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Bacilli consortia positively regulates agronomic and growth traits in rice

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Abstract
In our study, soil samples from the rhizosphere of various uncultivated weeds were collected from fifteen different locations of Gujarat. Heat treatment was given at 65°C for 20 minutes prior to initial screening for spore-forming *Bacilli* spp. Among them, 20 nitrogen-fixing (NFB), 27 phosphate solubilizing (PSB) and 15 potassium mobilizing (KMB) isolates were screened primarily. After molecular identification only *Bacilli* isolates were further selected and characterized. Three superior *Bacillus* isolates were selected from each category by secondary screening. All three isolates for nitrogen fixation were found *nif* positive, while *Bacillus megaterium* (NAUN2) showed maximum 2.04 µmol/ml ammonia production followed by *Bacillus sp.* (NAUN1) and *Lysinibacillus macroides* (NAUN3) with 0.64 and 0.99 µmol/ml, respectively. *Bacillus sp.* (NAUP1) gave maximum 401.94 µg/ml phosphate solubilization on Pikovaskya broth media after third day while *Bacillus sp.* (NAUP2) and *Brevibacillus sp.* (NAUP3) showed 376.74 and 308.16 µg/ml phosphate solubilization, correspondingly. *Lysinibacillus macroides* (NAUK2) showed maximum 228.14 µg/ml potassium mobilization on Aleksandrov after the twentieth day while *Lysinibacillus macroides* (NAUK1), *Bacillus megaterium* (NAUK3) and *Bacillus sp.* (NAUP1) had effective 196.91, 158.18, 59.33 µg/ml potassium mobilization, respectively. Interestingly, no selected *Bacilli* isolates from the consortia were found to have inhibitory effect on other isolates in compatibility test. Out of the nine *Bacillus* isolates NAUK1, NAUK2, NAUK3 were found to be effective against rice pathogen *Magnaportha oryzae* on PDA plates. All the nine isolates were checked for IAA and siderophore production. Isolate NAUN1, NAUP3, NAUK1, NAUK2 and NAUK3 showed 37.2%, 17.9%, 33.6%, 38.9%, 48.2% siderophore production on CAS medium after 72 hrs, respectively. Isolate NAUP3 showed maximum 146.68 µg/ml IAA production while NAUN1, NAUN3, NAUP1, NAUK2 and NAUP2 showed 99.37, 99.37, 114.57, 101.46, 93.48 µg/ml IAA production, respectively after the seventh day. Rice seeds were inoculated with individual isolate and different *Bacilli* consortia, which significantly improved growth parameters as compared to control in pot study after 60 days of sowing. 100% RDF + NPK consortia gave significantly increased root length (11.65%), shoot length (10.28%), no. of leaves (17.39%), leaf area (11.68%) and chlorophyll content in leaf (4.76%). Similar trends were observed when the data was compared with absolute control. The spore forming *Bacilli* consortia was able to survive a wide range of temperature and pH fluctuations and found effective N-fixers, P-solubilizers, K-mobilizers, siderophore producers, IAA producers with antagonistic activity against rice pathogen *Magnaportha oryzae.*

Keywords: *Bacilli*, NFB, PSB, KMB, rhizosphere

Introduction
Plants grow in complex environmental conditions having plenty of microorganisms. Soil microorganisms show effects on plants ranging from favorable effects caused by many soil microorganisms to hazardous effects caused by plant pathogens. The exact mechanisms by which PGPR enhances the plant growth promotion is not known but it influences the plant growth in many ways, some of which are (i) Increasing solubilization of nutrients thereby providing bioavailable forms of potassium, phosphorus, nitrogen and other nutrients and trace elements [1]. (ii) Aiding or enhancing a symbiotic nitrogen fixation [2] or indirectly affecting symbiotic nitrogen fixation, nodulation or nodule occupancy [3]. (iii) Affecting the concentration of plant growth promoters and production of phytohormones like auxins, cytokinins and gibberellins (vi) Synthesis of antibiotic, fungicidal compounds and other pathogen-depressing substances such as siderophores, cyanide and chelating agents that protect plants from diseases [4]. (v) These organisms can also increase plant tolerance to various environmental adverse conditions and stress like high temperature, flooding [5],

salt stress [6] etc. (vi) Rhizobacteria containing ACC-deaminase increased growth and yield of plants. Historically, several approaches for restoring the soil health and maximizing plant growth have been used in agriculture. Such approaches include amending soil with organic materials, using crop rotations, and using cover crops in between growing seasons. The main issue from the modern agriculture perspective is that adding large amounts of organic material in the soil, using cover crops, and crop rotations are not economically feasible, especially in high production regions such as Gujarat. A more practical and cost-effective alternative approach is to increase the soil microbial populations by providing the treatment of plants and soils with cultured microbial communities. One restraint of the biofertilizer is that most of the time there is large variation in soil salinity, pH, and an atmospheric temperature where most of the biofertilizers cannot tolerate such harsh environmental conditions that lead to the gradual loss and washout of microbes from the soil. Hence, plants treated with beneficial *Bacilli* typically exhibit enhanced "nutrient utilization efficiency". This response means that at a given level of soil fertility, plants treated with *Bacilli* acquire more nutrients from the soil and that results in higher levels of key nutrients in plant tissues. The demonstration that microbial inoculants can increase nutrient utilization efficiency and ultimately results in reduced levels of chemical fertilizers' use. Several case studies support the notion that applied fertilizers could be reduced by 25% when useful bacterial cultures are applied to plants and that the quality of plant growth and yields can be maintained at levels equivalent to those that result with full fertility rates.

