Chapter 3

Microbe-Based Strategy for Plant Nutrient Management

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Abstract

The rapid industrialization and urbanization of developing countries such as India have encroached on cultivable lands to meet the demands of an ever-increasing population. The altered land use patterns with increased fertilizer use has increased crop yields with leaching of major portion of the applied nutrients from the soil. Nitrates and phosphates are the agricultural pollutants that are discharged into aquifers due to anthropogenic reasons causing severe environmental and health problems. Production of these nutrients requires energy and finite resources (rock phosphate, which has gradually depleting reserves). An alternative management strategy would be to sequester excess nutrients within a biomass that is reused for agriculture. Two discrete enriched microbial consortia with the potential of simultaneous nitrate and phosphate sequestration upon application as biofertilizer restricted them within the plant root zone, ensuring prevention of eutrophication through leaching while making it available for uptake by plants. The nutrient accumulated biomass enhanced the crop yield by 21.88% during mung bean cultivation with maintained elemental content and other nutritional qualities. The major drawback of conventional biofertilizer application (slow release and action) could be overcome using this formulation leading to environmental protection, crop yield enhancement and soil fertility maintenance post-cultivation.

Keywords: nitrate accumulation, plant growth promotion, phosphate accumulation, phosphatase activity, microbial consortium

1. Introduction

In developing countries like India, rapid industrialization and urbanization have led to encroachment of cultivable lands. The agricultural practices are being gradually modified to increase the food production so as to meet the need of the ever-increasing population. The significant increase in the use of inorganic and organic fertilizers as well as alterations in the land use pattern has led to high yield of crops. But the major disadvantage that emerged out of such practices is the gradual leaching of nutrients and harmful chemicals in the soil and water. Nitrate is one such common agricultural pollutant discharged into the aquifers. Other potential sources of nitrate are the geological processes like eruptions, flood and land silting, irregular rainfall and stream flow patterns, natural process of plant decay and organic residues, anthropogenic sources of land practices, traditional agricultural practices like dry farming, marginal irrigation, large scale flood plain farming and application of fertilizers, leaching from paddy and tea cultivation, sewage infiltration, reuse of agricultural land for human settlement, industrial chemical spills and landfill leachates [1–10]. Nitrate pollution has thus emerged as a global problem and happens to be the second most dangerous pollutant after the pesticides [11, 12]. In marine environment, it induces plankton bloom destroying the native flora and fauna of the region [13]. In humans, it causes condition known as methemoglobinemia (blue baby syndrome) in infants and disorders of central nervous system, cardiovascular system as well as gastrointestinal system while posing to be carcinogenic [14].

The permissible nitrate level in ground water (10 mg/l for NO_3–N and 45 mg/l for NO_3) has been demarcated by "United States Environmental Protection Agency (EPA)." Some of the conventional methods for nitrate removal from water include distillation, reverse osmosis and ion exchange. These processes are quite complex as well as expensive which limits their application during scale up of processes. Bioremediation appears as a desired alternative [15–17], but the major limitation for its application is the longer retention time as compared to the physicochemical processes. Lately the membrane technology of denitrification has been blended with biological immobilization techniques to achieve efficient operation. This combination helps minimize the associated problem while making the process economically viable [18]. Electro bioremediation where effect of electric field is observed on pollutant reduction has also been studied [19–21]. Nitrate reduction by biological means has been reported to be carried out in fluidized expanded bed bioreactors [22], submerged membrane bioreactor [23], continuous flow bioreactors [24] as well as packed bed reactor [25] with PVS tubes [26], alginate [27], K- Carrageenan [28] and microbial cellulose [29] as

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immobilization matrices. It could either be through assimilatory or dissimilatory pathway. An alternative pathway of nitrate removal is through nitrate accumulation as evident in Isolates of **genus** Beggiatoa, Thiomargarita and Thioploca, as well as one species of Bacillus [30].

Phosphate is another essential plant growth nutrient which is lost in wastewater from domestic, industrial (dairy as well as detergent) and agricultural sectors [31]. It also causes eutrophication upon seepage into the surface and ground water bodies. Phosphate is derived from rock phosphate whose reserves are limited [32]. Thus, it is desirable to sequester the phosphate from the wastewater for reuse instead of indiscriminate use of rock phosphate [32]. Phosphate accumulation is already reported in bacteria, but nitrate accumulation in bacteria is relatively rare. It is in the genus *Beggiatoa*, *Thioploca* and *Thiomargarita* that nitrate accumulation is observed in intracellular vacuoles [33–35]. Only recently nitrate accumulation from wastewater has been reported in the genus *Bacillus* [36]. Since nitrate and phosphate are both essentials for agriculture, but only a small fraction (12–30%) [7] of the applied nutrients is utilized by the plant, thus it becomes essential to trap these nutrients for reuse as well as environmental protection.

