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Original Research Paper

Macronutrients and their associated bacterial genera in the soils of Anaimalai block in Tamil Nadu for sustainable vegetable crops cultivation

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ABSTRACT

Meticulous analysis on intertwined interaction among the soil nutrients and microbial communities brings fruitful outcomes on horticulture. This study focuses on identifying well compatible bacterial genera in enhancing soil primary macro-nutrients for sustainable vegetable crops cultivation. The biochemical tests were executed for identification of bacterial genera. Eventually, mathematical models among available NPK nutrients (Nitrogen, Phosphorus and Potassium) and identified bacterial genera were derived to determine most suited bacterial genus for nutrients inhibition. This Study reveals the present nutrient's status of soils at Anaimalai block covering 12,424 ha of Coimbatore district in Tamil Nadu. Seven utilitarian bacterial genera were identified which inhibit the plant nutrients. Among them, *Azotobacter, Arthrobacter* and *Achromobacter* actively inhibit available NPK in the soil. Present study of correlating soil nutrients with bacterial components enriches successful conservation of biosphere through adopting these innovative technologies in horticulture.

Keywords: Correlation, horticultural crops, macronutrients, soil beneficiary microbes, soil fertility, sustainable agriculture and vegetable production.

INTRODUCTION

Soil utilitarian bacteria act as a significant contributing factor for improving diminished soil fertility. Soil bacteria increase the crop production through their inherent characteristics of soil structure improvisation, production of hormones and enzymes, controlling pathogen and heavy metal. Nevertheless, the decline of organic fertilizers usage and soil fertility exploitation through agrochemical affects the horticultural crop yield rate (Malhotra et al., 2017). The incorporation of soil utilitarian bacteria as biofertilizers is needed to overcome the aforementioned issues. Statistical infusion in the soil nutrient and bacterial data analysis would bring out a realistic field study. From the extensive works of literature, it is found that various initiatives have been taken to improvise horticultural crops productivity through soil nutrient and bacterial analysis. Besides, beneficiary microbes such as *A. chroococcum, A. vinelandii, A. beijerinckii, A. paspali, A. armeniacus, A. nigricans, A. salinestri , Azospirillum, Cyanobacteria, Azolla, Gluconacetobacter diazotrophicus, Bacillus aerius, Bacillus amyloliquefaciens, Bacillus mucilaginous, Bacillus subtilis, Enterobacter* contributes for nitrogen fixation in the soil (Dhayalan and Karuppasamy 2021). *Pseudomonas chlororaphiswere, P. putida, P. entomophila, P.koreensis, P.luteola, P. simiae, P.stutzeri, Bacillus* sp., *Achromobacter, Acinetobacter, Aeromonas hydrophila, Arthroderma cuniculi, Aspergillus niger, Bacillus aerius, B. altitudinis, B. thuringenesis, Enterococcus casseliflavus, E. gallinarum, Lecanicillium psalliotae, Paenibacillus taichungensis, Serratia nematodiphila, Sphingomonas paucimobilis, Azotobacter etc.* are some of the soil phosphorus solubilizing microbes which are used in different horticultural crops for enhanced productivity (Mussa *et al*., 2018). *Pseudomonas azotoformans, Burkholderia, Bacillus mucilaginosus, B. edaphicus, B. circulans, Pseudomonas, Acidithiobacillus ferrooxidans, Paenibacillus* sp., and *Enterobacter hormoecheiin* are some of the beneficiary bacteria that induces the solubilization of potassium (Prajapati and Modi 2016). Correlation among the soil nutrients and their associated bacterial genera is a substantial research gap. Resultant of correlation leads gateway for precise decision making relevant to bio fertilization for horticultural crops.

MATERIALS AND METHODS

Sample site description

Extensive research work was performed recently in Anaimalai at Pollachi, which lies between 10.662°N 77.00650°E, located 40km to the south of Coimbatore, India. The study area consists of vegetable crops such as tomato, bhendi, brinjal, chilies, caspium, paprika, pumpkin, snake gourd, bitter gourd, cluster bean, potato, cabbage, cauliflower, onion etc., Twenty-seven soil samples were collected from the rhizospheric region of horticultural crops for macronutrients analysis.