Materials and methods

For the present study, the soil samples were collected from the rhizosphere of various uncultivated weeds lacking of any externally supplied nutrients from fifteen different locations of Gujarat as shown in Table 1. Weeds were directly uprooted and the soil stuck on the root tips was collected. Five samples were collected from each sampling site and pooled together to make the composite sample. Samples were collected in aseptic bags, labelled, and immediately transported under cold conditions (4ºC) to the lab until further processing for isolation of plant growth-promoting *Bacilli*.

Isolation, purification, and identification of NFB, PSB, and KSB

1g sample was suspended in 5 ml autoclaved NaCl saline. After sedimentation of solid particles, clear supernatant was collected in microfuge tubes. Dilutions were made up to 10^{-8} and heat treatment was given at 65°C for 20 minutes for the selection of spore-forming *Bacilli* [7]. After the heat treatment, 100µL of the diluted suspension was spread over in selective agar media plates.

Media

For the isolation of NFB, PSB, and KMB, Jensen's nitrogen-free (JNF) agar media, Pikovaskya (PVK) medium agar, and Aleksandrov agar media with mica powder (MP) as a sole source of potassium were used, respectively [8, 9, 10].

Isolation of NFB, PSB, and KMB

For the isolation of NFB, PSB and KMB, heat-treated diluted samples were spreaded over JNF, PVK, and Aleksandrov with MP agar media plates, respectively and growth characteristics observed after incubation at 30°C for 7 days. Positive isolates in the case of PSB and KMB developed transparent zones against the opaque background. Colonies were selected and isolated based on zone formed around the colonies [11, 12]; only those isolates were selected whose Solubilising Index (SI) was higher comparatively. Solubilising index (SI) of the isolates was determined [13]. Further isolates were inoculated into respective media and a decrease in the pH was again monitored.

Identification of the bacterial isolates

All the selected isolates were examined for Gram's reaction, as per the standard procedures [14].

Identification of the isolates using the Biolog system

Biolog carbon substrates utilization patterns Biolog GP2 MicroPlates (Biolog, Inc., Hayward, CA, USA) were inoculated in duplicate using the standard procedures and were incubated at 30-35ºC for 24-36 hrs. The optical density at 590 nm produced from the reduction of tetrazolium violet in each well was read after 12, 24, 36 and 48 hrs using the Biolog microplate reader in conjunction with the MicroLog software (Release version 4.0). The reaction profile of the tested strains was compared with that of the related bacteria in the Biolog 24 hr database using the Biolog UPGMA (Unweighted Pair Group Method using Arithmetic Average) cluster analysis program [15].

Molecular characterization of the rhizospheric isolates

The selected rhizospheric isolates were identified through 16S rDNA gene sequencing [16].

Quantitative estimation of NFBs

Ammonia production

All the *Bacilli* isolates were tested for ammonia production on peptone water broth using the method described by Cappuccino and Sherman [9].

nifH amplification

To select a suitable isolate having better nitrogen-fixing potential, screening of bacterial isolates obtained from 15 different regions, were done based on the presence of the *nifH* gene 360 bp fragment from extracted genomic DNA using Pol F - TGCGAYCCSAARGCBGACTC and Pol R – ATSGCCATCATYTCRCCGGA which is then followed by PCR amplification [17].

Secondary screening of PSBs

Quantitative estimation of solubilized P by *Bacilli* isolates were done by the vanadomolybdophosphoric yellow color method in pikovskaya's broth media containing 5000 µg/ml tricalcium phosphate [9].

Secondary screening of KMBs

The *Bacilli* isolates showing a better zone of solubilization on Aleksandrov agar plates were further checked for their capability to release K from broth media (with 1% muscovite mica) using method described by Sugumaran and Janarthanam [10].

Biochemical characterization of NFB, PSB, and KMB:

Selected isolates were biochemically characterized by different biochemical test which includes:

Siderophore production and quantification assay

All the *Bacilli* isolates competent in nitrogen fixation, phosphate solubilization, and potassium mobilization were

checked for siderophore production using method given by Schwyn and Neilands on the Chrome-Azurol S (CAS) agar medium while the quantification of siderophore production was done by the method described by Meyer and Abdallah Meyer using succinate broth media [18].

IAA production:
The selected *Bacilli* isolates were inoculated in LB medium supplemented with 1 $mgml^{-1}$ of tryptophan to determine IAA production by the method described by Brick *et al*. [19].

Compatibility test
Compatibility of all the *Bacilli* isolates as consortia was tested by simultaneously streaking all the isolates on Nutrient Agar (NA) medium on a single petri dish and incubated at 30°C overnight. If a single isolate inhibits the growth of any of the other isolates, it indicates the isolate does not qualify as consortia [20].

Antagonistic activities against rice pathogenic fungi
The antagonistic effects of all nine *Bacilli* isolates were checked against the fungal pathogen of rice i.e., *Magnaportha oryzae* For this, the *Bacilli* isolates were streaked at a distance of 3.5 cm from the rim of individual petri plate containing potato dextrose agar (PDA) medium and then 6 mm mycelial disc from a 7-day old PDA culture of *Magnaportha oryzae* was placed on the center of the petri dish and the plates were incubated at 30°C for 4-7 days [21].