In order to address this upcoming environmental challenge, an alternative plant nutrient management strategy was developed with the following approach: (i) isolation and characterization of microbial consortium with ability to simultaneously accumulate nitrate and phosphate; (ii) utilize these microbes to prevent nutrient leaching from soil; and (iii) utilize these microbes with intracellular accumulated nutrients as biofertilizer.

2. Consortia development and characterization

Nitrate broth (Himedia M439) was used as the medium of choice for isolation of nitrate reducing microbial consortium. Two types of inoculum were used under both aerobic and anaerobic condition (in an atmosphere of carbon dioxide and nitrogen) at 37°C. The first type was the soil from East Calcutta Wetland (ECW) (22°27′ N, 88°27′E) which is known as the world's largest waste dumping ground and natural waste recycling center [37]. The reason for selecting soil from East Calcutta Wetland as the inoculum was that it was expected to harbor microbes with rich diversity as well as bioremedial ability. Since cultivation is the ongoing practice in this area, efficient strains with potential for promoting plant growth are expected to inhabit this area. The other inoculum was the biomass from a low-level radioactive waste treating microbial biofilm bioreactor removing mainly nitrate [38, 39]. This was expected to contain efficient nitrate reducers/accumulators due to its constant exposure to nitrate. Nitrate removal from the medium by the bacteria was set as the primary criteria for the selection of consortium. After 48 h of incubation, the nitrate concentration [40, 41] in the cell-free medium was checked. Of the four different combinations tested, two consortia were found to be efficient: anaerobic consortium from ECW (NB1) and aerobic consortium from bioreactor biomass

(BN7). They demonstrated 96 and 97.44% nitrate removal in 12 and 4 h by NB1 and BN7, respectively [39]. Another interesting feature of BN7 was its simultaneous accumulation of nitrate and phosphate from medium.

Both the cultures were also tested for phosphate removing ability as per standard procedure [30, 32] and demonstrated 23.88 and 48.2% removal with 565 and 1.14mg per gram wet weight of polyphosphate in NB1 and BN7, respectively. NB1 reduced 75–90% nitrate within a pH range of 5–12 with the maximum at pH 10 while that of BN7 was a range of 6–11 [39]. The optimum temperature range for NB1 was 30–40°C and that for BN7 was 25–37°C [39].

The effect of metals [viz., zinc ($ZnSO_4$), cobalt ($CoCl{\cdot}6H_2O$), lead {$Pb(NO_3)$} and copper ($CuSO_4{\cdot}5H_2O$)] on the nitrate reduction efficiency of NB1 and BN7 consortia was checked at two different concentrations, that is, 0.1 and 0.5 mM. It was compared to the reduction in the absence of metal salts (control) in both cases. The experiments were repeated thrice. The aerobic culture exhibiting growth along with nitrate reduction in the presence of different metal salts was checked for metal accumulation within the biomass using energy-dispersive X-ray fluorescence (EDXRF) analysis [39, 40]. While chromium (Cr), strontium (Sr) and cadmium (Cd) salts were inhibitory for the growth of the anaerobic consortium NB1 even at a concentration of 0.1 mM, the consortium showed growth in up to 0.5 mM concentration of copper (Cu), lead (Pb), cobalt (Co) and zinc (Zn). Being an anaerobic consortium, it was better preserved as glycerol stock while retaining its nitrate removal activity up to 24 days rather than stab or lyophilized culture as compared to BN7 [39].

16S rDNA based molecular characterization of both the consortia were done as per prior report [42]. The sequences obtained were subjected to NCBI nucleotide BLAST analysis, and novel sequences were submitted to GenBank. These sequences were then subjected to phylogenetic analysis using neighbor joining method. The rarefaction curves were drawn, and the richness (Shannon diversity index) and evenness (equitability index) of the population were determined as per standard procedure [37, 43, 44]. Mothur analysis was conducted using the data.