Macronutrient analysis

The basic physical parameters such as pH, Electrical conductivity, and salinity were measured using pH meter (Elico LI 617), conductivity meter (Elico CM 183), and salinity meter respectively. Available nitrogen in the soil sample was analyzed using the Kjeldahl nitrogen analysis method. Total phosphorus was analyzed using the Bray-1 method for acidic soil samples and Olsen method for alkaline soil samples. Available potassium in the soil was estimated using Flame Photometer (Jenway PFP7).

Bacterial analysis

Enumeration of microorganism

Enumeration of bacteria were carried out through serial dilution method to reduce the microbial load and plated by pour plate method. Bacteria being isolated from the plates are incubated at 37°C in a bacteriological incubator.

Gram staining

Gram staining was done for the isolated bacterial cultures in a slide using Gram's iodine, decolorizing agent and safronin strain. The slides were observed under the trinocular microscope, the purple colors indicated gram positive bacteria reveals the presence of bacterial genera, *Arthrobacter* and most of slides observed pink color indicating the presence of *Azotobacter, Enterobacter, Citrobacter, Achromobacter, E. coli* and *Cornybacterium* which are characterized by gram negative. Further analyses were carried out to confirm the presence of initially traced bacterial genera. The cover slip was taken where its edge was coated with vaseline and the test samples were transferred to the cover slip which was placed over the cavity slide. The slide was viewed under 100X magnification and the organisms' characteristics being motile or non-motile were noted down.

Biochemical test for bacterial genera

For the confirmation of bacterial genera certain biochemical tests were carried. Indole test were carried out for the isolated bacteria culture in which a red colour ring or pink colour ring at the top reviling positive reaction, where yellow colour ring at the top indicate negative reaction. Indole test reveals that most of the identified bacterial genera except *Arthrobacter, Citrobacter* and *Enterobacter* exhibit the positive reaction.

Followed by the indole test, methyl red test were performed, Formations of red colour ring at the top indicate the positive reaction which reveals the existence of *Azotobacter, Citrobacter, Achromobacter, E.coli and Cornybacterium* and formation of yellow colour ring at top shows the negative reaction which indicate the presence of *Arthrobacter* and *Enterobacter*. Further confirmation of bacterial genera was made by Simmons citrate utilization test, so as to determine the ability of the microorganism to use citrate as its sole source of carbon. From the Simmons citrate utilization test it was revealed that the survival of *Azotobacter*, *Citrobacter*, *Enterobacter and Achromobacter* were reverberated by means of green to blue colour changes in the bacterial medium specifying positive reaction. The existence of bacterial genera such as *Arthrobacter, Cornybacterium* and *E.coli* were revealed through green to yellow color changes of bacterial medium indicating the negative reaction (Ishiguro et al., 1979). Voges Proskauer tests were included to analyze the presence of identified bacterial genera at most precise manner. Voges Proskauer tests are used to demonstrate an organism's ability to convert pyruvate to acetoin. Formation of red colour ring exhibits the positive reaction which covey the existence of *Enterobacter* genera and yellow colour ring express the negative reaction exposing the survival of *Azotobacter, Citrobacter, Achromobacter, E.coli, Cornybacterium,* and *Arthrobacter*.

Bacterial genus interpretation

Based on the outcomes of various biochemical tests, identification of bacteria were made with the help of Bergey's manual of systematic bacteriology. Out of 11,669 different bacterial colonies 31% of *Arthrobacter*, 24% of *Citrobacter*, 17% of *Escherichia coli*, 13% of *Azotobacter*, 7% of *Enterobacter* and 5% of *Achromobacter*, was found as contributing bacterium for promoting soil nutrients. Total colonies of bacterial genus were identified by multiplying the average number of colonies with 10^5 as dilution factor (Table 1).