Evaluation of plant growth promotion in rice by inoculation of NPK fixing *Bacillus*
Healthy seeds of GNR-3 variety obtained from MCRS, NAU, Surat were washed thoroughly with distilled water followed by the surface sterilization process in laminar airflow using 0.1% $HgCl_2$ solution for 4 min followed by 70% ethanol for 10 min. Then, rice seeds were washed thoroughly with sterile distilled water. Surface sterilized seeds were coated with bacterial culture by incubation for 3 hrs followed by drying. Culture coated seeds were sown in the pot. All recommended practices and plant protection measures were adopted to obtain healthy plants. The observations were recorded viz, shoot length, root length, leaf area, no. of leaves, and chlorophyll content [22].

Statistical Analysis
A completely randomized design (CRD) was used for all experiments in this paper, with three replications for each treatment. Here the data represented are repeated at least twice with similar results. Statistical analysis of the various observations and measurements was carried out using analysis of variance (ANOVA).

Result and Discussion

Isolation of NFB, PSB, and KMB
As shown in Table 1, a total of 15 rhizospheric samples were collected from different uncultivated weeds across Gujarat such as Surat, Idar, Palanpur, Jamnagar, and Junagadh for the isolation of NFB, PSB, and KMB. As there was no added application of fertilizers, so the plants growing in that environment might be dependent on bacteria for the availability of nutrients for the growth of plants. Rhizospheric samples were diluted and heat treatment at 65ºC for 20 minutes was given, so the only spore-forming *Bacilli* survive which were further screened on selective agar media plates. Total 27 NFB, 20 PSB, and 15 KMB were picked and reinoculated into the same selective media agar plates to recheck its efficiency. From primary screening, the best 3 isolates from each category were further tested for its efficiency.

Table 1: Collection of samples from different sampling site.

Sample Identity	Plant	Area	Location
NAU1	*Echinochloa colonum*	Petrol pump, City Light Road, Surat	21.17° N, 72.80° E
NAU2	*Eragrostis pilosa*	Suvali beach, Surat	21.16° N, 72.62° E
NAU3	*Digitaria sanguinalis*	Plot No. 6113, Sachin GIDC, Surat	21.09° N, 72.86° E
NAU4	*Cyperus rotundus*	School parking wall, Adajan Surat	21.21° N, 72.79° E
NAU5	*Digitaria sanguinalis*	Dumping yard, Adajan, Surat	21.20° N, 72.78° E
NAU6	*Setaria verticillata*	Sumul dairy road, Surat	21.22° N, 72.84° E
NAU7	*Parthenium hysterophorus L.*	Juna bazar, Idar	23.60° N, 72.96° E
NAU8	*Gnaphalium indicum L.*	Tower, Idar	23.85° N, 73.00° E
NAU9	*Euphorbia hirta L.*	Town Hall, Idar	23.84° N, 73.01° E
NAU10	*Cassia sophera*	District Police Headquarter, Jamnagar	22.48° N, 70.05° E
NAU11	*Parthenium hysterophorus L.*	Shantinagar, Jamnagar	22.49° N, 70.06° E
NAU12	*Acanthospermum hispidum*	Green city, Jamnagar	22.43° N, 70.07° E
NAU13	*Acanthospermum hispidum*	Fort, Junagadh	21.52° N, 70.47° E
NAU14	*Cassia sophera*	Baug, Junagadh	21.51° N, 70.45° E
NAU15	*Acanthospermum hispidum*	APMC, Palanpur	24.15° N, 72.44° E

Characterization of rhizobacteria
All 9 isolates were assessed for gram reactions as shown in Fig. 1. All the isolates were found gram-positive rod shape which is a distinctive characteristic of *Bacilli* isolates.

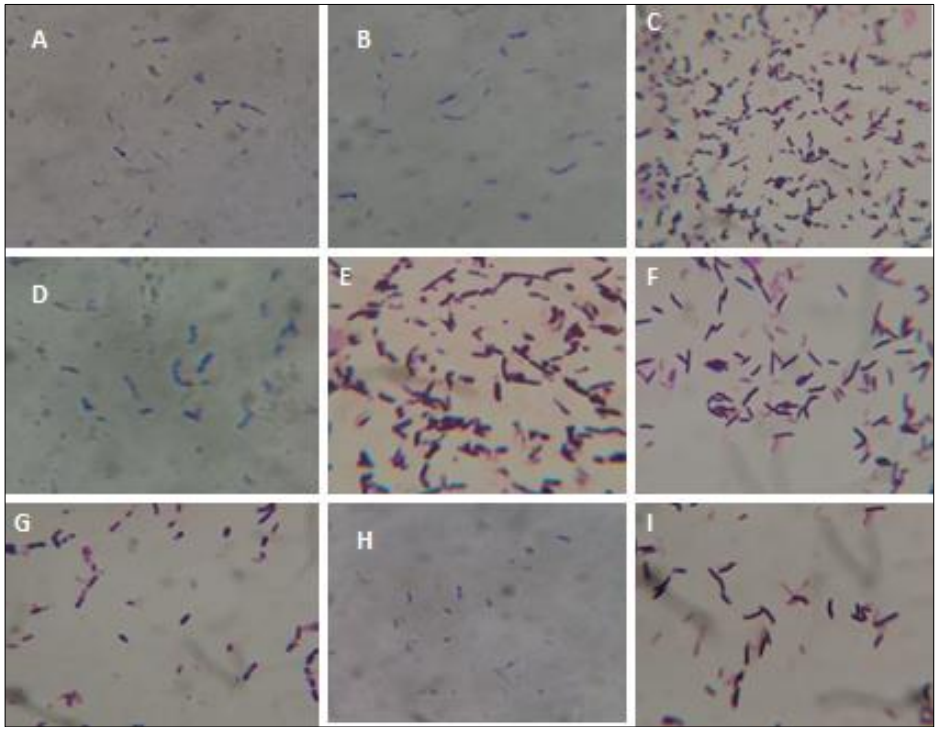

Fig 1: Gram staining of selected *Bacilli* isolates. (A. NAUN1, B. NAUN2, C. NAUN3, D. NAUP1, E. NAUP2, F. NAUP3, G. NAUK1, H. NAUK2, I. NAUK3)

