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IMPACT OF 16s rRNA GENE SEQUENCING BACTERIA AS BIOFERTILIZER AND BIOCONTROL AGENT ON RICE PLANT (VARIETY: SUGANDHA) ISOLATED FROM SELECTED GANGETIC REGION OF NORTH BIHAR

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Abstract : The soil acts as a reservoir for millions of microorganisms.This study aimed at isolating and characterizing plant growth-promoting bacteria as well as assessing their ability to promote plant growth. The present investigation was aimed towards isolation and purification of the strains from soil, their characterization , identification and further establishment of their biofertilizer potential on rice (Oryza sativa) plant. Five sampling sites namely S1 (Banana field soil, Hazipur), S2 (Near Gandhi Setu, wetland area), S3 (Sediment pond), S4 (Vaishali) and S5 (Sonepur) were selected. These isolates showed a wide range of temperature for growth between 4̊ C to 55̊ C which suggests its potential as biofertilizer. The maximum reproductive growths and size of fruits were obtained when the plants were grown in soil inoculated with S1 (C), S2 (F) and S3 (R) bacterial combinations. The molecular identification was carried out by16s rRNA sequencing. On the basis of BLAST alignment of the sequenced nucleotides the isolates S1(C), S2(F) and S3(R) were identified as different strains of Brevibacillus borstelensis and Paenibacillus dendritiformis, respectively. The isolates S1(C) and S2 (F) appear to be different strains of Brevibacillus borstelensis. The reported literatures suggest that the B. borstelensis acts as plant growth promoting bacteria whereas; P. dendritiformis as a biocontrol agent. All the three strains i.e. S1(C), S2 (F) and S3(R) favor rapid plant growth and inflorescence, thus forming a mixture of PGPR and biocontrol agents which in turn not only promote plant growth but also add to the plant vigor in fighting against diseases.

***Keywords*: Biofertilizer potential, Molecular identification, Brevibacillus borstelensis, Paenibacillus dendritiformis, biocontrol.**

1. INTRODUCTION

Microbiological techniques, especially in suppression of crop diseases, production of plant growth promoting substances and augmentation of nutrient recycling, offer a powerful tool in modern agriculture. Biofertilizer has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes [12]. In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies. The utilization of microbial products has several advantages over conventional chemicals for agricultural purposes. Neither toxic substances nor microbes themselves will be accumulated in the food chain. Self-replication of microbes circumvents the need for repeated application. Properly developed biocontrol agents are not considered harmful to ecological processes or the environment [11]. The application of microbial fertilizers in practice, somehow, has not achieved constant effects. The mechanisms and interactions among these microbes still are not well understood, especially in real applications [13] The term PGPR appears in 11 citations from the 1980s in the USDA's Agricola Electronic Database (National Agricultural Library, Washington, DC); during the first half of the 1990s the term appears in 34 citations, and during the second half of the decade, 72 citations. Likewise, the term biofertilizer appears to have come into common use in the scientific literature in the late 1970s. The Agricola Electronic Database indicates approximately 60 citations per decade which use the term biofertilizer during the 1980s and 1990s. This survey demonstrates the trend of an increasing rate of research on PGPR, but is not an absolute measure of the activity in the area. Although there is evidence of PGPR having biofertilizing effects on forest tree species [4]. The focus in this literature review will be on crop species. When the existence of a microorganism increases the growth of plants by

