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Microbial Diversity in Rhizosphere Soils of Tropical Tuber Crops: Utilization for Pathogen Suppression and Growth Promotion

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Abstract

Tuber crops are the third most important food crops, after cereals and legumes. There are many pathogenic microbes, which affect the production of tuber crops. Fungal and viral pathogens attack most of the tuber crops. However, there is much variability among the crops in their susceptibility to pathogens. Similarly, rhizosphere soil has a large diversity of the microbial community, which possesses plant growth-promoting activity as well as pathogen suppression. In the present study, the culturable microorganisms (fungi, bacteria and actinomycetes), in the rhizosphere of sixteen varieties of tropical tuber crops *viz*., sweet potato, elephant foot yam, cassava and yam were enumerated and compared; their role on suppression of *Sclerotium rolfsii,* the pathogen that causes collar rot in elephant foot yam was studied, and the ability of the organisms for growth promotion, nitrogen fixation, P and K solubilization was assessed, and the efficient antagonistic organisms were characterized by amplifying 16S rRNA. The microbial population varied significantly among the crops and varieties. Among one hundered and fifty organisms isolated and screened for pathogen suppression, ten bacterial and twelve actinomycete isolates completely inhibited the mycelial growth in the preliminary round of screening. Eight bacterial isolates chosen for further study based on consistency in pathogen suppression, exhibited IAA production while seven isolates showed nitrogen fixation. None of the bacterial isolates showed the formation of P or K solubilization zones in the selective media. Molecular studies based on 16S rRNA gene sequencing revealed that the most efficient antagonistic bacterial isolates with growth promotion potential were *Bacillus* spp. The six *Bacillus* species identified were *Bacillus siamensis*, *B. amyloliquefaciens*, *B. pumilus*, *B. halotolerans*, *B. subtilis and B. altitudinis*. Six out of eight efficient bacterial isolates were obtained from rhizosphere soils of sweet potato. The organisms, *B.amyloliquefaciens* and *B.subtilis* can serve as excellent bio-agents for pathogen suppression and growth promotion in tuber crops ecosystem.

Key words: Rhizosphere, *Bacillus*, sweet potato, nitriogen fixation, *Sclerotium rolfsii*

Introduction

Elephant foot yam (*Amorphophallus paeonifolius* (Dennst.) Nicolson) is an important tuber crop of tropical and sub-tropical countries, which offers an exceptional reach as a cash crop because of its high production potential (50-80 t/ha), market acceptability, medicinal properties and lucrative economic returns. *Amorphophallus* is susceptible to a number of diseases often causing heavy loss to the crop (Misra et al., 2005). Among the field diseases of elephant foot yam, collar rot caused by *Sclerotium rolfsii* Sacc. is the most destructive and common disease prevalent in all the elephant foot yam growing areas. Managing the diseases through the application of chemical pesticides have been increasingly curtailed. The heavy and widespread application of chemical pesticides has created the public's growing concern for the human health conditions and the environmental pollution associated with pesticide usage. In addition, reasons such

as the development of pesticide-resistant strains of pathogens and the lack of continuous approval of some of the most effective fungicides have motivated the search for alternative approaches. Furthermore, organic production is gaining an increasing share of the vegetable market since it is promoted and perceived by consumers as healthier and safer for the environment. But, organic growers have limited options for managing diseases of tuber crops since most of the effective fungicides, fumigants and seed treatments are synthetic, toxic and potentially polluting (Veena et al., 2013). This necessitates the development of an eco-friendly approach for managing diseases. The use of microorganisms as bio-control agents has provided a very promising alternative and less hazardous method for plant disease control (Cook, 1985). The rhizosphere is the region of soil that is immediately near to the root surface and that is affected by root exudates. Microorganisms that can grow in the rhizosphere are ideal for use as bio-control agents, since the rhizosphere provides the front-line defense for root against attack by pathogens. The microbial activity in the rhizosphere is essential for plant functioning as it assists the plant in nutrient uptake and offers protection against pathogen attack (Berendsen et al., 2012).

