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Research Paper

DIFFERENTIAL CAPACITY OF NUTRIENT MOBILIZATION OF MICROBIAL FLORA FROM A CHEMICAL FERTILIZER APPLIED AND BIOFERTILIZER APPLIED RHIZOSPHERE

Sandra Yesudasan Miranda and Ayona Jayadev

P. G. Department of Environmental Sciences,
All Saints' College, Thiruvananthapuram.

Abstract

Soil provides a very good environment for the proper growth of microbes such as protozoa, viruses, fungi and bacteria. Some microorganisms are able to colonize soil surrounding plant roots and are called 'the rhizosphere microorganisms' or rhizoflora. Rhizobacteria have the ability to multiply and colonize plant roots at all stages of plant growth, in the presence of a competing microflora. They also act as biocontrol agents by antibiotic production, or preventing the deleterious effects of xenobiotics by degradation (rhizoremediators). This work focuses on the isolation and screening of rhizospheric bacteria from the rhizosphere microflora supplemented with chemical and biofertilizers with an objective of comparing the activities shown by the two. The activities between rhizobacteria of a leguminous plant as well as that of a non-leguminous plant treated are also compared. The isolated strains were also screened for ammonification, nitrification and phosphate solubilisation activities. Microbial activity in soil was measured by soil respiration method. To assess the rhizosphere effect R: S ratio was also estimated. The overall results show that the biofertilizers support more microbial growth than the chemical fertilizers. These results support the application of biofertilizers instead of chemical fertilizers.

Key words: biofertilizer, chemical fertilizer, rhizobacteria, rhizosphere, R: S ratio.

INTRODUCTION

Soil and soil microbes provide a suitable atmosphere for plant growth and the microbes play essential role in food chain and in elemental cycling of carbon, nitrogen, sulphur, phosphorus, etc. [37]. Due to the presence of microbes, soil is not an inert static material. Cultivated soil has relatively much more microbes than the fallow land. Soil fauna like fungi and bacteria are the highest portion of microorganisms inhabiting in the rhizosphere [30]. The rhizosphere is the soil zone surrounding the plant roots and which helps for physical, chemical and biological properties of soil [29]. The root exudates in the rhizosphere region provide amino acids and growth factors required by soil bacteria. The root colonizing bacteria which inhabiting the rhizosphere and form symbiotic relationships with many plants is popularly known as rhizobacteria. Root free soil outside the rhizosphere which is not penetrated by plant roots is commonly known as bulk soil [13]. Here microbial populations are low in number compared to rhizospheric soil.

Based on the studies about rhizosphere and the effect of root exudates to the microbes (particularly bacteria), the term rhizospheric effect/phenomenon was come into existence for the first time. Then later Katznelson [1] introduced the term R/S ratio (Rhizosphere : soil ratio) to express the rhizospheric effect. The ratio of microbial population per unit weight of rhizosphere soil (R), to the microbial population per unit weight of the adjacent non-rhizosphere soil (S) is the R: S ratio. Type and moisture content of the soil, temperature, age of the plant, etc. are the factors which influence the rhizospheric effect. R:S ratio is decreasing with increase in soil depth [14].

In this paper, special focus was given to biofertilizer added and chemical fertilizer added leguminous and non-leguminous plants separately. Biofertilizers are absolutely environmental friendly fertilizers. They prevent damages to natural sources and help to some extent, in cleaning the nature from precipitated chemical fertilizers. Biofertilizer is also a good carrier material for nutrients and microorganisms [10].

Many studies revealed that drought, cold, pests and diseases withstanding capacity of plants can be improvised with the help of Nitrogen (N), Phosphorus (P) and Potassium(K) [39] [36]. The average soil contains about 0.05 % (w/w) phosphorus but the available phosphorus to plants is very low and it is only 0.1% of the total soil phosphorus because of poor solubility and its fixation in soil [12]. Anthropogenic addition of phosphorus fertilizers may cause toxic effect to plants and may also lead to fresh water eutrophication [32]. Here lies the importance of microorganisms which have the capacity to solubilize organic and inorganic phosphorus into plant available form. These microbes are living in rhizosphere, root surface or it may be also seen in connection with roots.

PSB secrete organic acids and phosphatases and the organic acids convert inorganic phosphates into monobasic and dibasic ions which is absorbable by plants. This process is known as mineral phosphate solubilization [24] [27] [40]. The role of phosphatases is to improve mineralization (hydrolysis) of organic phosphorus. So, PSB has an essential role in plant nutrition to increasing the uptake of phosphorus [34].

