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Full Length Research Article

Isolation and Biological Analysis of Plant Growth Promoting Factors from Vegetable Cultivate Soil Samples of Namakkal District, Tamil Nadu, India

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Abstract

Plant growth promoting factors (PGPF) is a wide range of compounds that have been identified as having a positive influence on the growth and development of plants. The aim of this study was to isolate the native isolates from vegetable cultivate soil samples Namakkal District, Tamil Nadu, India. The PGPF potential of these bacteria was assessed in vitro and a representative isolate was found using biochemical analysis. Totally 25 bacterial isolates of 8 genera were observed, among them, Bacillus genus were predominant and followed by Pseudomonas sp. In the present study, 44% and 20% of isolates showed the positive for siderophore and HCN production respectively, among the 8 genera, most of the Azotobacters and Rhizobium were also found to show positively for nitrogen fixation. In addition, 48%, 36% and 68% of isolate were producing the protease, cellulose, and amylase respectively. Among the 9 factors 8 factors harboring isolates as *Lysinibacillus macroides,* which was confirmed with 16srRNA sequencing analysis. Overall, using isolates to promote plant growth can be beneficial to the health of vegetables and the environment.

***Key words*:** PGPF, Siderophore, IAA, Azotobacters sp, Rhizobium sp, *Lysinibacillus macroides*

Chemical fertilizers are routinely used to provide essential nutrients to the soil-plant system all over the world. Chemical fertilizers, particularly N fertilizers, have considerable drawbacks in terms of cost, accessibility, and environmental impact in modern agriculture. As a result, it is essential to find alternative strategies to guarantee competitive crop yields, provide environmental safety and protect the agro-ecosystem while maintaining long-term ecological balance. Globally, microbial inoculants or PGPR are becoming more widely used in intensive agriculture as a means of developing sustainable agricultural production.

Rhizobacteria that promote plant growth aggressively colonies the rhizosphere and plant roots to increase plant growth and yield when added to seeds or crops [1]. The release of metabolites that directly stimulate growth best explains the plant growth promoting (PGP) function of the PGPR. There have been several proposed methods to explain how PGPR helps the host plant. Among them are: (a) the capacity to produce phytohormones or plant growth regulators, (b) the enhancement of asymbiotic relationships, (c) the solubilization of inorganic mineralization and/or other nutrients and (d) the antagonistic effect against phytopathogenic microorganisms [2].

Many studies have clearly demonstrated how PGPR improves the growth and yield of various crops, especially cereals [3], under varying environmental conditions, as interest in beneficial rhizobacteria associated with vegetables and fruits have recently increased [4]. Number of bacterial isolates of Bacillus sp and Pseudomonas sp, Azospirillum, Azotobacter, Klebsiella, Enterobacter were widely reported PGPR genera. Pahari *et al*. [5] utilized the *Bacillus* sp and *Pseudomonas* sp for as PGPR.

More recently, Mengistie and Awlachew [6] conducted experiments on wheat under pot and field condition to examine the effect of PGPRs (*Bacillus* sp) on the growth and yield of Tomato. Civelek and Yildirim [7] also use as PGPRs of *Bacillus* sp for improving the cultivation of cauliflower. Understanding the distribution and diversity of indigenous bacteria in the rhizosphere of specific crops requires knowledge of indigenous bacterial populations, their characterization and identification. In order to achieve desired crop production, region-specific microbial strains can be used as growth promoting/enhancing inoculums to replace chemical fertilizer-based agricultural practices [3]. The goal of the study was to separate the native isolates from vegetable cultivated soil samples Namakkal District, Tamil Nadu, India. The PGPF potential of these

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bacteria was assessed in vitro and the representative isolate was found using 16S rRNA sequence analysis.