The ability of plants to use beneficial microbes is a part of its capacity to build a protective cover that enhances pathogen control in the rhizosphere. However, only recently, attention has been given to identify and utilize effective microbial consortia that can mediate induced systemic resistance (ISR) in hosts. Pathogens encounter antagonism from rhizosphere microorganisms before and during primary infection and also during secondary spread in the roots. In the suppressive soil to pathogens, microbial antagonism of the pathogen is especially great, leading to substantial disease control. Although pathogen-suppressive soils are rare, those identified are excellent examples of the full potential of biological control of soil borne pathogens. Most widely studied microorganisms with antagonistic activity against plant pathogen and with beneficial effect on plant growth belongs to bacterial genera *Bacillus*, *Pseudomonas*, *Rhizobium* and fungi *Trichoderma* (Ongena and Jacques, 2008; Lorito et al., 2010). Nitrogen fixing bacteria are found in association with plants (Klein, 2000). Phosphate solubilizing microorganisms (PSMs) are ubiquitous in soils and could play an important role in supplying P to plants in a more environment friendly and sustainable manner. Similarly, Potassium solubilizing bacteria are usually present in all soils, although their number, diversity and ability for K solubilization vary depending upon the soil and climatic conditions. In the present study, variability of rhizosphere organisms existing among various tuber crops such as cassava, sweet potato, yams and elephant foot yam were explored; the potent bacterial isolates were evaluated for their potential for pathogen suppression, plant growth promotion, nitrogen fixation and P and K solubilization. The efficient bacterial isolates were identified by 16SrRNA gene sequencing.

Materials and Methods

Sample collection

Sixteen soil samples were collected from the rhizosphere region of different varieties of sweet potato (Sree Arun, Kanhanhad, Bhu Sona, Bhu Krishna and Sree Kanaka), elephant foot yam (Sree Padma, Gajendra and Sree Athira), cassava (Sree Reksha, Sree Pavithra and Sree Swarna), lesser yam (Sree Latha and Sree Kala), white yam (Sree Priya and Sree Subhra) and greater yam (Sree Neelima) cultivated in the crop museum of ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram. Barring the varieties, Kanhanhad (Sweet potato) and Gajendra (elephant foot yam), all other varieties were released from ICAR - Central Tuber Crops Research Institute. The soil samples were collected and were packed in sterile polythene bags and labelled properly for further processing.

Enumeration of rhizosphere microbes

Enumeration of microbes from the collected soil samples was performed by serial dilution and plate count technique. Ten gram of rhizosphere soil sample (homogenized) was weighed and serially diluted in sterile distilled water. One ml of soil suspension from 10^{-4} to 10^{-6} were pour plated into Rose Bengal Agar medium for fungus, Ken Knight and Munaier's medium for actinomycetes and Nutrient Agar and Kings Medium B for bacteria respectively. The plates were incubated at 28 ± 2°C and 35 ±2°C for 1-5 days according to the kind of organism. Three replicates were kept for each dilution. The plates were observed at an interval of 24 hour and the colony count was taken and expressed in $cfug^{-1}$ soil. Colonies obtained from different varieties and different crops were compared. Colonies showing

different morphological characters were selected and purified by sub-culturing into respective media and were stored at 4°C.

Culture maintenance

The colonies of fungi, actinomycetes and bacteria were cultured individually on Rose Bengal Agar, Kenknight and Munaier's and Nutrient Agar media for further studies.

Screening of isolates against *Sclerotium rolfsii*

The morphologically distinct isolates were tested against the pathogen, *Sclerotium rolfsii,* the causal organism of collar rot disease in elephant foot yam. Three methods *viz*., dual culture method, production of diffusible metabolites and production of volatiles were used for selecting the isolates with antagonistic potential.

Dual culture method

Dual culture technique described by Skidmore and Dickinson (1976) was used for the study. This method involved the use of media supporting the growth of both type of organisms selected. Mycelial discs of the pathogen, *Sclerotium rolfsii* (5 mm) were taken from the edge of an actively growing fungal colony with a cork borer and placed in the centre of potato dextrose agar plate and test bacteria and actinomycetes were streaked on either side of the pathogen discs. In the case of fungi, a 5mm disc of fungi were placed on the one side and pathogen were placed on the other side of the potato dextrose agar plate. Plates incubated with the pathogen alone served as the control. Three replicates were observed constantly; the radial growth of the pathogen was recorded at an interval of 24 h until the growth of the pathogen in control plates covered fully (4 days). The percentage of inhibition was worked out as follows,

$I = (C-T/C) \times 100$

Where, I = percent inhibition; C = radial growth of pathogen (in mm) alone in the control plate; T = radial growth of pathogen (in mm) in the presence of test organisms (Edington et al., 1971).

Isolates showing high percent of inhibition were re-evaluated for their activity. Out of 150 organisms, eight organisms showing high and consistent percent of inhibition were taken and tested by the following methods.

Antibiosis test for production of diffusible inhibitory metabolites

The test was carried out using cellophane paper method described by Dennis and Webster (1971a). For this, cellophane paper were cut and sterilized in an autoclave at 121°C for 15 minutes and then each sterilized cellophane paper was aseptically placed over the potato dextrose agar medium taken in the Petri-plate. Eight potent isolated bacteria which, showed consistent reaction were taken, and streaked on the centre of the cellophane paper. The adhering bacteria were removed carefully on the second day of incubation and placed five mm mycelial disc of pathogen immediately on the medium at the centre previously occupied by the potent organisms. The radial growth of pathogen was recorded after every 24 hours for four days and compared with the growth in the control plate. Based on this, the percent inhibition of pathogen, if any was calculated as described earlier in case of dual culture method (Edington et al., 1971).