Table 1. Biochemical characteristic for the identification of bacterial species

S.No.	S.F. No	Latitude	Longitude	Total BC	GS	IT	MRT	SCUT	VPT	IB	CC
1	590	10.588153	76.885931	380	GNR	(+)	(+)	(+)	(-)	*Azotobacter*	157
2	592	10.587975	76.885421	200	GNR	(-)	(-)	(+)	(+)	*Enterobacter*	125
3	596	10.587718	76.885257	511	GNR	(-)	(+)	(+)	(-)	*Citrobacter*	425
4	600	10.588111	76.883713	561	GNR	(+)	(+)	(+)	(-)	*Achromobacter*	362
5	601	10.587909	76.884139	356	GNR	(+)	(+)	(-)	(-)	*E.coli*	208
6	602	10.587721	76.884218	312	GPR	(-)	(+)	(-)	(-)	*Cornybacterium*	215
7	606	10.587537	76.884144	326	GNR	(-)	(-)	(+)	(+)	*Enterobacter*	225
8	608	10.587163	76.884039	322	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	253
9	630	10.586454	76.882942	320	GNR	(+)	(+)	(+)	(-)	*Azotobacter*	257
10	637	10.587134	76.883235	320	GNR	(+)	(+)	(-)	(-)	*E.coli*	218
11	638	10.587643	76.883528	320	GNR	(+)	(+)	(-)	(-)	*E.coli*	218
12	641	10.587063	76.88287	312	GNR	(+)	(+)	(-)	(-)	*E.coli*	218
13	642	10.587491	76.882627	245	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	253
14	648	10.587277	76.882267	478	GNR	(-)	(+)	(+)	(-)	*Citrobacter*	425
15	649	10.587783	76.88216	520	GNR	(-)	(+)	(+)	(-)	*Citrobacter*	425
16	657	10.587926	76.882583	956	GNR	(+)	(+)	(+)	(-)	*Azotobacter*	557
17	656	10.588503	76.882551	288	GNR	(-)	(-)	(+)	(+)	*Enterobacter*	225
18	660	10.588254	76.883272	540	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	253
19	661	10.588261	76.883422	300	GNR	(+)	(+)	(-)	(-)	*E.coli*	218
20	702	10.587996	76.87914	96	GNR	(+)	(+)	(-)	(-)	*E.coli*	58
21	703	10.588698	76.87921	96	GNR	(+)	(+)	(-)	(-)	*E.coli*	68
22	721	10.589401	76.879324	96	GNR	(-)	(-)	(+)	(+)	*Enterobacter*	55
23	747	10.588187	76.877757	87	GNR	(-)	(-)	(+)	(+)	*Enterobacter*	72
24	750	10.587696	76.877974	956	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	553
25	751	10.587271	76.878479	956	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	651
26	756	10.587306	76.878045	956	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	253
27	758	10.587714	76.877569	859	GNR	(-)	(+)	(+)	(-)	*Citrobacter*	425

BC, Bacterial colonies; GS, Gram Straining; GNR, Gram negative- Rod; GPR, Gram positive- Rod; IT, Indole test; MRT, Methyl red test; SCUT, Simmons Citrate Utilization test; VPT, Voges Proskauer Test; IB, Identified Bacteria; CC, Colonies Count; (+), Positive; (-), Negative.

RESULTS AND DISCUSSION

Statistical Analysis

The resulted characteristics of soil were compared with TNAU standards. Pearson coefficients (Table 2.) were incorporated which represents the relationship among the nutrients measured and the associated microbes. Statistics which includes bacterial symmetry, bacterial counts, mean value, minimum bacterial count were interpreted by descriptive statistical analysis (Table 3).