In order to satisfy crop nutritional requirements, Nitrogen, Phosphorus and Potassium are externally added. Generally for this purpose chemical fertilizers are used because of their rapid action. However synthesis of these fertilizers are highly energy intensive process, and have long term impacts on the environment in terms of eutrophication, soil fertility depletion and carbon footprint. Moreover, plants can use only a little amount of these fertilizers and 76-90% added fertilizers rapidly become fixed in soils by re-precipitation [33].

The biggest issue facing the use of chemical fertilizers is none other than ground water contamination. Over use of nitrogen containing chemical fertilizers like urea produces ammonia emanation and this contributes to acid rain, groundwater contamination and ozone depletion by the release of nitrous oxide by denitrification process.

So by the increased use of these types of fertilizers in future, this problem may increase several fold in the coming decades. Such environmental concerns have led to the search for sustainable way of nutrition for crops like biofertilizers, phosphate solubilizing microorganisms (PSM), etc. In this regard, biofertilizers are more environmental friendly than chemical fertilizers.

MATERIALS AND METHODS

This work was done in order to study and compare nutrient mobilization efficiency, especially phosphorus and nitrogen mobilization by the microflora of the rhizosphere of chemical and biofertilizer applied leguminous plant, *Vigna radiata* (leguminous) and non-leguminous plant, *Capsicum frutescens*. The activity difference of microflora of rhizosphere of leguminous and non-leguminous plant is also analyzed.

The physico-chemical parameters of soil used for growing the study plants *Vigna radiata* and *Capsicum frutescens* were tested in pathology section of Kerala Agricultural University, College of Agriculture, Vellayani just to get an idea about the type of the soil the plants are to be grown.

For microbial analysis work the following soil samples were used:

(a) Rhizospheric soil from chemical fertilizer added *Vigna radiata*

(b) Rhizospheric soil from biofertilizer added *Vigna radiata*

(c) Rhizospheric soil from chemical fertilizer added *Capsicum frutescens*
(d) Rhizospheric soil from biofertilizer added *Capsicum frutescens*

Based on the colony morphology, a total of 18 strains distinct bacterial colonies were isolated and selected for further studies. The pure cultures of bacterial strains obtained for different samples were subjected to gram staining technique to categorize them into gram positive and gram negative [3].

Enrichment of microorganisms

The soil samples were enriched in nutrient agar plates using pour plate method after serial dilution. The plates were then incubated at 35°C– 40°C for 24 – 48 hours.

Isolation of pure culture

The morphologically distinct bacterial colonies observed on nutrient agar plates were subjected to streak plate method using sterilized inoculating wire loop on separate plates for isolation and identification of pure bacterial colonies from a mixed population. All plates were then incubated at same temperature and for time period as before indicated.

Morphological characterization of microorganisms

Purified microbial colonies were studied for 5 different morphological characters namely colour, margin of colony, surface form, surface texture and elevation [6]. The obtained microorganisms were subjected to Gram staining technique [11].

The pure cultures were tested for the following abilities (Table 1).

RESULTS AND DISCUSSION

The work was done to identify the capabilities of various microorganisms that can be isolated from a leguminous rhizosphere and a non-leguminous rhizosphere and to compare their efficiencies for the said activities and to find the effect of addition of a biofertilizer and a chemical fertilizer. The results obtained are presented below.

Analysis of Physico- Chemical parameters of soil

Chemical properties of soil are the most important among the factors that determine the nutrient supplying power of the soil to the plants and microbes.

In the present study analysis of physico- chemical parameters of the soil for the fertility status was analyzed and the results are given in (Table 2). The soil acidity was found towards an acidic range. This can be because of different anthropogenic and natural activities including leaching of chemicals, acid rains, decomposition of organic materials etc. [21].

The very low content of potash may be due to the soil management activities or the parent materials. Wakene [20] clearly reported that the variation in the distribution of K depends on the mineral present, particles size distribution, degree of weathering, soil management practices, climatic conditions, degree of soil development, the intensity of cultivation and the parent material from which the soil is formed.

Microbial Analysis

Enrichment and isolation of bacterial isolates

From chemical fertilizer added pea rhizosphere = 3
From biofertilizer added pea rhizosphere = 4
From chemical fertilizer added chilli rhizosphere = 4
From biofertilizer added chilli rhizosphere = 7

Gram Staining

Eleven gram negative bacterial strains were obtained. Out of which three were bacilli and eight were cocci. Seven gram positive bacteria were also obtained and out of which three were bacilli and four were cocci.

Screening of bacterial strains for Nitrogen fixing activity

Identification of Ammonifying Bacteria

In the present study the isolated bacterial strains were tested for ammonification and nitrification. The results are shown in Table 3.