Antibiosis test for production of volatile inhibitory compound production

The test was carried out by slightly modifying the sealed Petri-plate technique described by Dennis and Webster, (1971b). For this test, the isolates were inoculated on potato dextrose agar plates, the lids of the Petri-plate inoculated with antagonist were replaced by the culture of the pathogen on the potato agar medium plates. The plates were fixed with cello tape and incubated for 2-3 days. Pathogen alone kept in the similar way served as the control. The radial growth of pathogen was recorded at 24 hour intervals for four days and compared with the growth in control plates. Based on this, percentage inhibition of pathogen, if any was calculated as described earlier in case of dual culture method (Edington et al., 1971).

Indole Acetic Acid (IAA) production

Indole acetic acid production was detected as described by Brick et al., (2004). Bacterial cultures were grown for 72 hours in Jensen's broth containing 2 $mgml^{-1}$ L-Tryptophan at 35±2°C. The cultures were incubated at 35±2°C with agitation at 125 rpm for 48 hours. Fully grown culture (2 ml) was centrifuged at 1500 rpm for 1 minute. The supernatant (1 ml) was taken and mixed with 2 ml of Salkowski reagent (40 ml sulphuric acid,

67 ml distilled water, 2 ml 0.5 FeCl3.7H2O): incubated at dark for 20 minutes in room temperature. The absorbance of the resultant pink colour read after 20 minutes at 540 nm in UV visible spectrophotometer.

Test for identifying Nitrogen fixers

The medium used was Jensen's nitrogen-free medium (HIMEDIA). Nitrogen-fixing organisms are free-living bacteria, which grow well on a nitrogen-free medium (Jensen, 1951). The eight potent bacterial isolates were inoculated into the Petri-plate containing Jensen's medium and incubated at 35±2°C for 48-72 hours. Three replicates were maintained. The isolates grew successfully were noted.

Test for identifying Phosphate solubilizers

The medium used was Pikovskya agar medium. The eight potent isolates were inoculated on the Petri-plate containing Pikovskya medium and incubated at 35±2°C for 24-48 hours. Three replicates were maintained. Bacteria that produced zone in media by solubilising Tricalcium Phosphate (TCP) present in the medium were noted (Pikovskya, 1948).

Test for identifying Potassium solubilizers

Sucrose minimal salt K- limited medium was used. The eight potent isolates were inoculated into the Petri-plate containing SSK medium and incubated at 35±2°C for 48-72 hours, the colonies that shown zone around them were noted (Sheng et al., 2008). Three replicates were maintained.

Molecular characterization of the potent organism

Isolation and purification of genomic DNA

Eight potent bacteria identified were incubated overnight in NA broth and used for extracting genomic DNA. A portion of the culture (1.5 ml) was transferred to a microfuge tube and centrifuged at 12,000 rpm for 10 minutes. The pellet was collected and re-suspended in 400 µl TE buffer and vortexed. To this added 50 µl of 10% SDS and 20 µl of 20 $mgml^{-1}$ Proteinase K and incubated in water bath for 45minutes at 65°C. To this added 5 µl of 20 $mgml^{-1}$ RNAase and incubated in water bath for 30 minutes at 37°C. The contents were pipetted in and out many times using Micropipettes. It was extracted twice with 500 µl Phenol: Chloroform (1:1) and twice with 500 µl Chloroform by centrifugation at 12,000 rpm for 10 minutes. The supernatant was taken each time. 25 µl 5M NaCl and 1 ml 95% ethanol were added to precipitate for nucleic acids and centrifuged for 10 minutes at 12,000 rpm. Then the supernatant was discarded and pellet was dried. The DNA was re-suspended in 30 µl TE buffer stored at -20°C. The quality of the DNA isolated was checked by using Agarose Gel Electrophoresis.