Physical parameters and primary macronutrients

The study area is observed with clay loam, sandy clay loam and sandy loam types of soil. Loam soil is the mixture of sand, silt and clay having the pH value 4.5 to 6.5. Most of samples in the study area are reddish brown in color indicating fine soil texture that greatly helps in the horticulture. Soil pH value at Anaimalai ranges from 6.41 to 8.72. The overall pH result make a strong report that the pH values which falls above 6.5 in some field area is completely due to the other predominant factors such as water and agrochemicals. The bacterium plays a key role in maintaining the soil pH range (Hoorman, 2016). One of the identified genera *Citrobacter* count holds positive correlation with pH (Table 2). *Citrobacter* can maintain the soil pH ranges from 3 to 11 (Oliveira *et al.*, 2016). Electrical conductivity finds its value ranges from 0.08 to 0.9 and the highest EC recorded is 0.9 dS/m. Excess salinity causes a huge hindrance for horticulture (Habib et al., 2016). Beneficial bacteria lower the concentration of ethylene that directs to deduce the salinity stresses in horticulture farmlands. Utilitarian bacterial genuses identified were *Achromobacter* and *Azotobacter* which are positively correlated (Table 2) with Electrical conductivity. Field available nitrogen ranges from 128 to 265 kg/ha. Inspite of two bacterial

Table 2. Pearson correlation among soil available NPK and bacterial species

Pearson Correlations										
	Nitrogen	Phosphorus	Potassium	AC	AR	AZ	CI	CO	E.Coli	EN
Nitrogen	1	0.291	-0.405*	-0.097	-0.366	-0.016	-0.188	0.077	0.150	0.276
Phosphorus	0.291	1	0.322	-0.273	0.175	0.357	-0.239	-0.081	-0.059	0.003
Potassium	-0.405*	0.322	1	0.121	-0.170	-0.031	-0.058	0.186	-0.145	0.136
AC	-0.987	-0.273	0.121	1	-0.093	-0.061	-0.082	-0.038	-0.105	-0.081
AR	-0.366	0.175	-0.175	-0.093	1	-0.147	-0.198	-0.093	-0.255	-0.197
AZ	-0.016	0.357	-0.031	-0.061	-0.147	1	-0.129	-0.061	-0.166	-0.128
CI	-0.118	-0.239	-0.058	-0.082	-0.198	-0.129	1	-0.082	-0.224	-0.172
CO	0.077	-0.081	0.186	-0.038	-0.093	-0.061	-0.082	1	-0.105	-0.081
E.Coli	0.150	-0.059	-0.145	-0.105	-0.255	-0.166	-0.224	-0.105	1	-0.222
EN	0.276	0.003	0.136	-0.081	-0.197	-0.128	-0.172	-0.081	-0.222	1

*. Correlation is significant at the 0.05 level (2-tailed). AC, Achromobacter; AR, Arthrobacter; AZ, Azotobacter; CI, Citrobacter; CO, Cornybacterium; E.Coli, Escherichia Coli; EN, Enterobacter;

Table 3. Descriptive analysis of bacterial species.

	N total	Mean	SD	Sum	Skewness	Kurtosis	CV	Min	Median	Max
Achromobacter	27	13.40	69.66	362	5.19	27	5.19	0	0	362
Arthrobacter	27	82.07	175.8	2216	2.26	4.64	2.14	0	0	651
Azotobacter	27	35.96	118.6	971	3.80	15.28	3.29	0	0	557
Citrobacter	27	62.96	153.8	1700	2.09	2.59	2.44	0	0	425
Cornybacterium	27	7.96	41.37	215	5.19	27	5.19	0	0	215
E.Coli	27	44.66	84.91	1206	1.58	0.72	1.09	0	0	218
Enterobacter	27	26	64.10	702	2.59	5.94	2.46	0	0	225

SD, Standard Deviation; CV, Coefficient of Variation; Min, Minimum; Max, Maximum;

genus presences in the soil, soil available nitrogen indicates low status. This is due to the presence of *E. coli* which is not a nitrogen fixation bacterium and also due to other environmental factors. Nevertheless, the outcomes of the genus action in the soil still contribute 19% of available nitrogen to the plants. Available phosphorus ranges from 15 to 37 kg/ha in the field and fulfills the recommended level which is considered as major growth contributing factor in horticultural crops productivity. Two major bacterial genera *Arthrobacter* and *azotobacter* shows high positive correlation with phosphorus (Table 2).Various literatures show the importance of *azotobacter* and *Arthrobacter* in solubilizing the soil phosphorus (Banerjee *et al*., 2010). Potassium founds to be extracted at higher concentration by horticultural crops (Pimentel *et al*., 2015) which are in the range between 132 to 374 kg/ha. Soil bacteria ensure prolonged crop growth through its production of inorganic acids, acidolysis, polysaccharides and chelation (Archana et al., 2013). Contributing identified bacterial genus *Achromobacter* and *Enterobacter* shows positive correlation with available potassium level (Table 2).