MATERIALS AND METHODS

Collection of soil samples

The vegetable cultivates soil samples were collected from Namakkal area. Samples were placed individually in plastic bags and brought to Laboratory for isolation of bacteria. Rhizospheric bacteria were isolated from 1 g soil by serial dilution plating on Nutrient agar (Himedia, India) plates. The plates were incubated at 28 ± 2 °C 24 hours to 48 hours. Individual colonies were picked and streaked on NA plates for further purification.

Isolation of plant growth promoting factors producing isolates

All rhizobacterial isolates obtained were screened for different plant growth promoting traits. Each isolate was inoculated on modified Pikovskaya agar media with tricalcium phosphate (TCP) and incubated at 30±0.1°C for 5 to 7 days and observed the zone of clearance around the colonies which indicate as positive for phosphate solubilization.

IAA production was assayed using qualitative method developed by some modification of Bric *et al*. [8] method. Bacterial cultures were inoculated in nutrient broth with tryptophan (1mg/ml) wear incubated at 35 ± 2 °C for 7 days. Then 2 drops of orthophosphoric acid and 4 ml of Salkowski's reagent (50 ml, 35% perchloric acid; 1 ml 0.5mM of $FeCl_3$). The development of a pink colour indicated as IAA production.

The siderophore production was observed by the isolates was checked on solid CAS agar plates. The isolates were inoculated on to CAS blue agar media and incubated at 35 ± 2 °C for 72 hrs. Formation of yellow-orange halo zone around the colony indicated production and release of the siderophores on the agar plate [9].

For determination of ammonia production isolates were inoculated into peptone water forr 48 h at 35 ± 2°C. The 0.5 ml of Nessler's reagent was added in each tube. Development of brown to yellow color observed was a positive test for ammonia production [10].

Bacterial cultures were streaked on nutrient agar medium containing 4.4 gm/l of glycine. A Whatman filter paper No. 1 soaked in 0.5% picric acid solution (in 2% sodium carbonate) was placed inside the lid of a plate. Plates were sealed with parafilm and incubated at 35 ± 2ºC for 4 days [11]. Development of orange to red color indicated HCN production.

The nitrogen fixing bacteria can be isolated from the soil [12], using yeast extract mannitol selective culture media (YEM) with Bromthimol blue (BTB). The yellow halos around the colonies on blue were positive for nitrogen fixing bacteria.

The protease activity of the bacterial isolates was determined using the skim milk agar medium. The isolates were spot inoculated on skim milk agar medium, and after two days of incubation at 30 °C, proteolytic activity was assessed by clear zone around the colonies [13].

The isolates were screened for cellulase production by supplemented with 10 g l−1 carboxymethyl cellulose (CMC). After 48 hours incubation at 30 °C, plates were flooded with Congo red dye, and the clear halos formed surrounding the colonies indicated their cellulolytic activity [14].

The bacterial isolates were spot inoculated on starch agar (Beef extract 3.0, peptone 5.0, soluble starch 2.0, Agar 15.0, Distilled water 1liter) medium plates and incubated at 30°C for 48 hrs. At the end of the incubation period, the plates were flooded with iodine solution, the clear hole was observed around the colonies which indicate a positive for amylase production [15].

RESULTS AND DISCUSSION

Plant growth promoting factors (PGPF) refers to a wide range of compounds that have been identified as having a positive influence on the growth and development of plants. These range from microbial-derived compounds and plant hormones, to plant extracts, plant-derived nutrient sources, and even soil amendments. PGPF have been shown to improve crop yield, increase resistance to environmental stresses, and boost overall plant health. They can also help plants obtain essential nutrients, improve soil fertility, and prevent disease. In recent years, PGPF have become increasingly important in the agricultural industry as they offer a sustainable, cost-effective way of promoting crop growth and yield. The agro climatic conditions was important for plant growth especially; pH of the soil was important for plant growth, in this area, 5.5 to 8.8 of soil pH was presented, however nitrogen and phosphorus were not sufficient level in this area, therefore alternatives to agrochemicals for better growth and development of crop plants. So, this study was contacted to document the region-specific isolates for improve the growth of plant.