PCR amplification of 16S rRNA gene

For molecular identification of the isolates, the 16S rDNA gene regions were amplified using the polymerase chain reaction. In this reaction, 8F (5' AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' GGTTACCTTGTTACGACTT 3') were used as forward and reverse primers, respectively. The reaction was performed by using PCR mix, sterile water-9.5 µl; DNA-2 µl; Emerald Master mix- 12.5 µl; Primer (forward) -0.5 µl and Primer (reverse) -0.5 µl. The reaction was performed with an initial denaturation at 95°C (2 min 30 seconds) followed by 34 cycles of denaturation at 55.5°C (30 seconds), annealing at 72°C (1 min), extension at 72 °C (8 min) and a final extension at 72°C (10 min). Elution was carried out using Nucleospin® Gel and PCR Clean-up kit (Macherey-Nagel). Amplification products were resolved by agarose gel electrophoresis and photograph was scanned through the Gel Doc System (Alpha Imager, Alpha Innotech, USA) and sequenced with the same primers as for the PCR amplifications. The amplified gene products were sequenced by Agri-genome, Kochi, Kerala. The nucleotide sequence was determined by nucleotide BLAST (Basic Local Alignment Search Tool) search program of NCBI. The gene sequences were also submitted to GenBank® and accession numbers were obtained.

Statistical analysis

The data were statistically analysed using SAS statistical software (SAS 2010 – SAS Institute Inc., Cary, North Carolina, USA).

Results and Discussion

Enumeration of rhizosphere organisms

Plant associated microbes play a key role in plant health, a systematic study on how and to what extent plants can shape their own detrimental or beneficial microbiome is essential. The microbiome influences plant health and

development, playing a vital role at all stages of plant growth. For sustainable agriculture maintenance soil dynamic nature is of prime importance (Paustian et al., 2016). In the present study, the microbial population highly varied in the rhizosphere soils collected from different tuber crops and their varieties. Among the organisms enumerated, the bacterial population was the highest followed by actinomycetes and fungi population (Table 1). Among the rhizosphere soils of sixteen varieties of tuber crops analysed, maximum bacterial (313 x 10^6 $cfug^{-1}$ soil) and fungal (131 x 10^4 $cfug^{-1}$) populations were harbored by white yam variety, Sree Priya and the population was significantly high compared to all other varieties. The bacterial population in rhizosphere soils of the variety, Sree Priya was followed by sweet potato varieties, Bhu Krishna and Bhu Sona (27.3 x10^6 $cfug^{-1}$ soil and 25 x10^6 $cfug^{-1}$ soil respectively). Whereas, fungal population in rhizosphere soils of the variety, Sree Priya was followed by Sree Neelima and cassava varieties, Sree Swarna and Sree Reksha. Highest population of actinomycetes was noted in rhizosphere soils of Sree Arun (46.7 x10^6 $cfug^{-1}$ soil) and Sree Reksha(46.3 x10^6 $cfug^{-1}$ soil). On contrary to the high bacterial and fungal populations in rhizosphere soils of Sree Priya, least number of actinomycetes was noted in the variety. Irrespective of the varieties and types of tuber crops, the rhizosphere microbial population differed significantly. Microorganism numbers vary in and between different soil types and conditions, with bacteria being the most numerous. Bacterial population and their composition result from the interaction between soil type, plant species and its rhizosphere localization (Vieria and Nahas, 2005). Previous studies have demonstrated that the bacterial diversity in rhizosphere could be influenced by many factors, such as soil type, nutrition, management practice, soil properties, and varietal differences within a species, plant age, plant species and plant genotype (Buyer et al., 2010). Here, all the varieties of tuber crops were grown in the same field, which excludes the factors like difference in soil type, soil properties etc. It suggests that the variation in microbial population observed among varieties of tuber crops under this study would have influenced by plant species and plant genotype. There is an increasing evidence for the existence of genetic variation in the regulation of plant-microbe interactions (Wile et al., 2018). Microbial species and

Table 1. Variability in microbial population from rhizosphere samples collected from different varieties of tropical tuber crops

Crop	Variety	Bacteria (x 10^6 $cfug^{-1}$ soil)	Fungi (x10^4 $cfug^{-1}$ soil)	Actinomycetes (x 10^5 $cfug^{-1}$ soil)
Sweet Potato	Sree Arun	9.0 (6.83*)	10.3 (5.01)	46.7 (6.66)
	Kanhanhad	13.3 (7.07)	14.7 (5.16)	36.0 (6.56)
	Bhu Sona	25.0 (7.39)	5.7 (4.75)	35.3 (6.55)
	Bhu Krishna	27.3 (7.41)	5.3 (4.73)	23.7 (6.37)
	Sree Kanaka	7.3 (6.83)	7.3 (4.80)	39.3 (6.59)
Elephant foot yam	Sree Padma	19.3 (7.27)	6.0 (4.74)	25.0 (6.28)
	Gajendra	9.3 (6.79)	10.7 (5.02)	22.7 (6.33)
	Sree Athira	13.7 (7.02)	9.0 (4.90)	32.3 (6.50)
Cassava	Sree Reksha	13.0 (7.11)	16.7 (5.21)	46.3 (6.66)
	Sree Pavithra	6.7 (6.75)	2.0 (4.26)	17.7 (6.23)
	Sree Swarna	4.7 (6.66)	18.3 (5.26)	33.7 (6.51)
Lesser yam	Sree Latha	1.7 (6.20)	7.0 (4.84)	28.7 (6.45)
	Sree Kala	15.3 (6.92)	13.0 (5.10)	33.3 (6.51)
Greater yam	Sree Neelima	1.3 (6.10)	22.7 (5.35)	36.0 (6.55)
White yam	Sree Priya	313.0 (8.50)	131.0 (6.09)	2.3 (5.36)
	Sree Subhra	4.3 (6.48)	8.3 (5.01)	34.0 (6.53)
	SE (d)	0.256	0.136	0.115
	HSD at 1%	1.122	0.5944	0.5058