Identified Bacterial genera

Maximum of 362 colonies were found to be *Achromobacter* genus, which gives positive correlation with soil Electrical conductivity and Potassium. *Achromobacter* genus is capable of solubilizing 5.4 µg/ml of potassium in the soil at maximum extend (Gupta *et al*., 2016). Nevertheless, the genus finds negative correlations with nitrogen and phosphorus. *Achromobacter* induce ethylene hormones for contribution of soil available nitrogen (Bangash *et al*., 2021) and helps to expose to transient water stress in horticultural crops more specifically tomato and pepper. *Arthrobacter* genus ranges maximum of 651 in terms of colonies count at the selected study boundary. Distinctive nature of *Arthrobacter* in synthesizing plant hormones alleviates the phosphorus deficiency and stress developed by the salinity in the soil (Etesami and Glick, 2020). The genus finds positive correlation with soil phosphorus whereas shows negative correlation with other soil nutrients. *Arthrobacter* genus induces active mechanism against salt stress in the *Pisum sativum* (pea) crops. Maximum of 557 *Azotobacter* colonies count were identified which implies positive correlations with phosphorus, whereas negative correlation with other soil nutrients. Though *azotobacter* magnifies the nitrogen fixation in the soil through active production of phytopathogenic inhibitors, mathematical model implies negative correlation because of the presence of toxic inorganic fertilizers. *Azotobacter* due to its biological activity helps in increasing the phosphorus solubilization. Compared to *Arthrobacter, azotobacter* implies the indole acidic acids which are responsible for phosphorus solubilization (Aung *et al*., 2020). *Citrobacter* (425 colonies count) maintain the soil pH in recommended range because of its incredible biological activity (Oliveira et al., 2016). Maximum of 218 colonies were found to be *Escherichia coli* genera, which give positive correlation with soil nitrogen and negative correlations with other soil nutrients. *E.coli* is not a nitrogen fixing bacterium but possibly helps in nitrogen cycle by producing urea when *E. coli* utilizes ammonium. Potentiality of *E. coli* in producing iron-chelating compounds helps to promote the growth of potato crops. *Enterobacter* due to ACC deaminase activity induces nitrogen and potassium in the soil (Guo *et al*., 2020). Production of indole-3-acetic acids by the *Enterobacter* helps in phosphorus solubilization thus in turn multiple the yields of tomato, cucumber and pepper crops.

Organic fertilizer selection and dosage recommendations

Identified bacterial genera needs to be formulated for its survival so as to reach the soil for progress of crop productions. BioAtivo, Azotovit, Rhizotorphin, Azotobacterin, Ekophit, Mizorin®, Mamezo, Phylazonit-M, Symbion-N, CALOBIUM, Sardar Biofertilizers and Ferti-Bio are commonly available biofertilizers having identified genera in active state. Biofertilizers are usually bioformulated into two major type viz. liquid and solid with natural carriers.

CONCLUSION

This study implies the necessity of incorporating mathematical models to bring micro investigation on association of Insitu soil nutrient and its correlated bacterial genera so as to perceive the current nutrient status. *Azotobacter, Enterobacter, Citrobacter, Achromobacter, E. coli,* and *Arthrobacter* were some of the identified bacterial species that contribute to the soil primary macronutrients. This can be extempori-zing for organic fertilizer formulation consisting aforementioned identified bacterial genera and it is recommended to utilize those organic biofertlizers to attain massive yield in horticulture.

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