At the molecular level, NB1 was composed of novel organisms (GenBank JN626182-JN626198 and JN665074-JN665081) with closest identity in the ratio of 44:37:19 with *Pseudomonas* sp., *E. coli* and uncultured bacterium (**Figure 1a–c**) with poor diversity (Shannon diversity index 0.417) of evenly distributed population (equitability index 0.873). *Pseudomonas* sp. might be involved in nitrate removal as well as phosphate accumulation. BN7 on the other hand was composed of *Pseudomonas* sp.:*Azoarcus* sp.:uncultured bacterium: *Bacillus* sp. in the ratio of 20:31:46:3% in terms of 16S rDNA sequence similarity of its clones (GenBank GU644465 to GU644489). Like any enriched consortium in selective medium, BN7 reflected poor diversity (Shannon diversity index 0.39) of evenly distributed microbes (equitability index 0.83). Genus Pseudomonas and Bacillus were involved in phosphate accumulation and nitrate reduction [39].

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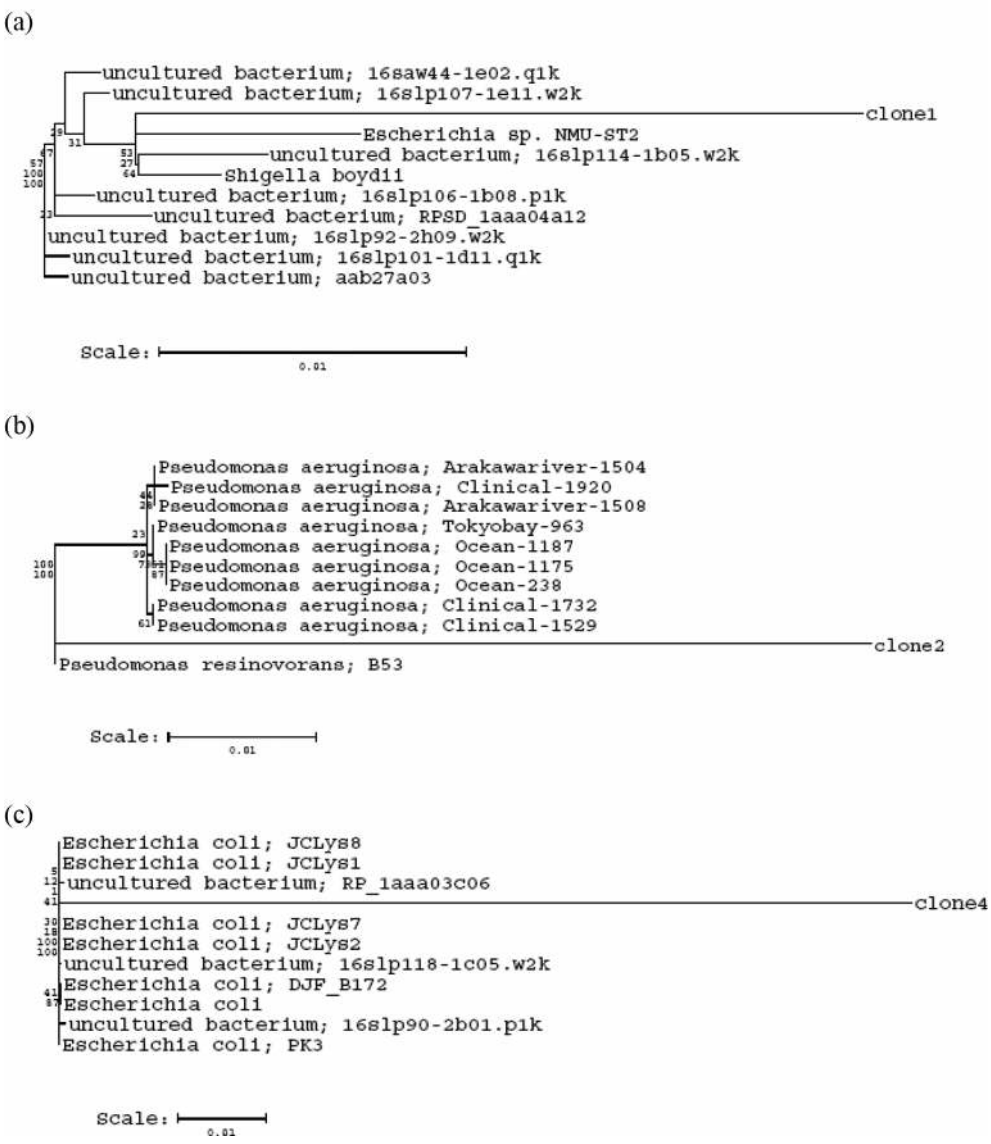

Figure 1. Phylogenetic trees constructed using neighbor joining method for the clones from the consortium NB1 showing maximum similarity with uncultured bacterium (a), Pseudomonas (b) and *E. coli* (c).