Identification of selected isolates using Biolog system

The Biolog system is one of the most authentic and versatile system for the identification of various microorganisms on the basis of its sugar and metabolic profile. On the basis of 26 different sugar utilization (as shown in Fig. 2) in the Biolog system for 36-48 hrs incubation, all isolates were found to be of *Bacilli* species (as shown in Table 2). Since none of the isolates had a cent percent similarity between them, all the isolates were considered distinct.

	NAUN1	NAUN2	NAUN3	NAUP1	NAUP2	NAUP3	NAUK1	NAUK2	NAUK3
D-Maltose									
D-trehalose									
D-cellobiose									
Gentibiose									
Sucrose									
D-Turanose									
D-Raffinose									
α-D-Lactose									
D-Melibiose									
β-Methyl-D-Glucoside									
D-Salicin									
N-acetyl-D-Glucosamine									
N-acetyl-β-D-Mannosamine									
N-acetyl-D-Galactosamine									
N-acetyl-Neuraminic acid									
α-D-Glucose									
D-Mannose									
D-Fructose									
D-Galactose									
3-Methyl glucose									
D-Fucose									
L-Fucose									
D-Rhamnose									
D-Sorbitol									
D-Mannitol									
D-Arabitol									
Myo-inositol									

RED=HIGHLY POSITIVE
YELLOW=MODERATE POSITIVE
BLUE=NEGATIVE

Fig 2: Sugar profile of all 9 isolates on Biolog system.

Table 2: Identification of bacteria by Biolog system sugar profile.

Sequence Name	Biolog Sequence Name	Blast Sequence Id	Blast Organism Name
NAUN1	*Bacillus zhangzhouensis*	MN647597	*Bacillus sp.*strain 01105
NAUN2	*Bacillus megaterium*	MN647598	*Bacillus megaterium*
NAUN3	*Lysinibacillus macroides*	MN647599	*Lysinibacillus macroides*
NAUP1	*Bacillus aryabhattai*	MN647600	*Bacillus sp.*strain ZK5
NAUP2	*Bacillus megaterium*	MN647601	*Bacillus sp.*strain 5SB2
NAUP3	*Brevibacillus agri*	MN647602	*Brevibacillus sp.*strain RA5U1
NAUK1	*Lysinibacillus macroides*	MN647594	*Lysinibacillus macroides*
NAUK2	*Lysinibacillus macroides*	MN647595	*Lysinibacillus macroides*
NAUK3	*Bacillus megaterium*	MN647596	*Bacillus megaterium*

Molecular characterization

Using 16S rDNA sequencing, the molecular identification of all the selected 9 isolates was carried out. First, the genomic DNA of all the isolates was extracted (Fig. 3-I) and then its 16s rDNA region was amplified using 27F and 1492R pair of primers. The sequences of the isolates were analyzed using NCBI BLAST online homology search program. The size of the amplified 16s region of isolates was 1500 kb. As shown in Table 2, the results were obtained and then 16s rDNA sequences were subjected to multiple sequence alignment using Clustal Omega v1.2.1 by EMBL-EBI. The multiple distance matrix was further used to construct the phylogenetic tree using Clustalw 2.1 Phylogeny with the neighbor-joining (NJ) method (Fig. 3-II).

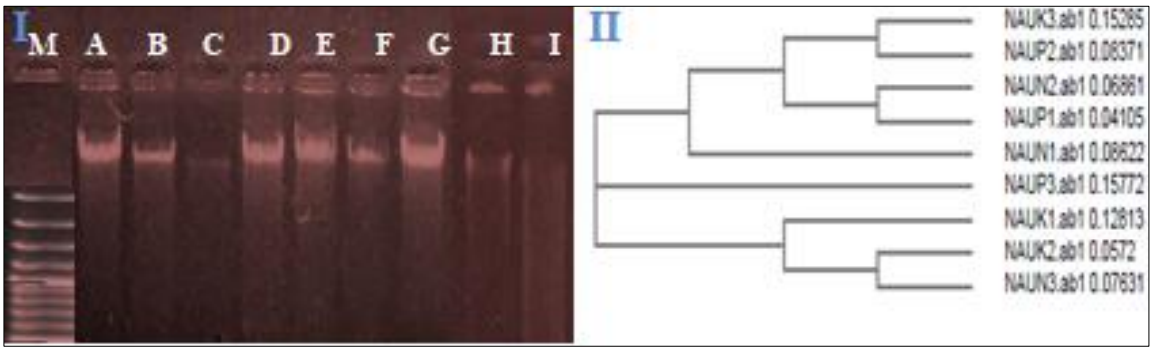

Fig 3: I) Bacterial genomic DNA isolated on agarose gel (1.2%) electrophoresis. (M. Marker-1 kb, A. NAUN1, B. NAUN2, C. NAUN3, D. NAUP1, E. NAUP2, F. NAUP3, G. NAUK1, H. NAUK2, I. NAUK3) II) Phylogenetic tree construction using Clustal Omega v1.2.1 by EMBL-EBI.

