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# Isolation and Characterization of Multi-trait PGPR from Banana (*Musa paradisiaca*) Rhizosphere

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**ABSTRACT**

*Plant Growth Promoting Rhizobacteria (PGPR) are the plant rhizosphere inhabiting bacteria that can promote plant growth by direct and indirect mechanisms. By considering usefulness of PGPR in sustainable agriculture, isolation and screening of multifaceted PGPR was carried out from banana rhizosphere soil samples. Total 54 isolates were obtained in pure culture from fourteen banana rhizospheric soil samples collected from different locations by considering diverse past cropping pattern. In vitro characterization data revealed that 72.22% showed antagonistic potential; 61.11%, 70.37% and 35.19% were positive for protease, amylase and lipase secretion respectively. In terms of increase in nutrient availability, 37.04%, 38.89%, 35.19% and 16.67% were showing positive phosphate solubilization, potash mobilization, zinc solubilization and nitrogen fixation respectively. Indole-3-acetic acid production was detected in 16.67% isolates and 24.07% were found positive for ACC deaminase activity. Two most potent isolates with multiple plant growth promoting characters were identified as Bacillus subtilis and Enterobacter cloacae. Therefore, exploring such multifaceted isolates in the sustainable way to solve the problems associated with overuse of synthetic chemicals in conventional agriculture. This is a step ahead for healthy food production along with enhancement of nutritional value of food.*

***Keywords:*** *Rhizospheric bacteria, Plant growth promotion, PGPR, Nutrient availability, IAA*

## INTRODUCTION

Banana is one of the important fruit crop grown worldwide for food in addition to source of beverages, medicines, fermentable sugars, flavouring, cordages, silage, fragrance, rope, fertilizers, garlands, shelter, clothing, smoking material, and numerous ceremonial and religious uses (Mohiuddin et al., 2014; Rana et al., 2018). However, banana plant is susceptible to different biotic and abiotic stresses (Santos et al., 2018) that largely impart its productivity. Many agrochemicals are available in the market that provides protection to the plant against these stresses. Use of these agrichemicals in the agriculture invites problems pertaining to the soil, plant, human and environment health (Sharma et al., 2018).

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Plant rhizosphere region is consisting of diverse and naturally occurring plant beneficial bacteria known as Plant Growth Promoting Rhizobacteria (PGPR). Such microbes have beneficial effect on plants and can promote its growth by different mechanisms such as biological nitrogen fixation (BNF), increasing bio-availability of nutrients, increasing root absorption area, enhancing beneficial symbiosis with host and combinations of above actions
(Apastambh et al., 2016). Therefore, application of PGPR in agriculture is an eco-friendly method for maintaining plant health and to promote plant growth. Mainly PGPR genera characterized are *Azotobacter, Azospirillum, Bacillus, Enterobacter, Klebsiella, Serratia,* etc. (Glick, 2012)

## MATERIALS AND METHODS:

**Collection of soil samples and isolation of PGPR**

Soil samples of approx. 500 g were collected, four from the each corner and one from the centre. A representative sample was made by mixing all five samples of the farm and was taken into lab for further study. Total fourteen banana rhizospheric soil samples were collected from different locations by considering diverse past cropping pattern.

Isolation of PGPR from collected soil samples was performed as per procedure given by Chakraborty et al., (2011) with little modifications**.** Briefly 1 g soil was mixed with 25 ml of distilled water followed by serial dilution and suspension was inoculated on nutrient agar (NA) plates. For pure culture isolation, well isolated colonies with distinct morphology were transferred on fresh plate by four sector streaking method.

**Screening of PGPR**

Different isolated bacteria were screened for their *in vitro* efficacy of plant growth promotion on the basis of different plant growth promoting parameters as follows:

**ACC deaminase activity**

ACC deaminase producer microbes were screened as per standard procedure given by Singh and Jha (2015). Pure cultures of isolates were streaked on DF (Dworkin & Foster) minimal medium containing ACC (3.0 mili-Molar) as sole source of nitrogen. Plates containing only DF minimal medium without ACC were kept as negative control and with 0.2% $(NH_4)_2SO_4$ as positive control.

**Phosphate solubilization**

Rate of phosphate solubilization was checked by inoculating bacteria on Pikovskaya's medium followed by measurement of zone index (Pikovskaya, 1948). Halo zone near colony due to insoluble tri-calcium solubilization was taken as an evidence of positive test

**IAA production**

IAA estimation was done using Salkowski method (Dobbelaere et al., 1999).

**Antagonistic potential**

Biocontrol ability of the isolates was evaluated against *Sclerotium rolfsii*. Efficiency of isolates was judged on per cent growth inhibition (PGI) method given by Vincent (1947).

**Nitrogen fixation**

Nitrogen fixation ability of isolates was judged by inoculating pure culture on nitrogen free Ashby's Mannitol Agar medium. Presence of growth after 48 hrs of incubation at 28°C was taken as positive test for nitrogen fixation.

**Potash mobilization**

Rate of potash mobilization was checked by incubating bacteria on glucose yeast extract calcium carbonate (GYC) medium. Halo zone after incubation was measured and taken as an evidence of potash mobilization.

**Zinc solubilization**

Zinc solubilization was determined on mineral salt agar medium amended with 1% ZnO (zinc oxide) substrate. The actively growing cultures were spot inoculated onto the medium, incubated at 28°C followed by measurement of solubilization zone (Venkatakrishnan et al., 2003).