*Values in parentheses are log transformed values

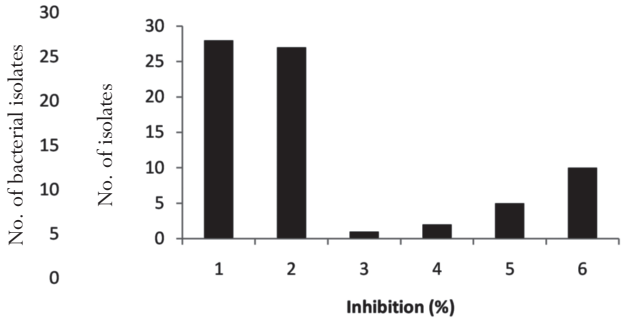
their population in the rhizosphere soil contribute considerably in maintaining health of the crops (Acosta et al., 2010). This includes changes in yield, timing of developmental phases and tolerance of biotic and abiotic stresses. Microorganisms affect plant tolerance to drought (Fitzpatrick et al., 2018) and disease (Bakker et al., 2018), enhance the plant's ability to acquire nutrients (Friesen et al., 2011) and impact yield (Busby et al., 2017).

The array of organisms also differed among the varieties. Maximum variability in types of bacteria was noted with Sree Priya (8.5) followed by Bhu Krishna (7.4) and Bhu Sona (7.39). Maximum number of fungal species were noted in Sree Priya (6.09) followed by Sree Neelima (5.35). The types of actinomycetes obtained from different varieties did not show much variation and all the varieties except Sree Priya showed six morphologically different types of actinomycetes. Morphologically dissimilar organisms were sub-cultured and there were 73 bacterial, 43 fungal and 34 actinomycetes isolates. The genera, *Bacillus* were the most dominant bacterial isolates. Whereas, *Trichoderma, Aspergillus* and *Penicillium* were the predominant fungi isolated from the rhizosphere.

Screening of isolates against *Sclerotium rolfsii*

a. Dual culture method

All the 150 isolates were screened for their ability to suppress mycelial growth of *S. rolfsii*. In preliminary screening, among the seventy three bacterial isolates screened, 75% of the isolates (55 isolates) showed <25% inhibition. 15 isolates exhibited >75% inhibition and 10 isolates among them completely arrested the growth of *S. rolfsii* (Fig.1). Out of the ten isolates, which had 100% inhibition in preliminary screening, seven were obtained from rhizosphere soils of sweet potato. Similarly, four out of 5 isolates, which showed inhibition between 75 to 99%, were also obtained from rhizosphere soils of sweet potato. Fungal isolates did not show good inhibition against the tested pathogen while one isolate (obtained from Bhu Krishna) had >50% inhibition (Fig. 2). Actinomycetes showed good inhibition and 12 out of 34 isolates tested completely inhibited the mycelial growth of the pathogen in the preliminary round of screening (Fig. 3). The twelve isolates were, 4 from sweet potato; 2 from elephant foot yam; 3 from cassava and 3 from yam rhizosphere soils.

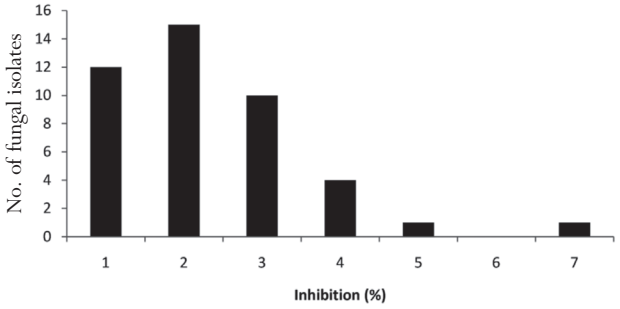


1-0, 2 - <25, 3- 26 to 50, 4 -51 to 75, 5- 76 to 99, 6- 100

Fig. 1. Percent inhibition of mycelial growth of *S. rolfsii* by bacterial isolates