Totally 25 bacterial isolates of 8 genus were observed, among them, Bacillus genus (*Bacillus subtilis, Bacillus cereus Bacillus amyloliquefaciens* and unidentified of one *Bacillus* sp) were predominant and followed by Pseudomonas sp (*Pseudomonas aeruginosa* and *Pseudomonas fluorescens*). Among the 8 geneus, 37.5% of gram positive and 62.5% of gram-positive isolates were observed. Similarly, in 2018, previous study also observed the bacterial consortium of PGPF producing isolates from Namakkal areas [16]. Singh *et al*. [17] at Banaras Hindu University found different bacterial communities from vegetable cultivated soil samples.

Presently, all isolates were screened for PGPF characters, each isolates contains a specific plant growth factor. The IAA, also known as auxin, is a hormone that regulates plant growth and is crucial for several physiological processes throughout plant development, including cell division and elongation, tissue differentiation and the beginning of new roots [18]. Presently 32% of bacteria produce the IAA and all isolates of *Azotobacter sp* were showed positively for IAA. This result was correlated with earlier study of Ponmurugan *et al*. [19]; they were also observed the IAA production in all *Azotobacter sp*. Not only this, other characters of PGPR Phosphate solubility and Ammonia also affect the growth of the plant if they are not formed. Presently 36% of ammonia producers and 32% of phosphate solubilizer were observed; among them 75% of *Azotobacter sp* were producing the ammonia and phosphate solubilization (Fig 1-2). This was contrary to earlier study of Ponmurugan *et al*. [19]; they were noted that both PGPF character were observed from all isolates of *Azotobacter sp*, simultaneously other genera of *Bacillus sp, Rhizobium sp,* and *Rhizobium sp* were showed positively for ammonia production and phosphate solubilization.

Siderophores have important applications in promoting plant growth, biocontrol activity, and several other ecological factors. It was created by different microorganisms in order to demonstrate the ferric iron's competency in the ferric hydroxide complex. In the present study, 44% of isolates were showed positive for siderophore production, among the 8 genera, *Pseudomonas* sp were predominately positive and most of the *Azotobacters sp* and *Rhizobium sp* were also found to show siderophore production. This is in accordance with the study report of Jenifer [20] in which siderophore production was

exhibited by free living rhizospheric isolates of Azotobacter (16.22%), *Pseudomonas* sp and *Bacillus* sp.

HCN, also known as hydrogen cyanide, is a chemical compound that has been acknowledged for its potential as a plant growth promoter. It has been suggested that HCN can act as a biocontrol agent against plant pathogens. According to earlier reports, rhizospheric isolates were protecting the several plants from root disease caused by soil borne fungi through HCN production [21]. Presently 20% of isolates account to positive for HCN namely *Azotobacters sp, Rhizobium sp, Pseudomonas aeruginosa, Serratia marcescens* and *Lysinibacillus sp.* Previous study of Ponmurugan *et al*. [19] also observed the HCN producing *Azotobacters sp* from Namakkal area. In 2017, Naureen *et al*. [22] found the HCN producing *Lysinibacillus sp* from maize cultivate soil samples.

Amongst the nutrients, nitrogen is the only nutrient, which plays a major role in the synthesis of chlorophyll, amino acids, and protein building block. The nitrogen-fixing bacteria play an important role in maintaining nitrogen levels in soil, which is essential for healthy plant growth. They also help to improve soil fertility and reduce the need for synthetic nitrogen fertilizers. Therefore, realizing the importance of this, an attempt was made to find out the nitrogen fixing bacteria from soil samples. The results of a nitrogen-fixing test showed that all 36% could fixation nitrogen and all isolates of *Azotobacters* and *Rhizobium sp* were showed positively for nitrogen fixing activity. Furthermore, *Lysinibacillus sp*, *Pseudomonas sp* and *Bacillus* sp also showed positive for nitrogen fixation. In recently Shameem *et al*. [23] found the nitrogen fixing *Rhizobium mayense* from groundnut soil samples.