Mothur analysis revealed saturation of screening of the consortia which were different from each other (**Figure 2**; **Tables 1** and **2**).

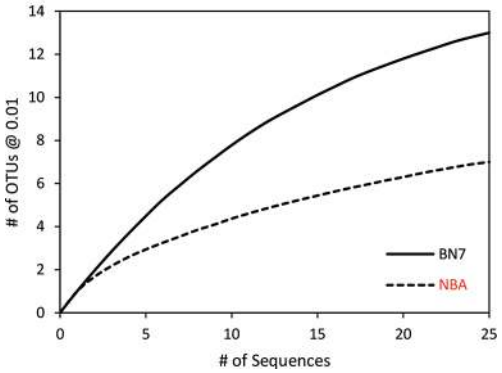

Figure 2. Rarefaction curve drawn for the consortium BN7 and NB1 reflecting saturation of screening for both the consortiums.

Comparison	dCXYScore	Significance
BN7-NB1	0.0206	<0.0001
NB1-BN7	0.0121	<0.0001

Table 1. Libshuff comparison showing that both libraries have a very different community structure.

Diversity index @ 0.01	BN7	NB1
N	25	25
S	13	7
Simpson (1/D)	18.75	3.03
95% LCI	12.90	1.96
95% HCI	34.32	6.69
Shannon (H)	2.47	1.41
95% LCI	2.22	0.99
95% HCI	2.72	1.82
H_{max}	2.84	1.67
Chao	15.00	8.00
95% LCI	13.29	7.09
95% HCI	26.96	17.68
Ace	16.25	10.08
95% LCI	14.49	7.45
95% HCI	20.07	28.24

Diversity index @ 0.01	BN7	NB1
Jackknife	18.00	10.00
95% LCI	11.80	5.20
95% HCI	24.20	14.80

Table 2. Diversity indices calculated for both the consortia.

3. Soil leaching

An experimental tub of dimension 18 cm × 12 cm × 17 cm (l × b × h respectively) (**Figure 3**), with surface area of 216 cm^2 and volume 3672 cm^3 filled up with 8.095 kg of soil, was set up for studying nitrate leaching in soil. In order to study the leaching process, outlets were made along the breadth of the tub at different heights of 3, 7, 11, 15 and 17 cm from the surface of the soil which facilitated in sample collection which in turn were assessed for the nitrate concentration [37, 38].

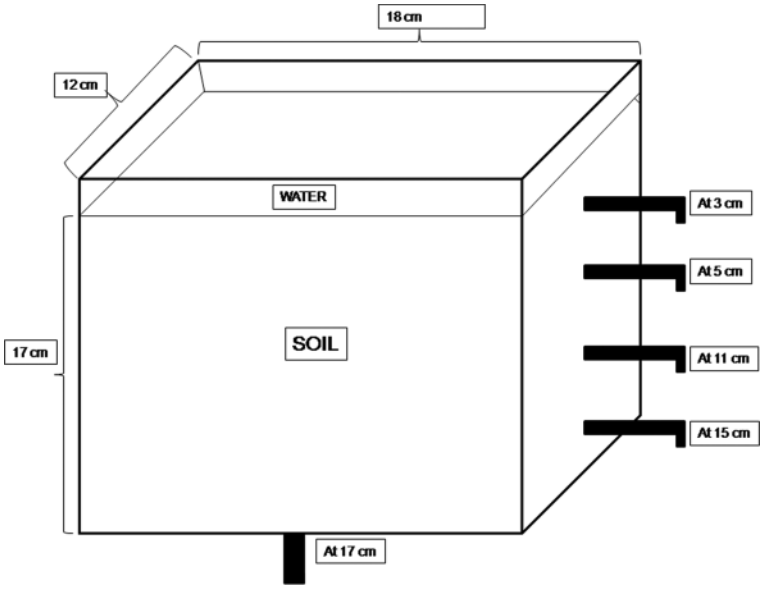

Figure 3. Schematic representation of the apparatus (soil filled tub) used for soil leaching experiment.