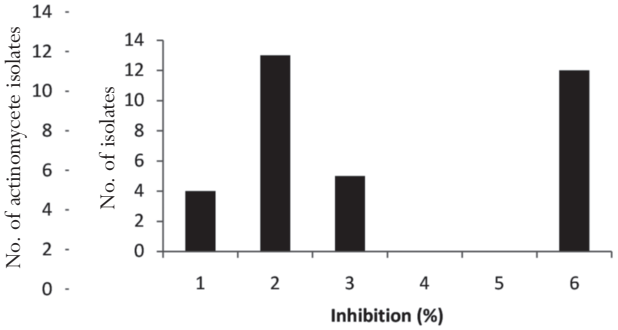
Production of diffusible and volatile inhibitory metabolites by the potent isolates

The isolates that showed a high percentage of inhibition on repeated screening by adopting dual culture method were tested for their ability to produce diffusible and volatile inhibitory metabolites against the pathogen (Fig. 4 and Table 2). The selected 8 bacterial isolates showed



1-0, 2- 1 to 10, 3- 11 to 20, 4 -21 to 30, 5- 31 to 40, 6- 41 to 50, 7- 51 to 60

Fig. 2. Percent inhibition of mycelial growth of *S. rolfsii* by fungal isolates



1-0, 2- <25, 3- 26 to 50, 4 -51 to 75, 5- 76 to 99, 6- 41 to 50, 7- 51 to 60

Fig. 3. Percent inhibition of mycelial growth of *S. rolfsii* by actinomycetes isolate

Table 2. Comparative inhibition shown by the bacterial isolates under different methods of screening of antagonistic potential

Isolates	Crop	% Inhibition (Dual culture)	% inhibition (Diffusible metabolites)	% inhibition (Volatile production)
KD1	Sweet potato	88.8	90.0	0.0
KD7	Sweet potato	88.8	26.6	5.5
KR3	Sweet potato	98.8	64.4	0.0
KR1	Sweet potato	100.0	93.3	0.0
KR4	Sweet potato	87.7	81.1	0.0
SA5	Sweet potato	88.8	55.5	27.7
SP5	Cassava	86.6	43.3	0.0
AP4	Elephant foot yam	100.0	83.3	0.0

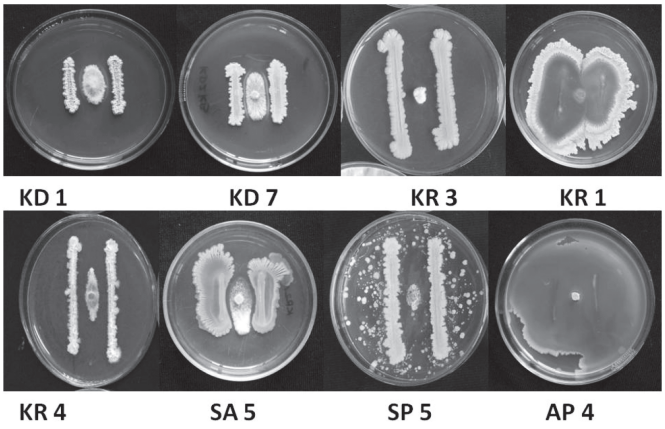


Fig. 4. Mycelial growth inhibition of *S. rolfsii* as affected by bacterial isolates (dual culture)

consistency in their reaction by showing antagonism to the target pathogen by these methods also. Among the three methods adopted for screening, maximum inhibition was obtained in dual culture. *Bacillus* species can produce various kinds of diffusible and volatile compounds with strong inhibitory activity against plant pathogens (Hossain et al., 2016). Volatile compounds can promote plant growth with antifungal activity (Arrebola et al., 2010). But, in the study, the volatiles produced by the isolates contributed least to the suppression exhibited by the isolates.

Production of Indole Acetic Acid

The isolates, which showed excellent antagonistic potential, were further evaluated for their efficiency for plant growth promotion, nitrogen fixation and P and K solubilisation. Isolates with these additional qualities will be a boon for organic cultivation since their application will help the growers to skip chemical fertilizers along with synthetic pesticides. The production of auxin (IAA) has been recognized as an important factor in direct plant-growth-promoting abilities of rhizosphere bacteria. All the eight potent bacterial isolates were able to produce IAA. The concentration of IAA produced by the bacterial isolates ranged between 113-187µgml^{-1} (Fig. 5). There was significant difference in the quantity of IAA produced among the isolates. The isolate KR 3 produced highest amount of IAA whereas isolates KD 1 and SP5 produced very low amount of IAA in culture medium. IAA is produced by many plant growth promoting rhizobacteria (PGPR) such as *Pseudomonas* and *Acinetobacter* strains which result in enhanced uptake of iron, zinc, magnesium, calcium, potassium and phosphorous by crop plants. Indole-3-acetic acid (IAA) is the most abundant endogenous auxin, which has roles in stem elongation and root growth. The auxin level is usually higher in the rhizosphere, where high percentage of rhizosphere bacteria is likely to synthesize auxin as secondary metabolites because of the rich supplies of root exudates. They stimulate the proliferation of lateral roots that increase nutrient absorbing surfaces and results in better assimilation of water and nutrients from the soil. This in turn significantly increases the shoot and root length of plants (Egamberdieva, 2011).