replacing soil nutrients (e.g. by biological N_2 fixation (BNF)) or making nutrients more available (e.g. by solubilization of phosphates) or increasing plant access to nutrients (e.g. by increasing root surface area), as long as the nutrient status of the plant has been enhanced by the microorganism.The substance that was applied to the plant or soil containing the microorganisms, is referred to here as a biofertilizer. Not all PGPR can be considered biofertilizers. Bacteria that promote plant growth by control of deleterious organism are biopesticides, but not biofertilizers. Interestingly some PGPR appear to promote growth by acting as both biofertilizer and biopesticides. For example, strains of *Burkholderia cepacia* have been shown to have biocontrol characteristics to *Fusarium spp.*, but also can stimulate growth of maize under iron-poor conditions via siderophore production [2]. Interest in biological control has increased recently fuelled by public concerns over the use of chemicals in the environment in general, and the need to find alternatives to the use of chemicals for disease control. Modern methods for analysing microbial community structures may prove particularly valuable to help define the key organisms or groups of organisms responsible for natural disease control mechanisms as well as for monitoring the spread and impact of introduction of specific biocontrol agents or other management practices on natural microbial populations [3]. A large part of South Bihar is, nevertheless, rain fed and the soil is especially suited for paddy and pulses. Although a lot of efforts have been made by scientists of different Agricultural University to develop varieties that best suit the soil of Bihar, very little effort has been made to investigate the soil microflora that supports and promotes the growth of crops even without addition of fertilizers, especially of the most fertile Indo-Gangetic plains.

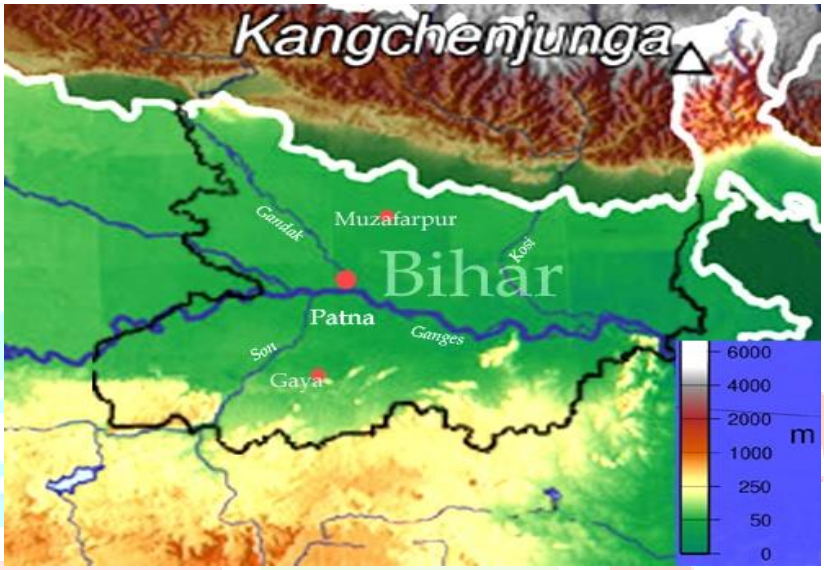

Fig. 1.1. The topographic map of Bihar

Thus the Gangetic planes of Bihar need immediate attention to isolate and screen microbes that help in enhanced soil fertility and decomposition of agricultural wastes. This will not only help generating the database of indigenous microbes but also play a major role in poverty alleviation through involvement of rural masses.

Fig.1.2. The district map of Bihar showing the Gangetic regions selected for the microbial diversity and their PGPR potential studies

Fig.1.3. The GPS map of sampling sites in Vaishali district (Bihar) of Gangetic region showing different site locations by S1, S2, S3, S4 and S5.

Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. The rhizosphere concept was first introduced by Hiltner to describe the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities [6]. The original concept has now been extended to include the soil surrounding a root in which physical, chemical and biological properties have been changed by root growth and activity [10]. A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates [9] creating a very selective environment where diversity is low [5]. Since bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization [1]. Rhizobacteria inhabit plant roots and exert a positive effect ranging from direct influence mechanisms to an indirect effect. So, the bacteria inhabiting the rhizosphere and beneficial to plants are termed PGPR [7]. In the last few years, the number of PGPR that have been identified has seen a great increase, mainly because the role of the rhizosphere as an ecosystem has gained importance in the functioning of the biosphere. Various species of bacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Enterobacter*, *Arthrobacter*, *Bacillus* and *Serratia* have been reported to enhance the plant growth [8]. There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism; suppression of plant diseases (termed bioprotectants), improved nutrient acquisition (biofertilizers), or phytohormone production (biostimulants). Inoculant development has been most successful to deliver biological control agents of plant disease i.e. organisms capable of killing other organisms pathogenic or disease causing to crops. Various bacteria which are predominantly studied and increasingly marketed as the biological control agents includes the genera *Bacillus*, *Streptomyces*, *Pseudomonas* and *Agrobacterium*. Thus, exploring these bacterial communities and utilizing their potentials for agricultural purpose can provide a new dimension for future research. Besides, there are also growing concerns over possible health and environmental consequences of using increasing amounts of mineral fertilizers and chemical pesticides which have led to strong interest in alternative strategies to ensure yields and protection of crops. Use of microbial inoculants for biofertilizer/PGPR in agriculture represents an attractive environment friendly alternative. The use of PGPR offers an attractive way to replace chemical fertilizers, pesticides, and supplements. Most of the isolates result in a significant increase in plant height, root length, and dry matter production of shoot and root of plants. They also help in the disease control in plants. Some PGPR especially if they are inoculated on the seed before planting, are able to establish themselves on the crop roots. This new approach to farming often referred to as sustainable agriculture. The present investigation focuses on the screening of effective strains on the basis of their potential for plant growth promoting activity under pot experiments. All three bacterial isolates identified previously as S1(C), S2 (F) and S3(R) individually as well as in consortia were tested for on rice (*Oryza sativa*), a common crop of Bihar for their evaluation for biofertilizer potential. The comparative analysis of vegetative and reproductive plant growth patterns of rice (*Oryza sativa*), from germination of seeds to maturation of fruit body in pot experiments were done.

II. Materials and methods

Sources of sample collection

The isolates were obtained from the soil of Gangetic region of North Bihar. The next step was the screening of isolates to select bacterial strains with biofertilizer potential. They are mostly differentiated on the basis of structure and development of cells, morphology, responses to culturing, physiology and biochemistry. Their colony on agar media produce spores, the later representing the preparative phase. The majority of microorganisms, when grown on solid media, grow into colony representing a massive cluster of cells of organisms which are capable of growing together in a single complex colony. Microscopic examination of the organisms from a colony should reveal only a single type of cells. Differential staining procedures, such as Gram stains are useful for establishing that the colony does not contain a mixture of different microbial types.

Experimental approaches

Pot experiments on Oryza sativa sugandha (rice) for the determination of biofertilizer potential of bacterial isolates

The soil for the pot experiments was collected from A.N. College, Patna garden and sterilized by autoclaving to remove any microbes already available in soil. The sterilized soil was used as control wherever applicable. The commercial biofertilizer was obtained from Taru Mitra Biofertilizers (procured from Taru Mitra center, East Boring Canal road). The three bacterial isolates from Gangetic soil namely S1 (C), S2 (F) and S3 (R) were used as nutrient broth both individually and in consortia. Three types of soil combinations containing control soil, control soil with market procured biofertilizer (Taru Mitra biofertilizer) and control soil with bacterial isolates S1(C), S2(F) and S3(R) both individually as well as in consortia form were prepared and filled in separate labeled pots. The pots were immediately sowed with the rice seeds under exposed sunlight condition. All the plants were grown in similar natural environmental conditions. The comparative evaluation of vegetative as well as reproductive growth patterns of plants grown in different soil combinations were made periodically for more than 120 days. Crop development data were collected on an average interval of 10-23 days from the sowing of seeds to maturation of new seeds. The various observed parameters to determine both vegetative as well as reproductive plant growth patterns were date of sowing, date of germination, number of plants germinated, number of leaves, length of leaves, colour of leaves, length of plants, growth of inflorescence, number of fruits, quality of fruits, number of seeds. The data were recorded, tabulated and compared to determine the biofertilizer potential of isolates.