The experiment was carried out in four sets. For the first set (control), leaching of nitrate from soil in the presence of the native soil microbial population was tested. For this, water was poured into the soil filled tub. As the water seeped down, samples were collected from

each outlet and analyzed for nitrate concentration [37, 38]. For the second and third set, the soil was inoculated with 100 ml of seed culture of BN7 and NB1, respectively. The system was left for 48 h for the consortium to colonize in the soil. Finally after 48 h, the leaching experiment was repeated as reported above to assess the nitrate released from the soil into the seepage water collected at different heights as a result of the interaction of soil native microbial population with the applied microbial consortia separately. For the fourth set, the combination of BN7 and NB1 in 1:1 ratio was applied and the experiment was repeated as in case of set two and three. The leaching of nitrate with and without external microbial consortium application was analyzed from the above experiments. This study was repeated thrice. In case of control, the soil interaction with the native microbial population as reflected through nitrate leaching was analyzed. In case of BN7 and NB1, these consortia were applied separately and the mixed impact of these consortia with the existing native soil microbial population was studied on the extent of nitrate leaching in water with traversed soil depth. In case of NB1 + BN7, the joint interaction of all the three consortium on nitrate leaching in soil was analyzed. From the results, it was observed that the application of the mixed formulation prevented leaching of nitrate from the soil resulting in decrease in the incidences of eutrophication due to soil nitrate leaching as documented in **Table 3**. It results in substantial reduction in nitrate leaching.

Level	**Concentration of nitrate in seepage water at different levels in ppm**							
	Distance from soil surface (cm)	**Control**	**BN7**	**Difference in concentration (fold change)**	**NB1**	**Difference in concentration (fold change)**	**BN7 + NB1**	**Difference in concentration (fold change)**
A	3	0	92.34	–	0	–	0	–
B	7	4.8	5.4	12.5	0	−100	0	−100
C	11	28.25	255.53	804.53	123.68	337.8	0	−100
D	15	75.1	425.7	466.84	154.82	106.15	4.36	−94.2
E	17	110.65	1160.27	948.59	120.6	8.99	12.83	−88.41
Correlation coefficient	–	0.94	0.82	–	0.88	–	0.79	–

Table 3. Tabular representation of the nitrate leaching from soil in the presence of different microbial consortia.

The correlation coefficient values indicate strong correlation between the depth of soil traversed by the applied water and the extent of nitrate leached in the presence of all the four treatments. Moreover, the prevention of leaching was complete at 11 cm of soil depth, indicating immobilization of nitrate in that zone. If this nitrate is made available to plants then this being the root zone for most of the plant, the productivity is expected to rise and the soil fertility is expected to be maintained. Also the phosphate accumulated inside as polyphosphate upon being released could be solubilized by the phosphatase released by the bacteria and made available to the plants. Both these phenomena are expected to strengthen the ability of this consortium (NB1 + BN7) to function as a biofertilizer. The nitrate and phosphate concentration in agricultural runoff could also be reduced by these microbes.

4. Plant growth promoting activity

Production of phytostimulator like ammonia, hydrogen cyanide (as plant protector), indole acetic acid, gibberellic acid (as plant hormones), phosphatase (to solubilize inorganic phosphate) and siderophore was tested for both the consortiums as per standard procedure [45]. NB1 produced 5.2 mg/100 ml and BN7 produced 1.64 mg/100 ml of ammonia with no hydrogen cyanide and siderophore production by either of them. Indole acetic acid (550 μg/ml) was produced by NB1 only. Both NB1 and BN7 produced enzyme phosphates, which were quantified to be 9.12 and 8.7 U/ml, respectively, with a final pH change to 4.11 and 6.3.

Since the consortium (NB1 + BN7) possessed plant growth promoting characters and also prevented leaching from soil, thereby making soil nutrients available to plants, both (NB1 and BN7) were tested for its effect on germination following soil application at the time of sowing, and the data were analyzed as per the standard protocol [45]. The data represent the combined effect of the native soil microbial population with the applied consortium. In order to assess the effect of only the combined consortia (NB1 + BN7) on germination in mung bean, the germination trial was repeated in germination tray using sterile soilrite mix kel006 (soil-free medium by Keltech Energies Limited, Bangaluru, India) and compared with that of control (uninoculated sterile soilrite). Application of either consortium improved the germination percentage, germination index and vigor index relative to the untreated control (**Table 4**).