Secondary screening of NFB

Ammonia Production

Ammonia production has an important role to play in the accumulation of nitrogen and helps in promoting root, shoot growth along with biomass production which ultimately accelerates plant growth [9]. However, the production of ammonia also contributes to the antagonistic potential of rhizobacteria. In the following study, all three *Bacilli* isolates were efficient ammonia producers but the intensity varied significantly among the isolates. The ammonia production was observed in the range of 2.04 µmol mL^{-1} to 0.64 µmol mL^{-1}. The average ammonia production by all three *Bacilli* was 1.23 µmol mL^{-1} (Fig. 4).

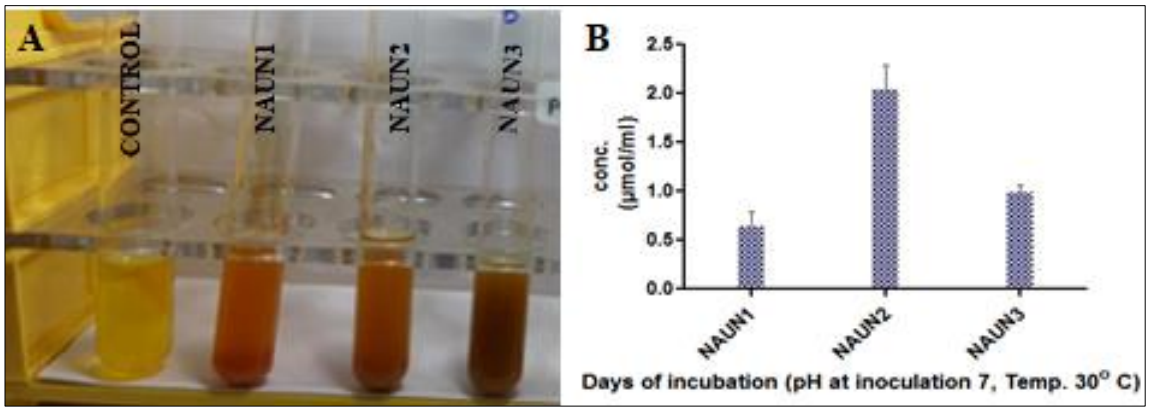

Fig 4: A) Ammonia production in peptone broth. B) Ammonia quantification on spectrophotometer at 450 nm.

***nifH* gene**

The *nifH* gene encodes the iron (Fe)-protein subunit of nitrogenase which is an integral part of a nitrogenase enzyme complex and is highly conserved among all nitrogen-fixing microorganisms. *nifH* is one of the earliest characterized and best known functional genes [23], its amplification using degenerate primers is a handy tool for confirming nitrogen-fixation potential of the isolates [24]. *nifH* PCR amplification was indicative that all 3 isolates NAUN1, NAUN2, and NAUN3 can fix nitrogen, produced the expected 360-bp amplification product (Fig. 5).

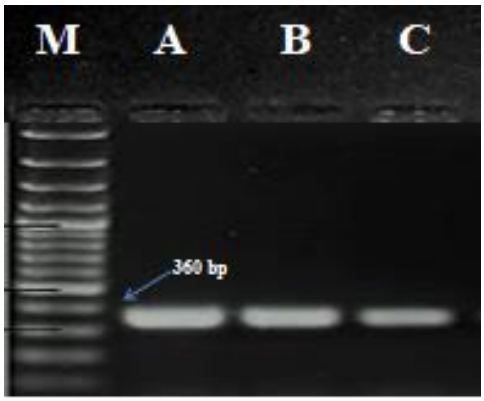

Fig 5: *nifH* gene PCR amplification (M. Marker-100 bp, A. NAUN1, B. NAUN2, C. NAUN3)

Phosphate solubilization

Microbes enhance the P availability to plants by mineralizing and solubilizing P in the soil [25]. The microbial biomass in soil contains a significant amount of P (typically 10-15 kg/ha, but as high as 100 kg P/ha), accounts for 2-5% of total P and around 10-15% of the soil organic P [26]. Plant growth substances produced by PSBs improve plant growth by their direct effects on plant metabolic processes. PSBs also induce the proliferation of lateral roots and root hairs that results in increased nutrient absorbing surfaces. Initially, the phosphate solubilization was less but as soon as the incubation period increased, all 3 *Bacillus* isolates exhibited the ability to solubilize calcium phosphate and help in making phosphorus available for plants to absorb by producing organic acids. As shown in Fig. 6, NAUP1 being the promising strain showed a maximum index of (5.23) followed by NAUP2 and NAUP3 which showed 5.08 and 4.95 SI respectively after the 7th day of incubation at 30°C on PVK agar medium. On PVK broth, NAUP1 being the most prominent showed maximum phosphate solubilization 401.94 µg/ml while NAUP2 and NAUP3 showed maximum phosphate solubilization 376.74 and 308.16 µg/ml respectively after 3rd day of incubation at 30°C and pH 7.

