	+ve
SB10	White, undulate, irregular, flat	Small rods	+ve	SB30	Sharp orange, entire, circular, convex	Long rods	+ve
SB11	White, undulate, irregular, flat	Minute cocci	-ve	SB31	Straw brown, lobate, irregular, flat	Small cocci	-ve
SB12	White creamy, serrate, irregular, raised	Minute cocci	-ve	SB32	Creamy white, entire, circular, raised	Medium rods	-ve
SB13	Brownish, entire, irregular, slightly raised	Medium rods	+ve	SB33	Light red, entire, circular, raised	Medium rods	-ve
SB14	Light brown, entire, circular, convex	Medium rods	+ve	SB34	Light orange, entire, circular, convex	Small oval shaped cells	-ve
SB15	Faint white, lobate, irregular, convex	Small rods in pairs	-ve	SB35	Bright white, serrate, irregular, flat	Small cocci	-ve
SB16	White, serrate, circular, flat	Long rods	-ve	SB36	White, entire, circular, convex	Small rods	-ve
SB17	Yellow, circular, entire, convex	Minute cocci	+ve	SB37	Faint white, serrate, irregular, flat	Medium rods	-ve
SB18	Gummy white, entire, circular, convex	Minute rods	-ve	SB38	Yellow, entire, circular, convex	Medium rods	-ve
SB19	White, serrate, irregular, raised	Irregular cocci	+ve	SB39	White, entire, circular, convex	Cocci	-ve
SB20	Creamy faint white, lobate, irregular convex	Small rods	-ve	SB40	Sharp white, lobate, irregular, raised	Big rods in pairs	-ve

Table 3: (continue): Morphological characters of bacterial endophytes isolated from roots of brown sarson

Isolate	Colony morphology	Cell morphology	Gram stain	Isolate	Colony morphology	Cell morphology	Gram stain
SB41	Faint white, lobate, irregular, flat	Medium rods	-ve	SB61	Brown, serrate, irregular, flat	Small oval shape cells	-ve
SB42	Light brown, entire, circular, raised	Cocci	-ve	SB62	Deep orange, entire, circular, convex	Small cocci	+ve
SB43	Gummy white, serrate, irregular, raised	Long rods	+ve	SB63	Light brown, entire, circular, convex	Small rods	-ve
SB44	Yellow, lobate, irregular, convex	Long rods	+ve	SB64	Waxy white, entire, circular, raised	Medium rods	+ve
SB45	Waxy white, lobate, irregular, flat	Small rods	+ve	SB65	Gummy orange, entire, circular, convex	Small rods	+ve
SB46	White, lobate, irregular, flat	Medium rods	+ve	SB66	Sharp white, entire, circular, flat	Minute cocci	-ve
SB47	Bright white, lobate, irregular, raised	Small oval shape cells	-ve	SB67	Light brown, entire, circular, convex	Small rods	-ve
SB48	Straw brown, entire, circular, raised	Long rods	+ve	SB68	Gummy white, undulate, irregular flat	Minute rods	-ve
SB49	Gummy brown gummy, lobate, irregular, flat	Medium rods	+ve	SB69	Gummy, light reddish, entire, circular, flat	Medium rods	+ve
SB50	Gummy brown, serrate, irregular, flat	Cocci	-ve	SB70	Gummy white, entire, circular, convex	Small rods	-ve
SB51	Faint yellow, entire, circular, convex	Long to medium rods	-ve	SB71	Light brown, entire, circular, flat	Big rods in pairs	+ve
SB52	Faint brown, entire, circular, convex	Medium rods	+ve	SB72	Light orange, entire, circular, convex	Long rods	-ve
SB53	Faint white, lobate, irregular, flat	Minute rods	-ve	SB73	Bright orange, entire, circular, flat	Minute cocci	-ve
SB54	Light brown, undulate, irregular, raised	Medium rods	-ve	SB74	Sharp white, entire, circular, convex	Small oval shape cells	-ve
SB55	Brown, undulate, irregular, umbonate	Small rods	-ve	SB75	Faint white, undulate, irregular, raised	Medium rods	+ve
SB56	Orange, entire, circular, convex	Small rods	-ve	SB76	Gummy white, lobate, irregular flat	Minute cocci	-ve
SB57	Faint white, entire, circular, convex	Small rods	-ve	SB77	White, circular, entire, flat	Medium rods	-ve
SB58	Brown, entire, circular, flat	Long rods	-ve	SB78	Gummy orange, entire, circular, convex	Cocci	-ve
SB59	Light orange, entire, circular, raised	Minute rods	+ve	SB79	Gummy white, circular, entire, raised	Long rods	+ve
SB60	White, undulate, irregular, umbonate	Long rods	-ve	SB80	Bright white, entire, circular, convex	Small rods	+ve
				SB81	Straw brown, entire, circular, convex	Cocci	-ve

Table 4: Metabolic properties of isolated bacterial endophytes

Carbon source	Bacterial endophyte exhibiting metabolic properties
Glucose metabolism	All +ve
Galactose metabolism	All +ve
Maltose metabolism	All +ve except: SB4, SB23, SB32, SB34, SB38, SB40, SB42, SB44, SB52, SB54, SB56,SB60, SB63, SB71, SB74, SB76
Sucrose metabolism	All +ve except: SB3, SB5, SB6, SB8, SB17, SB20, SB22, SB26,SB59, SB60, SB61, SB65, SB67, SB69, SB73, SB74, SB75, SB76, SB78, SB80, SB81
Isolates with +ve metabolism for tested carbon sources *viz.*glucose, galactose, maltose and sucrose	SB1, SB2, SB7, SB9, SB10, SB11, SB12, SB13, SB14, SB15, SB16, SB18, SB19, SB21, SB24, SB25, SB27, SB28, SB29, SB30, SB31, SB33 SB35, SB36, SB37, SB39, SB41, SB43, SB45, SB46, SB47, SB48, SB49, SB50, SB51, SB53, SB55, SB57, SB58, SB62, SB64, SB66, SB68, SB70, SB72, SB77, SB79

Table 3: Production of indole-3-acetic acid by isolated bacterial root endophytes

*Isolate	IAA produced (µg/mL)	*Isolate	IAA produced (µg/mL)	*Isolate	IAA produced (µg/mL)	*Isolate	IAA produced (µg/mL)
SB2	3.13	SB20	10.64	SB41	11.51	SB64	16.55
SB5	12.33	SB21	5.25	SB42	4.05	SB67	10.11
SB6	4.86	SB22	3.09	SB43	9.36	SB68	2.64
SB7	2.94	SB26	5.36	SB46	5.92	SB69	9.79
SB9	10.74	SB28	19.54	SB51	11.05	SB70	2.20
SB12	9.72	SB29	7.86	SB55	8.45	SB71	5.73
SB13	9.55	SB30	8.05	SB56	8.28	SB73	9.92

SB14	11.84	SB31	7.65	SB57	6.23	SB74	6.92
SB15	5.41	SB32	8.17	SB58	13.19	SB76	5.64
SB18	8.22	SB34	7.92	SB60	16.64	SB77	6.68
SB19	9.76	SB39	2.70	SB63	5.23	SB79	8.05
Overall Mean : 8.15; C.D ($p \leq 0.05$) : 0.49; C.V% : 0.45 ; SE(m) : 0.06							

*All other isolates did not produce IAA

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