Results and Discussion

The bacterial isolates from soil of Gangetic region of Vaishali district of Bihar were earlier evaluated on agar plates for their biochemical and physiological characteristics. In this investigation they are further tested for their biofertilizer potential on rice (*O. sativa*) through pot experiments. The analysis is based on the comparative studies of vegetative and reproductive growth patterns of plants growing from germination of seeds to maturation of fruit body in pots containing different soil combinations explained in the experimental section.

There was no difference in the pattern of germination of plants sown in all the soil combinations which suggests that all the isolates and commercial biofertilizer have no visible effect on seed germinations. The vegetative growth patterns of plants grown in soil with individual isolates and with S1(C) and S2 (F) bacterial combinations, with S1(C) andS3(R) bacterial combinations and with S1(C), S2 (F) and S3(R) bacterial combinations were better than plants grown both in sterilized (control) soil and with Taru Mitra biofertilizer combination (Fig. 3.1 to 3.5) (Table 3.7). Similarly, the reproductive growth patterns of plants were also found to be better than both control plants as well as plants growing in control with Taru Mitra biofertilizer combination for all the microbes both individual as well as in different consortia combination of microbial strains used (Fig. 3.4 and 3.5) (Table 3.8). Although, the maximum reproductive growths were obtained when the plants were grown in soil inoculated with S1 (C), S2 (F) and S3 (R) bacterial combinations as shown in Fig. 3.5. Interestingly, the best plant growth in terms of plant height was recorded for the plants growing in control with strain S3(R) combination (Fig. 3.6.A) which was subsequently reflected in S1(C)+S3(R) and S1(C), S2(F) and S3(R) combinations too. The strain S1(C) also showed quite promising results in terms of maximum number of leaves and leaf length (Fig. 3.6. B and C). The fruit bodies of the plants growing in control with strain S1(C), S2(F) and S3(R) combination was most healthy with maximum number of seeds (Fig. 3.5). This was followed by the fruit body of plants growing in control with strain S1(C) and S2 (F) combinations. The third position was occupied by the plants growing in control with strain S1(C) and S3(R) combinations. All the above stated reproductive growth was better than both control plants as well as plants growing in control with Taru Mitra combinations.

Thus, this investigation confirms that the selected microbial isolates (bacteria) can be used as biofertilizer for better growth of *Oryza sativa* (rice) plants. The combination of microbes tested has shown better results as compared to the biofertilizer available in market. It will be economically a better option resulting in better yields. Use of these biofertilizers will be eco-friendly option. Characterized microbes can enhance agricultural potential of soil of Bihar. This will help in identifying local microbes which can be exploited for agricultural productivity. The characterization of microbes will also help in identifying enzymes produced by the various strains isolated from the soil of Bihar.

III. FIGURES AND TABLES

Fig.3.1. First observation of plants growing in control, control with Taru Mitra biofertilizer and control with bacterial isolate combinations. The bacterial isolate combinations were: S1(C), S2 (F), S3(R), S1(C)+S2(F), S1(C)+S3(R) and S1(C)+S2(F)+S3(R) as A, B, C, A×B, A×C and A×B×C, respectively.

Fig.3.2. Third observation of plants growing in control, control with Taru Mitra biofertilizer and control with bacterial isolate combinations. The bacterial isolate combinations were: S1(C), S2 (F), S3(R), S1(C)+S2(F), S1(C)+S3(R) and S1(C)+S2(F)+S3(R) as A, B, C, A×B, A×C and A×B×C, respectively.

Fig.3.3. Fifth observation of plants growing in control, control with Taru Mitra biofertilizer and control with bacterial isolate combinations. The bacterial isolate combinations were: S1(C), S2 (F), S3(R), S1(C)+S2(F), S1(C)+S3(R) and S1(C)+S2(F)+S3(R) as A, B, C, A×B, A×C and A×B×C, respectively.