Germination trial data	**Treatment set**		
Parameter	**Control**	**BN7**	**NB1**
Germination percentage	74.07 ± 22.45	98.15% ± 3.21	92.59 ± 8.49
Germination index	39.77 ± 9.39	75.95 ± 11.87	82.47 ± 11.23
Vigor index	1639.06 ± 366.67	1925.38 ± 490.02	1959.3 ± 632.25

Table 4. Represents data for germination trial with and without consortium application.

Even without any supporting microbes in the soil-free medium (Soilrite mix), this combination (NB1 + BN7) enhanced *Vigna radiata* (mung bean) germination (98%) as compared to the control (78%).

The consortia (NB1, BN7, NB1 + BN7) were further tested during pot trial (at Maulana Abul Kalam Azad University of Technology, India) and field trial for *Vigna radiata* var Samrat (developed by Indian Institute of Pulse Research, Kanpur, India) from Feb 2013 to May 2013 (spring/summer cultivation). The culture was applied only once at the time of sowing. For field trial, randomized block design with four replicates was carried out at Bidhan Chandra Krishi Viswavidyalaya Seed farm, Kalyani, Nadia, West Bengal, India as well as at State Department of Science and Technology facility, Salt Lake, Kolkata, West Bengal,

India. The sowing was done in the north south orientation in February 2013. The seeds post-germination were subjected to thinning on the 8th day post-sowing such that each 1 m^2 area contains a total of 40 plants (4 rows of 10 plants each). The inoculum applied on the day of sowing for field trial was 3.68×10^9 cells per plot (1 m × 1 m). The following parameters were monitored: plant height, number of branches, 50% flowering, 100% flowering, number of flowers, pod initiation, number of pods/plant, pod length, weight/pod, seeds/pod and weight of 100 seeds. In order to compare the data of the above-mentioned agronomic parameters as well as yield with that of conventional agriculture, simultaneously four (1 m × 1 m) plots were treated with chemical fertilizer. The chemical fertilizer (12.59 g) was applied in the ratio of N:P:K equals 20:40:40 (urea:single super phosphate:murated potash) for each 1 m × 1 m area. The total yield per hectare for each of the applications was monitored with respect to control (unfertilized). When applied together (NB1 + BN7) in field trials, the consortium significantly improved plant growth as compared to separate application (**Table 5**).

Parameters	Treatments				
	Control	**NB1**	**BN7**	**NB1 + BN7**	**Chemical**
Height of plants (cm)	37.86 ± 4.79	38.87 ± 10.27	40.25 ± 9	38.99 ± 6.79	31.34 ± 8.57
Number of branches	7.8 ± 0.63	7.9 ± 0.8	8.2 ± 1.3	8.9 ± 0.99	8 ± 1.41
Number of pods per plant	4.12 ± 3.09	10.25 ± 3.87	12.89 ± 4.98	11.85 ± 6.23	3.87 ± 2.69
Pod length (cm)	6.33 ± 0.86	7.65 ± 0.67	7.71 ± 1.31	8.07 ± 1.12	7.83 ± 1.05
Weight per pod (g)	0.41 ± 0.12	0.58 ± 0.23	0.53 ± 0.18	0.77 ± 0.22	0.53 ± 0.11
Seeds per pod	4 ± 1.58	4 ± 0.83	5 ± 1.15	7 ± 1.3	10 ± 0.83
Weight of 100 seeds (g)	3 ± 0.005	3.7 ± 0.45	3.59 ± 0.86	4.34 ± 0.46	4.27 ± 0.01

Table 5. Agronomic parameters for mung bean cultivation following chemical and biofertilizer application as compared to control (unfertilized) condition.

For every parameter, the combined application of NB1 + BN7 exhibited a better effect. Notably, the calculated yield per hectare was highest for NB1 + BN7 (2582.5 kg/ha) followed by chemical fertilizer (2017.5 kg/ha), BN7 (1802.5 kg/ha), NB1 (799.6 kg/ha) and the control (710.05 kg/ha). Thus, it offers potential advantage in meeting the increased food requirement in today's limited availability of land for agriculture. In addition, the consortia NB1 + BN7 also maintained soil fertility as revealed during the pot trial (**Table 6**).