Nitrogen fixation

Nitrogen fixation can be carried out by several associative and free-living microorganisms in the

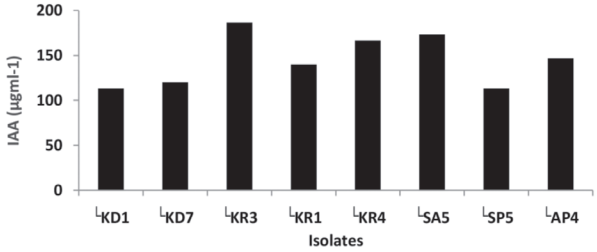


Fig. 5. Indole acetic acid production by bacterial isolates

rhizosphere of plants and it is recognized to play an important role in nitrogen nutrition of plants (Boddey et al., 1996). Jensens Medium is recommended for detection and cultivation of nitrogen-fixing bacteria. Results showed that except the isolate KR 1, all other isolates were capable of fixing nitrogen by showing good growth in Jensen's medium (Table 3). The plant associated microbes play a key role in plant health. However, a systematic picture of how and to what extent plants can shape their own detrimental or beneficial microbiome remains to be drawn. The organisms in the rhizosphere regions of the nutrient efficient tuber crop varieties may be explored thoroughly to establish the relationship between plant–microbe interactions and nutrient efficiency.

Phosphate solubilization

Phosphate solubilizing bacteria (PSB) are beneficial bacteria capable of solubilizing inorganic phosphorus from insoluble compounds (Chen et al., 2006). Phosphorous solubilization ability of rhizosphere microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition. However, the result of the present study showed that none of the isolates were able to form clear zone around the colonies on Pikovskya medium indicating their inefficiency to solubilize Phosphate.

Potassium solubilization

There is considerable population of bacteria in soil and in plant rhizosphere with ability to solubilise Potassium. Solubilization of K by KSB from insoluble and fixed forms is an import aspect regarding K availability in soils. K solubilization is carried out by wide range of saprophytic bacteria, fungal strains and actinomycetes (Ahmad et al., 2016; Bakhshandeh et al*.,* 2017). In the present study, none of the isolates tested were able to form clear zone around the colonies on SSK medium indicating their inefficiency to solubilize Potassium.

Table 3. The nitrogen fixation, phosphate solubilization and potassium solubilization activity of the bacterial isolates

Isolates	Nitrogen fixation	Phosphate solublization	Potassium solubilization
KD 1	+	-	-
KD 7	+	-	-
KR 3	+	-	-
KR 1	-	-	-
KR 4	+	-	-
SA 5	+	-	-
SP 5	+	-	-
AP 4	+	-	-

Molecular characterization of potent organism

Bacterial isolates were identified by 16S rRNA gene sequencing. The genomic DNA of 8 potent bacteria was extracted and amplification of 16S rRNA gene was performed with the total DNA isolated with primers forward 8F and reverse 1492R primer for 34 cycles. By comparing with 1kb plus marker, the size of the DNA band is approximately 1500bp (Fig 6). The phylogeny and the family of the strains were accessed using BLAST search. The sequences were submitted to Gen bank and accession numbers were obtained. Identification of the isolates revealed that all the isolates come under the genera *Bacillus* (Table 4). Numerous species of the genus *Bacillus* have been identified as plant-growth promoting

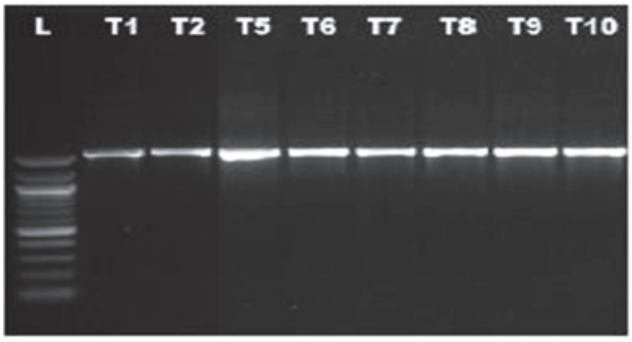


Fig. 6. Amplification of bacterial isolates Lane L- marker, T1 to T10 bacterial isolates