Fig.3.4. Sixth observation of plants growing in control, control with Taru Mitra biofertilizer and control with bacterial isolate combinations. The bacterial isolate combinations were: S1(C), S2 (F), S3(R), S1(C)+S2(F), S1(C)+S3(R) and S1(C)+S2(F)+S3(R) as A, B, C, A×B, A×C and A×B×C, respectively.

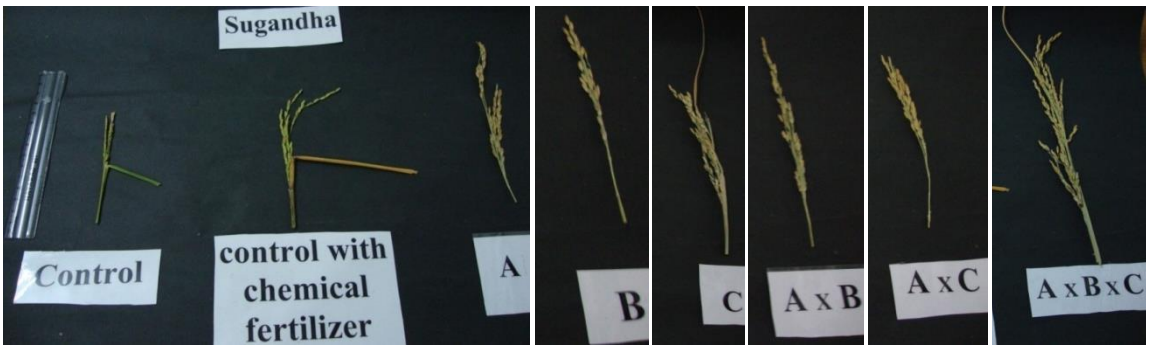

Fig.3.5. Exposed fruit bodies of plants growing in control, control with Taru Mitra biofertilizer and control with bacterial isolate combinations. The bacterial isolate combinations were: S1(C), S2 (F), S3(R), S1(C)+S2(F), S1(C)+S3(R) and S1(C)+S2(F)+S3(R) as A, B, C, A×B, A×C and A×B×C, respectively.