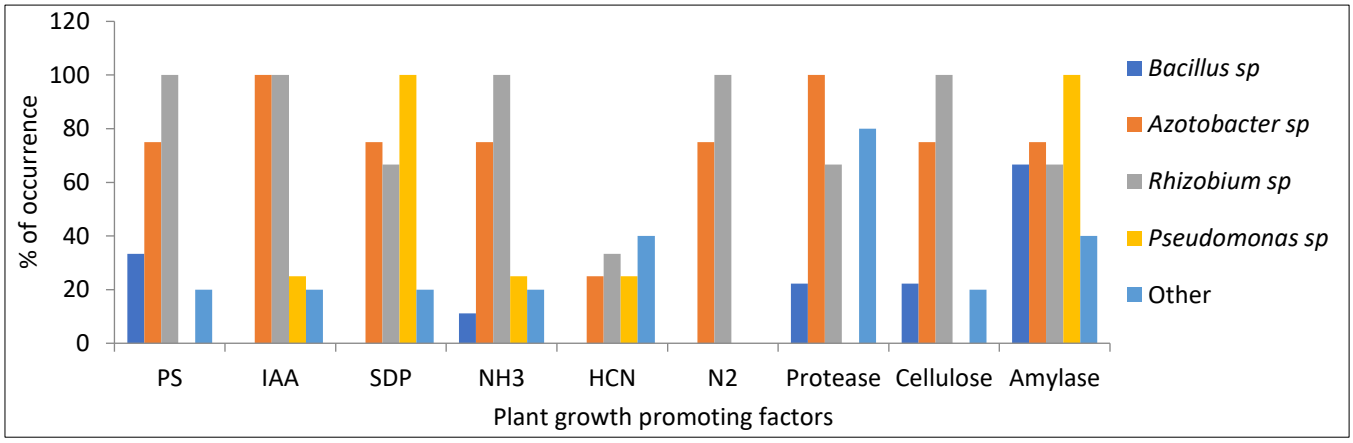

Fig 1 Identification of plant growth promoting traits in various bacterial isolates