In addition, each consortium (NB1, BN7, NB1 + BN7) could remove hydrocarbons such as metacil, pesticide and servo (lubricant) from the soil, suggesting that it has potential use in oil spill bioremediation.

Test parameters	Treatments				
	Unused soil	**Control**	**NB1**	**BN7**	**NB1 + BN7**
pH (1:2.5)	6.4	6.2	6.8	7.2	7.3
Conductivity (1:5) ds/m	0.091	0.086	0.108	0.13	0.079
Alkalinity (mg/kg)	225	187.5	225	225	187.5
Sodium (mg/kg)	156.67	150.16	138.25	119.05	168.65
Potassium (mg/kg)	69.9	60.25	44.46	54.43	76.11
Phosphate (mg/kg)	52.71	39.22	31.56	44.13	60.37
Amonical nitrogen (mg/kg)	87.5	73.5	89.25	70	99.75
Kjeldahal nitrogen (mg/kg)	96.25	82.25	85.75	78.75	108.5
Nitrate (mg/kg)	36.7	28	34.3	32.8	44.4
Nitrite (mg/kg)	27.2	20.8	25.4	24.3	32.9
Hydrocarbon (%)	0.136	0.041	0.004	0.004	0.09
Bulk density (g/cc)	1.11	1.05	1.12	1.16	1.11
Particle density (g/cc)	2.55	2.42	2.43	2.53	2.61
Pore space (%)	59.39	59.21	55.81	57.08	59.92
Water holding capacity (%)	53.25	56.13	50.4	50.52	52.94
Organic carbon (%)	1.36	1.23	0.95	0.82	1.91
Organic matter (%)	2.34	2.12	1.64	1.41	3.29
Available nitrogen (mg/kg)	113.75	105	117.25	99.75	138.25
Available potassium (mg/kg)	63.3	51.12	34.41	41.96	53.51
Available phosphorous (mg/kg)	17.2	12.8	10.3	14.4	19.7
Moisture (%)	2.91	2.7	1.89	1.65	2.76
Sand (%)	28.2	31.6	38.2	39.1	33.9
Silt (%)	43.4	42.5	36.8	37.5	37.5
Clay (%)	28.4	25.9	25	23.4	28.6
Textural classification	Clay loam	Loam	Loam	Loam	Loam

Source: Refs. [48–52].

Table 6. Soil nutritional quality analysis pre- and post-cultivation of mung bean during pot trial using standard methods..

5. Seed quality analysis

The seeds were lyophilized for 24 h and manually ground in the mortar and pestle; 0.2 g ground material was pelleted using Pelletizer (Technolab, Kbr Press) at 110 kg/cm^2. The mineral content of the pellets was assessed using energy-dispersive X-ray fluorescence (Jordan Valley EX–3600) analysis as per reported protocol [46, 47] at University Grant Commission-Department of Atomic Energy facility, Kolkata Center, India (**Table 7**).

Elements mg/kg (ppm)	Control	NB1	BN7	NB1 + BN7	Chemical	p-Value	Recommended by USDA
Zn	37.21 ± 2	44.57 ± 2.05	27 ± 3.02	29.06 ± 2.43	34.23 ± 2.58	0.04	26.8
Fe	68.34 ± 2.25	71.92 ± 1.66	68.45 ± 6.89	70.71 ± 0.57	67.21 ± 4.41	0.04	67.4
Mn	12.42 ± 0.44	12.74 ± 1.56	13.65 ± 1.43	15.46 ± 1.50	13.30 ± 0.64	0.02	10.35
Cu	13.30 ± 0.45	15.19 ± 0.56	15.66 ± 1.02	14.62 ± 1.39	14.49 ± 1.30	0.21	9.41
P	4242.09 ± 475.2	4604.71 ± 50.2	2429.97 ± 619.20	3741.01 ± 481.4947	1416.79 ± 574.18	0.003	3670.00
K	13,538.33 ± 491.76	13,830.88 ± 415.3	9651.83 ± 1546.293	11,807.17 ± 773.6117	10,943.22 ± 1349.72	0.18	12,460.00
S	2165.53 ± 288.35	2341.02 ± 63.25	1692.56 ± 199.5616	2037.44 ± 118.75	1575.90 ± 118.02	0.05	NA
Ca	2034.13 ± 149.41	2071.45 ± 214.95	1650.99 ± 410.549	1714.23 ± 79.81	1777.90 ± 396.11	0.04	1320.00

The commercially available fertilizer (Urea: Single Super Phosphate: Murated Potash) was applied in ratio of N:P:K equals 20:40:40 whereas in case of microbial biomass (N:P—2.52:1.51), 3.68 × 10^9 cells were added per plot (1 m × 1 m). The lyophilized seeds were manually grounded, and 0.25 g of the powder was converted into pellet and was analyzed by EDXRF for mineral content.