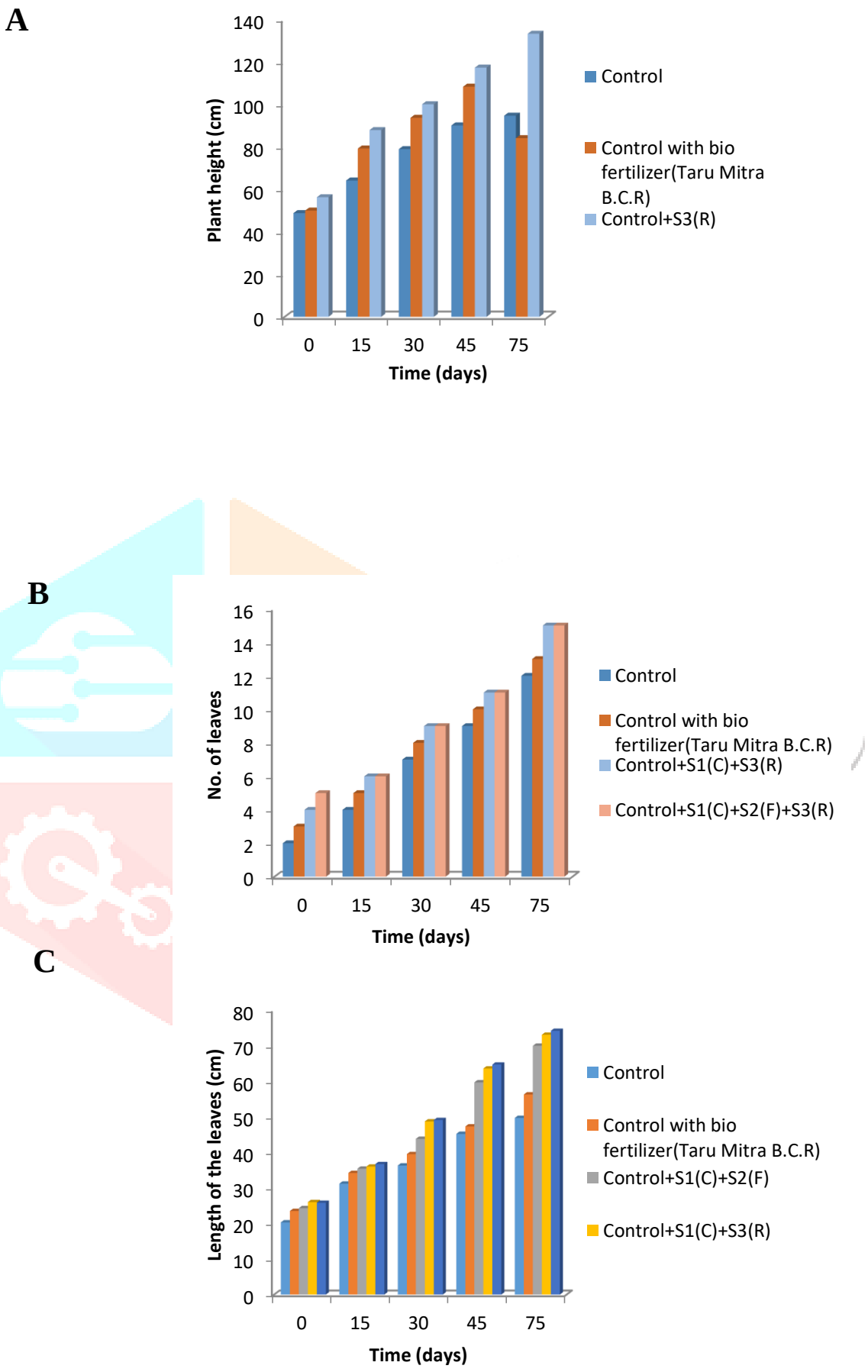

Fig. 3.6. A, B and C Graphs showing differences in vegetative growth patterns between the plants grown in soil with and without commercial biofertilizer and soil supplemented with bacterial isolates.

Table 3.7. The comparative effect of different soil combinations on vegetative growth of *O. sativa sugandha* to establish the biofertilizer potentials of selected bacterial isolates.

		Nature of soil							
	Observed Parameters	**Control**	**Control with biofertilizer (Taru Mitra B.C.R)**	**Control+ S1(C)**	**Control+ S2(F)**	**Control + S3(R)**	**Control+ S1(C)+S2(F)**	**Control+ S1(C)+S3 (R)**	**Control+ S1(C)+S2(F)+S3(R)**
	No. of plants germinated	10	6	6	6	6	6	6	6
Observation Day (1st day)	No. of leaves	2	3	3	3	3	3	4	5
	Leaf length (cm)	20.3	23.5	22.2	20.3	19.8	24.3	26	25.8
	Colour of leaves	Green	Green	Dark green	Green	Green	Dark green	Green	Dark green
	Plant length (cm)	48.6	49.8	60.3	45.1	56.1	54.5	55.3	52.8
Observation Day (15th day)	No. of leaves	4	5	5	5	5	5	6	6
	Leave length (cm)	31.2	34.2	32.2	30.3	29.8	35.4	36	36.7
	Colour of leaves	Green	Green	Dark green	Green	Green	Dark green	Green	Dark green
	Plant length (cm)	64	79.1	76.3	58.6	87.8	84	79.6	81.6