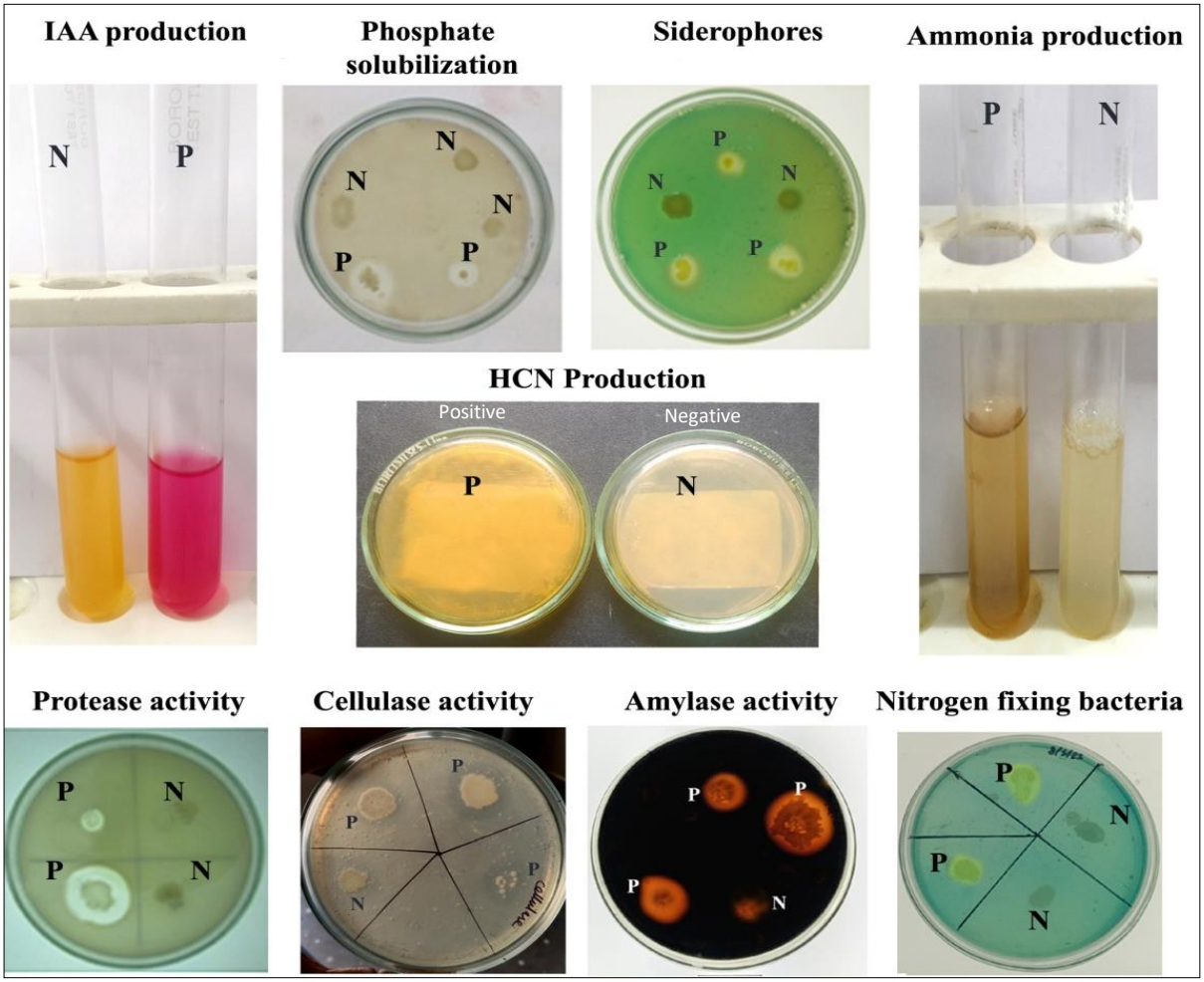

Fig 2 Isolation of PGPF producing bacterial isolates

Enzymes are proteins that act as catalysts and speed up chemical reactions in the body. They help break down large molecules into smaller ones and can also help in the uptake of certain nutrients, such as nitrogen, phosphorus, and potassium. Enzymes also help with the synthesis of hormones and other plant growth regulators, which can promote plant growth and development. Additionally, enzymes can help break down toxins and waste products, which can lead to better soil health and improved plant growth [24]. In the present study, 48%, 36% and 68% of isolates were producing the protease, cellulose and amylase respectively. Among the obtained 8 genera, highest prevalence of enzymes was in *Azotobacters* and *Rhizobium sp*. From the overall characterization, most of the plant growth promoting factors harbor in *Lysinibacillus sp*, except cellulose other plant growth promoting factors were present in this isolate.