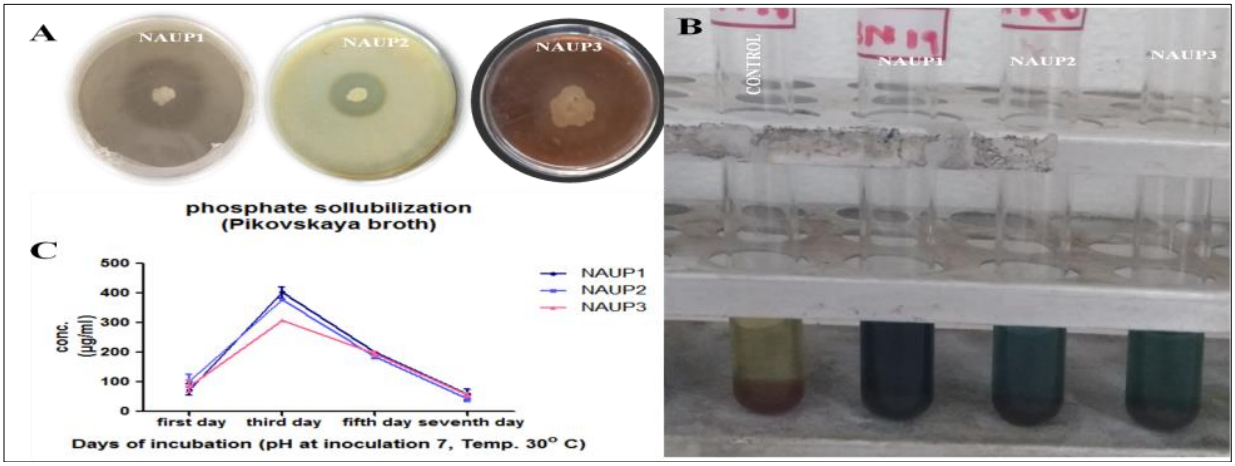

Fig 6: A) & B) Phosphate solubilization on PVK agar and broth. C) Graph representing quantification of phosphate solubilization at different time interval

Potassium mobilization

KMBs mobilize potassium to help plant in its growth promotion and also protect the plants from salinity injury by enhancing its growth-related characters such as stomatal conductance, electrolyte leakage and lipid peroxidation. Plant inoculated with KMBs also accumulates more type and number of soluble carbohydrates in leaves under salinity, which helps the plant to overcome osmotic stress [10].

NAUK2 being the promising KMB strain showed a maximum SI of (3.85) followed by NAUK1, and NAUK3 that showed 3.42, and 3.08 SI respectively after the 7th day of incubation at 30°C and pH 7 as indicated in Fig. 7, on Aleksandrov agar medium with mica powder as a sole source of potassium. NAUP1 being the most desirable showed a significant 2.92 potassium mobilization index along with its above mentioned phosphate solubilization characteristic. The amount of K released from the mica powder in the broth by the selected isolates was studied at 7, 15 and 20 days after incubation (DAI) in lab condition. As the days of incubation progresses, the amount of released K improved and the highest amount of released K observed at 20 DAI for all four different potassium mobilizers. The maximum K mobilization was observed in the case of NAUK2 i.e., 228.14 µg/ml while all other selected strains showed K mobilization above 50.00 µg/ml.

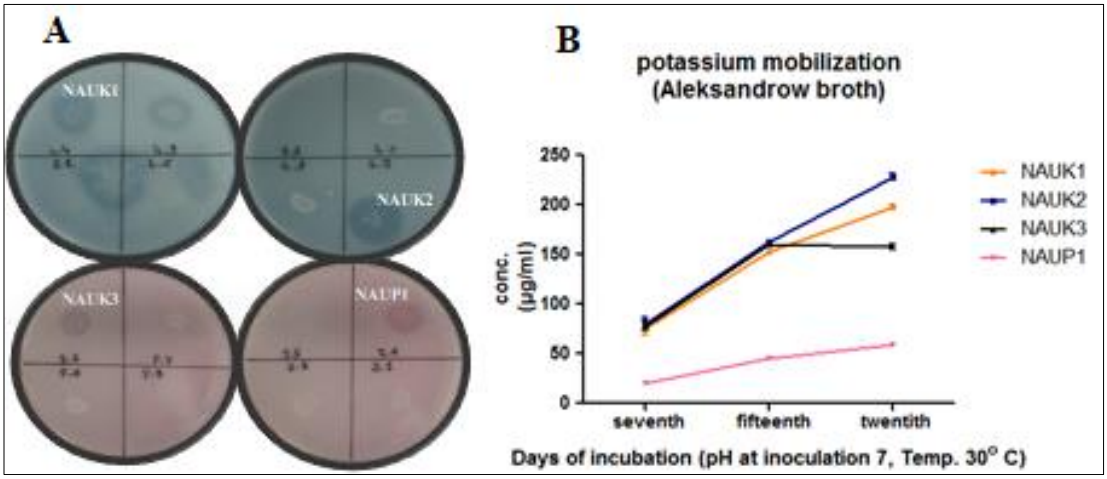

Fig 7: A) Potassium mobilization on Aleksandrov agar. B) Graph representing quantification of potassium mobilization at different time interval

IAA production

IAA is the core member of auxins' family produced by plants as it has an important role to play in numerous plant activities such as embryo development, leaf formation, root initiation and development, phototropism, geotropism, fruit development, abscission. Etc. IAA helps in the enhancement of root length with an increase in the number of root branches, root hairs and root laterals that aid in the uptake of nutrients from surrounding [27]. Here, all the *Bacilli* isolates were evaluated for their competence to synthesize IAA in minimal medium supplemented with trp+ at 30°C and pH 7. NAUP3 showed 51.68 µg/ml IAA production after 24 hrs of incubation as shown in Fig. 8. As the incubation time progresses, the IAA production increases to maximum 146.68 after 5th day of incubation. Then the amount of IAA production in the broth decreases, the reason for such fluctuations could be the production of IAA degrading enzymes by the cells which are inducible in the presence of IAA or utilization of IAA by the cells as a nutrient during late stationary phase. Average 109.16 µg/ml IAA production was observed by 6 different *Bacilli* isolates which belongs to NFBs, PSBs, and KMBs.