Table 7. Represents the elemental content of the seeds grown during control (unfertilized), chemical fertilizer as well as biofertilizer treatment.

The nutritional quality analysis like moisture [IS:4333(Part-II):2002], total protein (AOAC 920.87), available carbohydrate (AOAC 986.25), fat (AOAC 963.15), energy (Analytical Chemistry of Food by CS James:1995), ash content (AOAC 941.12), sugar (AOAC 923.09) and fiber (AOAC 985.29) content was carried out at SGS India Private Limited, Kolkata, India as per standard protocol (**Table 8**).

The statistical validation for the variation in elemental content of the seeds grown using varying treatments was carried out using single-factor ANOVA in Microsoft excel 2007. Here, the two hypotheses were as follows: null hypothesis H_0: no difference in elemental content with difference in treatment; alternative hypothesis H_1: significant difference in elemental content with difference in treatment. The level of significance was fixed at 5%. Based on a single-factor ANOVA, a significant variation was observed in the elemental content of the seeds produced after the treatments, especially in the Zn, Mn and Cu content between the control and NB1 + BN7 seeds. This clearly suggests that the consortium produces more elementally

stable seeds. However, the overall nutritional quality of the seeds was maintained regardless of the treatment. The consortium exhibited similar trends for *Cicer arietinum* (chick pea) and *Abelmoschus esculentus* (ladies finger) cultivations.

Parameters	Treatment				
	Control	**NB1**	**BN7**	**NB1 + BN7**	**Chemical**
Energy value (kcal/100 g)	335.06	332.55	335.37	332	333.51
Total carbohydrate (g/100 g)	56.75	55.99	55.89	55.40	56.37
Protein (g/100 g)	23.61	23.46	23.19	22.86	23.79
Moisture (g/100 g)	14.85	15.87	16.19	16.82	15.46
Total ash (g/100 g)	3.86	3.73	3.64	3.87	3.98
Crude fat (g/100 g)	0.93	0.95	1.09	1.04	0.85
Total sugar (g/100 g)	3.20	3.13	2.95	3.07	3.20
Total dietary fiber (g/100 g)	15.65	15.38	15.18	14.99	15.18

Table 8. The nutritional quality of the seeds following cultivation under control (unfertilized), chemical fertilizer as well as consortium (NB1, BN7, NB1 + BN7) treatment.

6. Conclusion

The aim of this study was to develop an alternative strategy for plant nutrient management through microbial intervention. The objective of prevention of leaching of nitrate from soil was achieved through application of a 1:1 mixture of NB1 and BN7. It also ensured retention of nitrate within the root zone of soil. Being accumulators of nitrate and phosphate as well as producers of phytohormones with phosphatase activity, they could enhance germination while making the phosphate available for plant uptake. Thus, a single combination has the desired properties of a biofertilizer like phytohormone production, supplying of nutrients (nitrate and phosphate) resulting in higher yield of nutritionally enriched seeds. The unique selling points of this bioformulation are as follows: (i) its 21.88 times greater productivity (in case of mung bean) as compared to chemical fertilizer application and (ii) maintenance of soil fertility post-cultivation. Hereby, the remaining objections of multinutrient sequestration and reuse were effectively achieved. The wide range of pH and metal tolerance makes these consortia suitable for environmental application under varied conditions. These unique features of BN7 as well as NB1 + BN7 have been filed as Indian Patents 518/KOL/2011 dated April 11, 2011 and 203/KOL/2013 dated Feb 21, 2013. By this method, the nitrate concentration from

agricultural runoff could be reduced substantially by using these microbes. All these properties point towards the future application of this innovation for bioremediation through nutrient sequestration from agricultural runoff as well as effluents and its reuse as biofertilizer with potential for environmental protection and agricultural sustenance.

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