All the bacterial strains isolated from pea rhizosphere treated with biofertilizer showed greater production of ammonia when compared to bacterial strains isolated from chemical fertilizer treated pea rhizosphere (Table 3).

In the case of bacterial strains isolated from chilli rhizosphere applied with biofertilizer, only two strains (OC6 and OC7) showed ammonification activity. Out of the five strains isolated from chemical fertilizer applied chilli rhizosphere only one strain (CC3) showed ammonification activity but their activity was found to be minimum (Table 3).
These results show that there is a considerable decrease in the activities of bacteria isolated from a chemical applied site and a biofertilizer applied site. These findings support the earlier observations that the application of chemical fertilizers lowers the capacities of microorganisms whereas biofertilizers help the microbes in their activities.

Identifying bacteria involved in nitrification

Nitrite production

In the present study the selected strains were analyzed for nitrite production and the results are shown in Table 3. None of the bacterial strains showed nitrite production. It may be due to the lack of oxidase enzymes in the selected bacterial isolates.

Nitrate production

Under anaerobic conditions, some bacteria are able to use nitrate (NO3-) as an external terminal electron acceptor. This kind of metabolism is analogous to the use of oxygen as a terminal electron acceptor by aerobic organisms and is called anaerobic respiration. Nitrate is an oxidized compound and there are several steps possible in its reduction. The initial step is the reduction of nitrate (NO3-) to nitrite (NO2-). In the present study the isolated strains were screened for nitrate production and the results were showed in Table 3.
Almost all the bacterial strains isolated from both pea and chilli rhizosphere showed nitrate production activity except those isolated from chemical fertilizer treated rhizospheres which showed a slight reduction in nitrate production. This may be due to the nature of the bacterial strains which vary in their ability to perform these reactions.
The present study reveals the presence of ammonifying and nitrifying bacteria seems to be decreased in chemical fertilizer applied soils and plants compared to the biofertilizer added ones. The addition of chemical fertilizers may affect soil health and which in turn affects the bacterial population. There are so many reports which show that the application of chemical fertilizer harms the natural microbes present in soil [16]. Prasad [41] reported that biofertilizers accelerate certain microbial processes in the soil or rhizosphere which augment the extent of availability of nutrients like nitrogen, phosphorus etc in a form easily assimilated by plants.The bacterial strains isolated from non leguminous rhizosphere also showed significant reduction in ammonification and nitrate production activity with that of leguminous rhizosphere. It may be because of leguminous roots. Biofertilizer application also favors plant nutrient uptake and rhizosphere microbial activities. The spatial localization of roots is important when nutrient is distributed heterogeneously [28].

Identification of Phosphate Solubilizing Bacteria

Halo zones/ clear zones surrounding the bacterial isolates is considered as phosphate solubilization zone and which is selected as positive [8]. In the study period several attempts were made to isolate Phosphate Solubilizing Bacteria (PSB). But only one bacterial isolate (OP4) with the said activity was obtained. Only OP4 was able to solubilize Tri Calcium Phosphate (TCP) in solid culture state.
But it was not considered for further quantitative analysis since it showed less than 3 mm phosphate solubilization zone. The isolates exhibiting 3 mm or more halo zone only will be considered for further quantitative analysis. At both low and high pH values, availability of phosphorus to the microbes is low which cen be due to the said observation. (in the present study, the soil sample having strongly acidic pH of 5.5). Acidic soil condition causes immobilization of soil phosphorus which may cause unavailability of phosphorus to the microbes. The proper phosphate solubilization pH for bacteria which is suitable to solubilize TCP is 7.2.
The basis for phosphate solubilization relies on organic acid production by the microbes which will solubilize the calcium phosphate used in the medium [18] [19]. The nature of the acid produced is also important [14]. Fasim *et al*. [23] suggested that bacterial isolates solubilize

phosphates only if the medium contains glucose. In the present study, the medium provided to isolate PSB contained glucose but due to the acidic pH of the soil (5.5) the organic acid production may be blocked. There are several factors related to phosphate solubilization and those may be nutritional, physiological, or it may be the culture growth conditions [15]. The study conducted by Sujatha *et al.* [26] revealed that some bacteria can easily solubilize di-calcium phosphate than tri-calcium phosphate. Bacteria are famous for their mineral solubilization properties, but in the present study about the case of phosphate solubilization their performance is poorly established. But based on the type of organisms involved, the degree of phosphate solubilization can also change.

Soil respiration

The microbial activity in soil was measured by soil respiration method in raw soil, biofertilizer added soil and chemical fertilizer added soil for two weeks and the results are shown in Table 4. From the table it is clear that the amount of CO_2 after one week was high and after that it decreased. It reveals, during the second week period the growth of the microbes come to stationary phase and thus the microbial activity gets reduced. So the first week time was considered as optimum growth period for the growth of the microbes [17] [31].