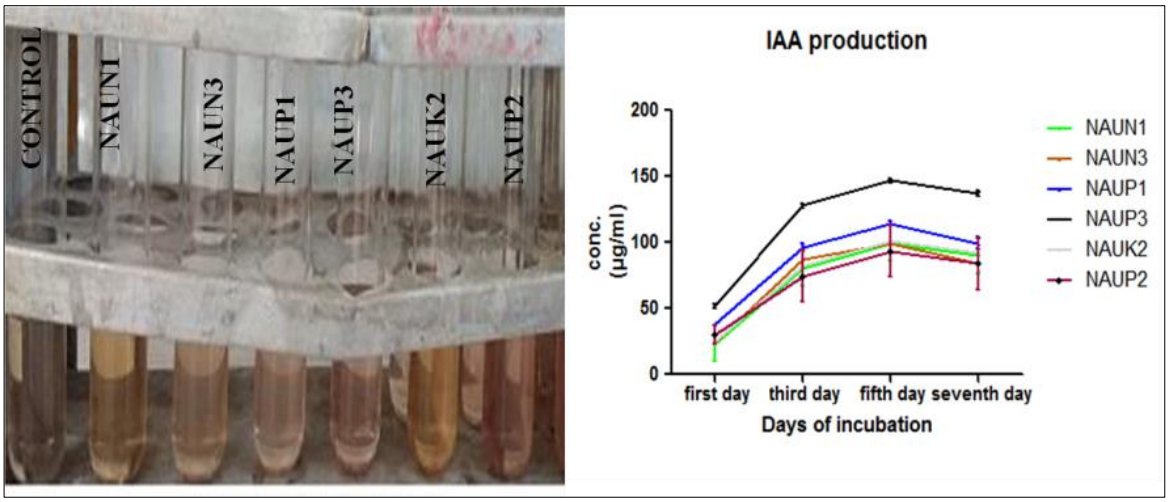

Fig 8: A) IAA production by the selected rhizosphere *Bacilli*. B) Graph representing quantification of IAA production at different time interval

Siderophore production

Siderophores chelate iron from mineral phases by the formation of soluble Fe^{3+} complexes that can be absorbed by energy-dependent membrane transport system and finally make soluble Fe3+ available to plants. It also binds with the Fe3+ in the rhizosphere and efficiently prevents the propagation of fungal pathogens by depriving them of available iron [19, 28]. In the following study, a shown in Fig. 9, five *Bacilli* isolates exhibited the ability of siderophore production in the range from 17.89% to 48.15% siderophore unit on CAS solution after 48 hrs of incubation at 30°C and pH 7 which implies that these isolates were competent of preventing the growth of deleterious pathogens in rhizosphere soil. Out of them, three isolates showed more than 30% siderophore unit production while NAUK3 showed highest i.e., 48.15% siderophore unit production.

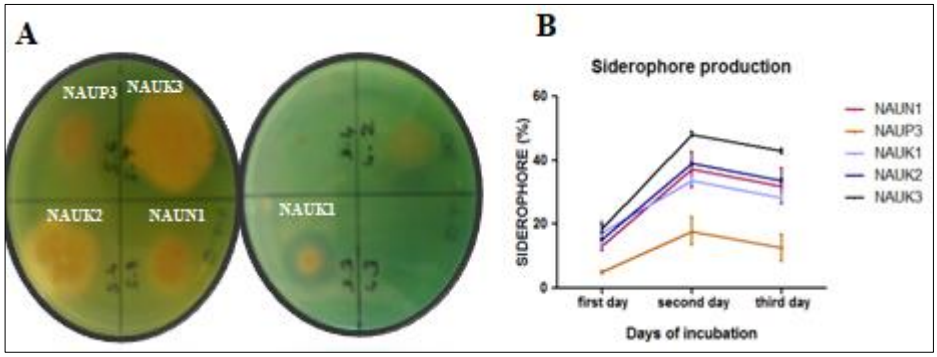

Fig 9: A) Siderophore production by the selected rhizosphere *Bacilli*. B) Graph representing quantification of siderophore production at different time interval

Antifungal metabolite production and compatibility test

The antifungal metabolite production by rhizosphere *Bacilli* isolates was recorded as a reduction in the growth of plant pathogenic fungi *Magnaportha oryzae* and three isolates *Bacillus sp.*strain 01105, *Lysinibacillus macroides*, and *Bacillus megaterium* found to inhibit the radial growth of the test fungi as shown in the Fig. 10 (A). The reduction of growth may be due to the production of volatile antifungal compounds that alter the physiological activities of pathogenic fungi resulting in inhibition of sclerotial activity and ascospore production by the pathogen [29].

None of the *Bacillus* isolate inhibits the growth of the other *Bacilli* isolates as shown in the Fig. 10 (B). The luxuriant growth of all the isolates found in the presence of other isolates which indicates that the consortia of all nine *Bacilli* isolates is ideal for its application as biofertilizer.