		Nature of soil							
	Observed Parameters	**Control**	**Control with biofertilizer (Taru Mitra B.C.R)**	**Control + S1(C)**	**Control+ S2(F)**	**Control + S3(R)**	**Control+ S1(C)+S2(F)**	**Control + S1(C)+ S3(R)**	**Control+ S1(C)+S2(F)+S3(R)**
	No. of plants germinated	10	6	6	6	6	6	6	6
Observation Day (30th day)	No. of leaves	7	8	8	8	8	8	9	9
	Leave length (cm)	36.3	39.5	38.2	36.3	39.2	43.8	48.7	49.1
	Colour of leaves	Green	Green	Dark green	Green	Green	Dark green	Green	Dark green
	Plant length (cm)	78.8	93.6	96	75.6	100	97	103.3	101.3
Observation Day (40th day)	No. of leaves	9	10	10	10	10	10	11	11
	Leave length (cm)	45.2	47.3	46.2	50.8	53.2	59.7	63.6	64.7
	Colour of leaves	Green	Green	Dark green	Green	Green	Dark green	Green	Dark green
	Plant length (cm)	89.6	102.3	103.6	87.3	112.3	106	113	100.5
Observation Day (63rd day)	No. of leaves	12	13	13	13	13	13	15	15
	Leave length (cm)	49.7	56.3	55.2	61.7	65.2	70	73.1	74.2

	Nature of soil							
Observed Parameters	**Control**	**Control with biofertilizer (Taru Mitra B.C.R)**	**Control + S1(C)**	**Control+ S2(F)**	**Control + S3(R)**	**Control+ S1(C)+S2(F)**	**Control + S1(C)+ S3(R)**	**Control+ S1(C)+S2(F)+S3(R)**
Colour of leaves	Green	Yellowish Green	Dark green	Green	Light Yellow	Dark green	Green	Dark green
Plant length (cm)	90	108.3	104.3	98.3	117.3	112	114.3	104.3

		Nature of soil							
	Observed Parameters	**Control**	**Control with biofertilizer (Taru Mitra B.C.R)**	**Control + S1(C)**	**Control+ S2(F)**	**Control+ S3(R)**	**Control + S1(C)+S2(F)**	**Control+ S1(C)+S3(R)**	**Control+ S1(C)+S2(F)+S3(R)**
Observation Day (88th day)	No. of leaves	13	14	14	14	15	15	17	17
	Leave length (cm)	55.3	62.2	61.3	71.2	75.3	80.1	83.2	85.1
	Colour of leaves	Green	Yellow	Dark green	Green	Yellow	Yellowish green	Green	Yellowish green
	Plant length (cm)	94.6	84	109	95.3	133.3	103.6	119.6	108

No. of pots: 8, day of sowing: 0 day, day of germination: 4th day, replantation in 8 pots: 14th day, day of 1st irrigation: 14th day.

Table 3.8. The comparative effect of different soil combinations on reproductive growth of *O. sativa sugandha* to establish the biofertilizer potentials of selected bacterial isolates.

		Nature of soil							
	Observed Parameters	**Control**	**Control with biofertilizer (Taru Mitra B.C.R)**	**Control +S1(C)**	**Control+ S2(F)**	**Control+ S3(R)**	**Control+ S1(C)+S2(F)**	**Control+ S1(C)+S3(R)**	**Control+ S1(C)+S2(F)+S3(R)**
Observation Day (63rd day)	Inflorescence	Yes	—	Yes	—	Yes	Yes	—	Yes
	No. of fruits	Yes	—	Yes	—	Yes	Yes	—	Yes
Observation Day (88th day)*	Inflorescence	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	No. of fruits	1	1	1	1	1	1	1	1
Observation Day (110th day)**	No. of fruits	1	1	1	1	1	1	1	2
	Fruits health	Good	Good	Good	Poor	Very Good	Very Good	Very Good	Excellent
	Germinated seed lengths (cm)	12	13.5	15	10.5	16.5	19.5	19	24

*The vegetative growth became static after 88th day.