The application of chemical fertilizer may affect the health of the plant and there by affect the microbial population in the rhizosphere. So the chemical fertilizer added soil exhibit least microbial activity (amount of carbon mineralized = 27.78 mg). The addition of biofertilizer enriches the nutrient quality of soil and transforms organic matter into nutrients that can be used to make plants healthy and productive. A healthy plant usually has a healthy rhizosphere which should be dominated by beneficial microbes.

It is well known that CO_2 production, transport and emission in soil depend on environmental factors such as aeration condition, soil temperature, soil moisture, supplies of organic carbon, fertilization, pH etc. [39] [25]. Therefore sound management of fertilization is must to ensure the soil quality.

R: S ratio

It is obvious that the rhizosphere microflora predominates as compared to non-rhizosphere ones. R: S ratio shown in Table 5 revealed that the microbial population was high in biofertilizer added rhizosphere compared to that of chemical fertilizer added one. R: S ratio gives a clear picture about the rate of microbial interaction with the plant roots. Egamberdiyeva [35] reported that the bacterial inoculation has a much better stimulatory effect on plant growth in nutrient deficient soil than in nutrient rich soil. Therefore the result is supportive for the present work.

In general, legumes exhibit evident rhizosphere effect than non-legumes. The quantitative difference in the microbial population of the rhizosphere from that of non- rhizosphere/ general soil is mainly due to root exudates in the rhizosphere region which supports more bacterial growth.

CONCLUSION

Chemical fertilizers will deteriorate soils and thereby destroying the natural soil ecosystem. This will lead to poor plant growth and reduced agricultural productivity. It will also make huge demand on water and deficiency in micro- nutrients. Biofertilizers help to increase the plant development as well as helps in the growth and soil rejuvenating activities of soil microbes.

Table 1. Characters analyzed and details of the tests carried out

TEST	REAGENTS/PROCEDURE	REFERENCE
Identification of Ammonifying Bacteria	Peptone broth and Nessler's reagent	[9]
Test for nitrite production	Ammonium sulphate broth and Trommsdorf's reagent	[38]
Test for nitrate production	Nitrite Broth (Nitrate Forming Broth) for testing nitrate production using Diphenylamine reagent	[38]
Isolation of Phosphate Solubilizing Microorganisms (PSM)	Pikovskaya's (PKV) agar medium supplemented with insoluble TCP	[2]
Measurement of microbial activity in soil	Soil respiration method	[7]
Estimation of R: S ratio	Quantitative estimation is required to determine the R: S ratio and assess the rhizosphere effect. Divided the values of CFUs of rhizosphere microorganisms with non-rhizosphere to get the R: S ratio.	[1] [5]

Table 2. Physico-chemical characteristics of soil

Parameter	**Reading**	**Rating**
Ph	5.5	Strongly acid
EC dSm^{-1}	0.04	Normal
Organic carbon (%)	2.2	High
Available phosphorus (Kg/ha)	29	High
Available potash(Kg/ha)	10	Very low

Table 3. The bacterial isolates showing ammonification and nitrification

Culture	**Production of Ammonia**	**Nitrate production**	**Nitrite production**
OP1	++	++	-
OP2	++	++	-
OP3	++	++	-
OP4	++	+	-
CP1	+	+	-
CP2	+	+	-
CP3	+	+	-
OC1	-	++	-
OC2	-	+	-
OC3	-	++	-
OC4	-	++	-
OC5	-	+	-
OC6	+	++	-
OC7	++	++	-
CC1	-	-	-
CC2	-	+	-
CC3	+	+	-
CC4	-	+	-

Note: '**-**' sign indicates no production, '**+**' sign indicates small amount of production and '**++**' sign indicates large amount of production.

Table 4. Soil respiration

Soil samples	Amount of carbon mineralized(mg)	
	I week	II week
Biofertilizer added soil	46.20	22.86
Chemical fertilizer added soil	27.78	16.92

Table 5. The R: S ratio

SOIL REGIONS	CFUs (×10^4/g)	R: S ratio
Non –rhizosphere (Chemical fertilizer added) Rhizosphere (Chemical fertilizer added chilli)	36 43	 1.2
Non –rhizosphere (Chemical fertilizer added) Rhizosphere (Chemical fertilizer added pea)	36 62	
Non –rhizosphere(Biofertilizer added) Rhizosphere (Biofertilizer added chilli)	42 103	
Non –rhizosphere(Biofertilizer added) Rhizosphere (Biofertilizer added pea)	42 112	 2.7

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