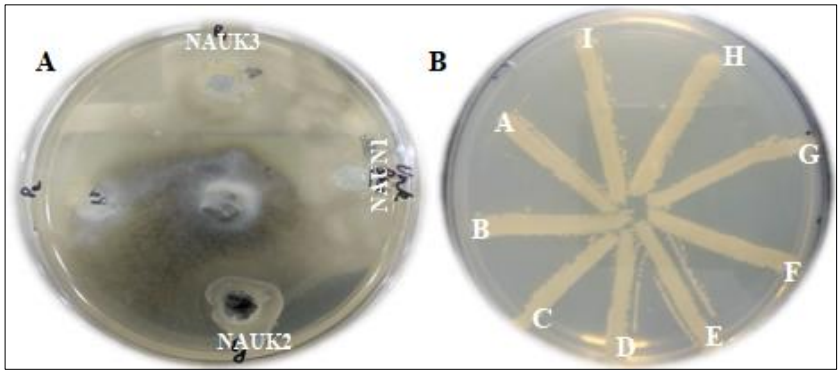

Fig 10: A) Antagonistic activity of selected rhizosphere *Bacilli*. B) Compatibility test of all rhizosphere *Bacilli*. (A. NAUN1, B. NAUN2, C. NAUN3, D. NAUP1, E. NAUP2, F. NAUP3, G. NAUK1, H. NAUK2, I. NAUK3)

Pot assay

All the NFB, PSB, KMB isolates as consortia were inoculated with rice seeds in pot experiment. The normal field soil along with 100% RDF was taken as a control.

Interaction of *Bacilli* consortia with rice

The rice GNR-3 seeds were surface sterilized and after treatment with isolated bacteria, sown in the pot. Continuous watering was done and after 60 days various growth parameters were observed a shown in Fig. 11. The parameters like shoot length, root length, no. of leaves, leaf area, and chlorophyll content were measured and increased growth parameters were observed for the NPK consortia treated rice plants as compared to the untreated plants (Table 3). For the rice shoot length after 60 days, the T6 i.e., treatment of rice seeds with NPK consortia along with 100% RDF gives maximum 16.12% increase in shoot length compared to control T3 i.e., 100% RDF, while T4 and T5 also gives significant 3.56% and 6.51% rise in the shoot length. For another morphological character, root length, the T6 gives the utmost 27.91% increase in shoot length compared to control T3; while T4 and T5 also give a considerable 6.90% and 18.41% rise in the root length. The treatment of *Bacilli* NPK consortia also found effective for the growth characters such as i.e., no. of leaves and leaf area compared to untreated plants. The T6 gives the highest 30.43% and 22.51% increase in no. of leaves and leaf area respectively compared to T3. Along with root length, shoot length, no. of leaves and leaf area, the treatment of selected rhizospheric *Bacilli* NPK consortia found significant in the case of chlorophyll content i.e., T6 gives noteworthy 17.65% rise compared to untreated control T3.

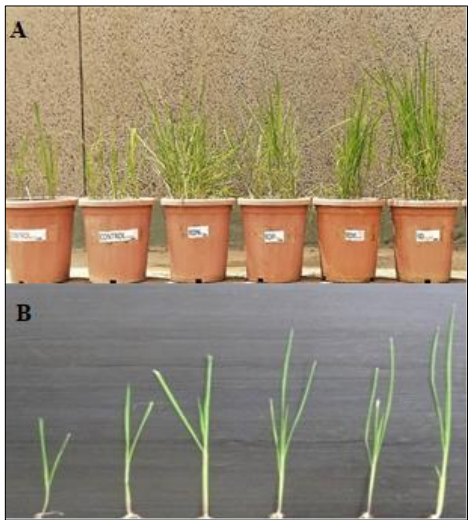

Fig 11: A) Rice seeds inoculated with *Bacillus* consortia. B) Shoot length after 60 DAI

Table 3: Physiological characters of rice after 6o days of inoculation

Treatments	Shoot Length (Cm)	Root Length (Cm)	No. of Leaves	Leaf Area (Cm^2)	Chlorophyll Content (mg/G)
T1-Soil	18.38 ± 0.95	5.35 ± 0.19	1.75 ± 0.50	0.36 ± 0.01	4.42 ±0.03
T2-RDF75%	28.58 ± 0.57	6.60 ± 0.14	2.50 ± 0.58	0.41 ± 0.01	4.88 ± 0.02
T3-RDF 100%	29.78 ± 0.38	7.43 ± 0.15	4.00 ± 0.00	0.45 ± 0.01	5.22 ± 0.02
T4-RDF100% + N consortia	30.88 ± 0.45	7.98 ± 0.10	4.25 ± 0.50	0.47 ± 0.01	5.55 ± 0.04
T5-RDF100% + NP consortia	31.85 ± 0.58	9.10 ± 0.14	4.75 ± 0.50	0.51 ± 0.01	6.04 ± 0.05
T6-RDF100% +NPK consortia	35.50 ± 0.64	10.30 ± 0.16	5.75 ± 0.50	0.58 ±0.03	6.34 ± 0.04
SEM	0.388	0.022	0.222	0	0.001
CD	0.925	0.223	0.7	0.021	0.05
CV %	2.136	1.925	12.298	3.113	0.62

Conclusion

Adding selected *Bacilli* consortia, from among the naturally occurring soil microbial community improves soil quality and results in increased plant growth. In our study, we isolated, characterized, and constructed *Bacilli* consortia for effective nitrogen fixation, phosphate solubilization, and potassium mobilization, which can tolerate a range of environmental fluctuations i.e., temperature, salinity, and pH. Along with its biofertilizer abilities, these isolates found significant IAA and siderophore producers as well as having prominent antagonistic activity against rice fungal pathogen *Magnaportha oryzae*. The *Bacilli* in consortia are compatible with each other and leads to an average 22.93% positive impact on different selected growth and yield attributing characteristics of rice compared to control. Hence, as we look to future strategies to allow both decreased use of fertilizers and maintenance of crop productivity, *Bacilli* inoculants have a clear role to play.

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