Table 4. Taxonomic affiliation of isolates and Gen bank accession number

Isolates	Identified organism	Accession number (NCBI)
KD 1	*Bacillus siamensis*	MH762179
KD 7	*B. siamensis*	MH819521
KR 3	*B. amyloliquefaciens*	MH819553
KR 1	*B. pumilus*	MH819554
KR 4	*B. amyloliquefaciens*	MH819556
SA 5	*B.halotolerans*	MH819557
SP 5	*B. subtilis*	MH819558
AP 4	*B. altitudinis*	MH824153

bacteria (PGPB) and/or biocontrol agents (BCA). The most commonly studied plant growth promoting bacteria (PGPB)/BCA are *B. amyloliquefaciens, B. licheniformis*, and *B. subtilis* (Khan et al., 2017). In the present study, 8 potent isolates belong to six species of *Bacillus* and the bio-control potential of all the 6 species were reported earlier. Seed treatment with *B. siamensis* showed that the bacterial isolate increased plant height by 14.66 to15.68%, fresh shoot weight by 34.5 to 65.09% and fresh root weight by 75.3 to 92.48% over the untreated control in tomato (Amanul Islam et al., 2019). *Bacillus amyloliquefaciens* exhibited predominantly antagonistic activities against a broad range of soil borne pathogens *viz*., *Fusarium oxysporum*, *Verticillium dahliae*, *F. solani*, *Phytophthora parasitica*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani* (Li et al., 2014). *B. pumilus* suppressed *Pseudoperonospora cubensis*, the pathogen of downy mildew of cucumber (El Gremi et al., 2013). *Bacillus* strains, which often produce a range of antimicrobial cyclic lipopeptides, including iturins, fengycins and surfactines antibiotics in the family iturin, especially iturin A, is most famous for biocontrol activity.

Due to the close symbiotic relationship with their microbiome, plants have recently been recognized as meta-organisms. Like humans and other eukaryotic hosts, plants also harbor a 'second genome' that fulfils important host functions including nutrient uptake (Ortiz et al., 2015), defense (Busby et al., 2016) and protection against biotic and abiotic stress. Microbiome plays a key role in reprogramming the defense responses of plants (Berg et al., 2015). All six species obtained in this study possess bio-control potential. However, considering the nature of various species to become human/animal pathogen, *B. amyloliquefaciens* (KR3 and KR4) and *B. subtilis* (SP5) can be effectively utilized for the management of different fungal diseases in tuber crops.

Conclusion

Rhizosphere microbes are occupied in many processes that decide agricultural soil productivity, including preservation of soil structure, nutrient recycling, disease control and degradation of pollutants. For sustainable agriculture maintenance, soil dynamic nature is of prime importance (Paustian et al., 2016). The dynamic nature of soil is a direct expression of soil microbes, bio-mineralization, and synergistic co-evolution with plants. From this study, it is clear that rhizosphere soils of tuber crops harbours a large number of microbes. It varied with the crop as well as varieties, even though all the crops and varieties were cultivated in the same field. The microbiome influences plant health and development, playing a vital role at all stages of plant growth. Microorganisms affect plant tolerance to drought (Fitzpatrick et al., 2018) and disease (Bakker et al., 2018), enhance the plant's ability to acquire nutrients (Friesen et al., 2011) and impact yield (Busby et al., 2017).

In the present study, it was found that a large number microbes are associated with sweet potato rhizosphere. Ranney et al. (2020) reported that studying sweet potato microbiome ecology may reveal paths to engineer the microbiome to improve sweet potato yield, and or combat sweet potato pests and diseases. They elucidated underpinnings of sweet potato microbiome community assembly, quantified microbiome composition variance within a single farm and revealed the variability in microorganisms associated with sweet potato. The rhizosphere organisms are capable of suppressing pathogens; promoting plant growth; nitrogen fixation; P and K solubilisation etc. *Bacillus* is the predominant genera of bacteria among rhizosphere organisms associated with the rhizosphere of tuber crops with plant growth promotion and pathogen suppression. The genus, *Bacillus* is dominant in the rhizosphere of sweet potato (Tangapo et al., 2018). Studies on the survival of *Bacillus* spp in the rhizosphere of tuber crops, soil moisture and pH requirement for the proliferation warrants detailed study. It is recommended that the bacteria, *B. amyloliquefaciens* and *B. subtilis* isolated from the rhizosphere of sweet potato and cassava may be utilized for bio-intensive management of diseases of tuber crops and growth promotion.

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