**At the observation day: 110th day, the plants started to wilt.

IV. Conclusion

1. On the basis of total growth pattern observation, results reveal that in the final stage the growth of plants growing in control with strain S1(C), strain S2(F) and strain S3(R) combination was fastest, followed by plants growing in control with strain S1(C) and strain S3(R) combination. The third position was occupied by plants growing in control with strain S1(C) and strain S2 (F) combination.
2. On the basis of total growth pattern observation, results reveals that the fruit body of the plants growing in control with strain S1(C), strain S2(F) and strain S3(R) combination was most healthy with maximum number of seeds. This was followed by the fruit body of plants growing in control with strain S1(C) and strain S3(R) combination. The third position was occupied by the plants growing in control with strain S1(C) and strain S2 (F) combination.

Thus, the comparative vegetative and reproductive plant growth patterns of *Oryza sativa* (Rice) both individually as well as in different combination of bacteria (consortia) with soil were interesting and very encouraging. Investigation of biofertilizer activity in sterilized soil inoculated with selected isolates revealed superiority of the selected isolates as biofertilizer over market available biofertilizer (Taru Mitra Biofertilizer). Based upon biofertilizer potentiality as growth promoting activity, the different consortia combinations of selected isolates were designated as the best plant growth promoting bacteria.

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References

[1] Antoun H, Kloepper JW (2001) Plant growth promoting rhizobacteria (PGPR). In: Encyclopedia of Genetics. Academic Press, New York, ed Brenner S, Miller JH, pp 1477–1480

[2] Bevivino A, Sarrocco S, Dalmastri C, Tabacchioni S, Cantale C, Chiarini L (1998) Characterization of a free-living maize rhizosphere population of *Burkholderia cepacia*: effect of seed treatment on disease suppression and growth promotion of maize. FEMS Microbiol Ecol 27:225–237

[3] Duineveld BM, Rosado AS, van Elsas JD, van Veen JA (1998) Analysis of the dynamics of bacterial communities in the rhizosphere of the chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. Appl Environ Microbiol 64:4950–4957

[4] Elo S, Maunuksela L, Salkinoja-Salonen M, Smolander A, Haahtela K (2000) Humus bacteria of Norway spruce stands: plant growth promoting properties and birch, red fescue and alder colonizing capacity. FEMS Microbiol Ecol 31:143–152

[5] Garcia JL, Probanza A, Ramos B, Manero FJG (2001) Ecology, genetic diversity and screening strategies of plant growth promoting rhizobacteria. J Plant Nutrition Soil Sci 164:1–7

[6] Hiltner L (1904) Uber neuere erfahrungen und probleme auf dem gebiet der bodenbakteriologie und unter besonderer berucksichtigungdetgrundungung und branche. Arb Deut Landw Ges 98:59-78

[7] Kloepper JW, Leong J, Teintze M, Scrhorth MN (1980) Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 286:885–886

[8] Kloepper JW, Lifshitz R, Zablotowicz RM (1989). Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–43

[9] Lynch JM (1990) The Rhizosphere. John Wiley & Sons Ltd, Chichester UK, ed Lynch JM, pp 458

[10] McCully M (2005) The rhizosphere: the key functional unit in plant/soil/microbial interactions in the field. Implications for the understanding of allelopathic effects. In Proceedings of the 4th World Congress on Allelopathy, Charles Sturt University, Wagga Wagga, NSW, Australia, International Allelopathy Society, ed Harper J, An M, Wu H, Kent J, pp 21-26

[11] Raaijmakers JM, Weller DM (1998) Natural plant protection by 2,4-diacetylphloroglucinol- producing *Pseudomonas* spp. in take-all decline soils. Molecular Plant-Microbe Interact 11:144–152

[12] Vessey J K (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255: 571–586

[13] Wu SC, Cao ZH, Li ZG, Cheung KC, Wong MH (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. 125:155–166