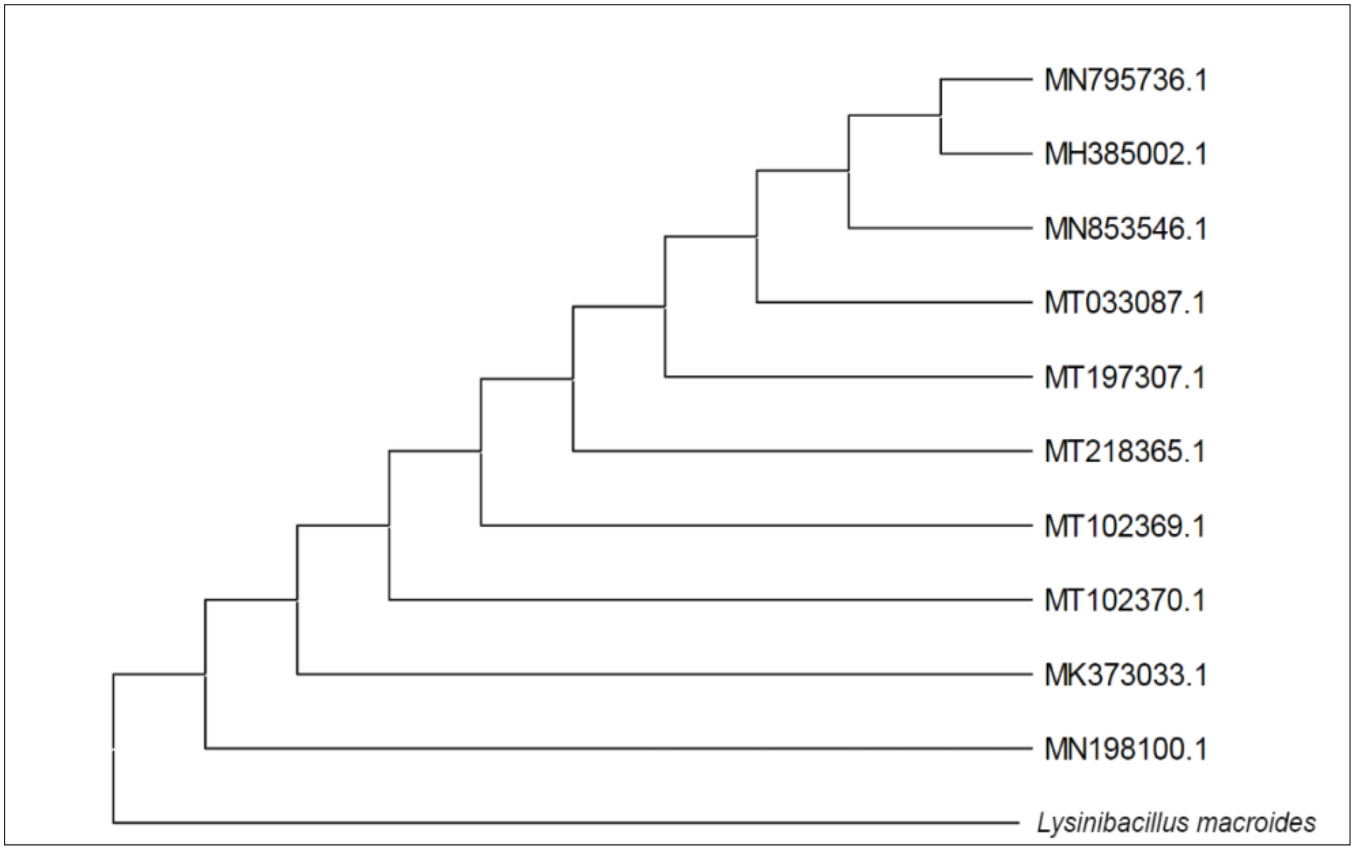

Fig 3 Phylogenetic tree analysis of *Lysinibacillus macroides*

The potential isolate of *Lysinibacillus sp* was known to be good plant growth promoters. This isolate may be considered as effective plant growth promoting bacterial strain; therefore, such isolate was subjected to 16srRNA sequence for specific species identification. The 16s rRNA genes were amplified using universal primers and the amplicans were sequenced in an automated gene sequencer. After the sequencing process, BLAST analysis was performed with existing 16srRNA bacterial sequence available in the nucleotide databases. The analysis revealed the homology of isolate. Sequencing and BLAST result revealed that the isolate was found to be very close to *Lysinibacillus sp* and had 100% sequence similarity with *Lysinibacillus macroides* (MN198100.1), (Fig 3).

CONCLUSION

The use of plant growth promoting factors producing isolates in vegetable cultivate soil samples has shown to be effective in enhancing the growth of vegetables. These isolates can increase the availability of nutrients in the soil, as well as improve the soil structure and microbial diversity. Overall, using isolates to promote plant growth in vegetable cultivate soil samples can be beneficial to the health of vegetables and the environment. Further research is being done on the commercialization and field use of integrated stable bio-formulations as efficient biocontrol methods.

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