**Extracellular enzyme production**

Extracellular enzymes proteases, lipases and amylases were detected in the medium with specific substrates i.e. casein, tributyrene and starch, respectively. Zone of clearance near

colony was taken as an evidence of positive test.

**Identification of isolates**

Potent PGPR isolates were identified by 16'S r-RNA sequencing method.

## RESULTS AND DISCUSSION

**Isolation of PGPR**

During growth and development, plant has to suffer from different stresses that pertain in its micro and macro environment that usually compromised plant productivity. Different biotic and abiotic stresses can cause growth deficiencies and also permanent damage or death of plant if it exceeds the plant tolerance limit (Mosa et al., 2017). Therefore, it is of utmost importance to minimize impact of these stresses on plant for increasing growth and productivity of the agriculturally important plants. Use of PGPR in the mitigation of such stresses and enhancement of plant productivity is the demanding technology in the modern and sustainable agriculture.

With the aim of isolation of PGPR from banana rhizosphere, total fourteen soil samples (OF-E, OF-N, RHRS-E, RHRS-N, IF-E, IF-N, EF-E, EF-N, SWM-E, SWM-N, BPF-E, BPF-N, CFA-E and CFA-N) were collected by considering diverse past cropping pattern. After dilution plate technique, well isolated colonies showing distinct colony morphology were selected and further purified by four sector method. Altogether, 54 PGPR isolates were obtained in pure culture form and were preserved at 4°C temperature for further studies.

***In vitro* screening of PGPR**

After isolation, bacterial isolates were screened for its efficacy of plant growth promotion (PGP) characters on the basis of different *in vitro* methods such as nitrogen fixation, phosphate solubilization, zinc solubilization, potash mobilization, ACC deaminase activity, IAA production, antagonistic potential, extracellular enzyme production, etc. (Table-1). Experimental data revealed that all the isolated possess variable abilities of plant growth promotion under *in vitro* conditions. Few isolates were multifaceted and showing majority of positive PGP characters tested under lab conditions.

Microorganisms of rhizosphere play a crucial role in the increment of nutrient availability and therefore, it should be considered as a mechanism of plant growth enhancement (Glick, 1995). Overall, in terms of increasing bioavailability of nutrients on lab medium, 20 (37.04%) isolates showed positive phosphate solubilization, 21 isolates were positive for potash mobilization (38.89%), 19 (35.19%) and 9 (16.67%) for zinc solubilization and nitrogen fixation respectively (Graph-1).

Apart from nutrient mobilization, few PGPR can increase growth of plant by the production of auxins that confers plant beneficial effect (Bal et al., 2013). Additionally, PGPR can also secrete ACC deaminase enzyme that hydrolyze 1-aminocyclopropane-1- carboxylic acid (ACC), the immediate biosynthetic precursor of the hormone ethylene into ammonia and α-ketobutyrate (Laslo et al., 2012), and thereby reducing the deleterious effect of plant growth retardant ethylene. Data indicated that out of total 54 isolates obtained from banana rhizosphere, 9 (16.67%) showed IAA production in medium amended with tryptophan in the range of 10.9 to 45.8 µg/ml. Additionally, 13 (24.07%) isolates were found positive for ACC deaminase activity.

Production of hydrolytic enzymes by PGPR is an indirect method of plant growth promotion due to its positive impact on biotic stress tolerance (Geetha et al., 2014). In this study, isolates were found to posses variable potential of hydrolytic enzyme production, 33 (61.11%), 38 (70.37%) and 19 35.19%) were found positive for protease, amylase and lipase production. Many PGPR also possess ability to control plant pathogens by antagonism such as siderophore production, antibiotic secretion and other mechanisms like induced systemic resistance (ISR) and systemic acquired resistance (SAR) in the plant (Beneduzi et al., 2012). Out of total 54 isolates tested for antagonistic potential, 39 (72.22%) isolates inhibited growth of *Sclerotium rolfsii* under

lab conditions when tested by dual culture method with Percent Growth Inhibition (PGI) in the range of 17.23% to 80.23%.

Gechemba et al. (2015) explored potential of 20 rhizobacteria associated with banana (*Musa* spp.) rhizosphere from seven banana farms in Juja, Kenya. Experimental data indicated that 19 had the potential to fix nitrogen, 18 had phosphate solubilization activities. Isolates were belonging to diverse genera i.e. *Pseudomonas, Bacillus, Staphylobacterium, Chryseobacterium, Streptomyces* and *Paenibacillus* spp. Therefore, it can be inferred that these banana rhizospheric bacterial isolates harbour plant growth promoting traits.

Many of the isolates of this study were showing multiple PGP traits and found more potent in *in vitro* conditions (Table-1). Out of all isolates, two isolates were most potent in terms of individual reactions and also showing multiple traits. Surprisingly, BPF-12 was showing highest phosphate solubilization and positive for all the plant growth promotion parameters tested under *in vitro* conditions, except nitrogen fixation. Isolate OF-2 was also good phosphate solubilizer as well as showing all the activities, except ACC deaminase and nitrogen fixation. Therefore, by considering multifaceted potential of OF-2 and BPF-12 in the different PGP character tested, both were further identified on the basis of 16s r-RNA sequencing method.

**Molecular identification**

By using 16'S r-RNA sequencing methods two most potent PGPR isolates were identified. Data indicated that OF-2 was *Bacillus subtilis* (Figure-1) and BPF-12 was belonging to *Enterobacter cloacae* (Figure-2).

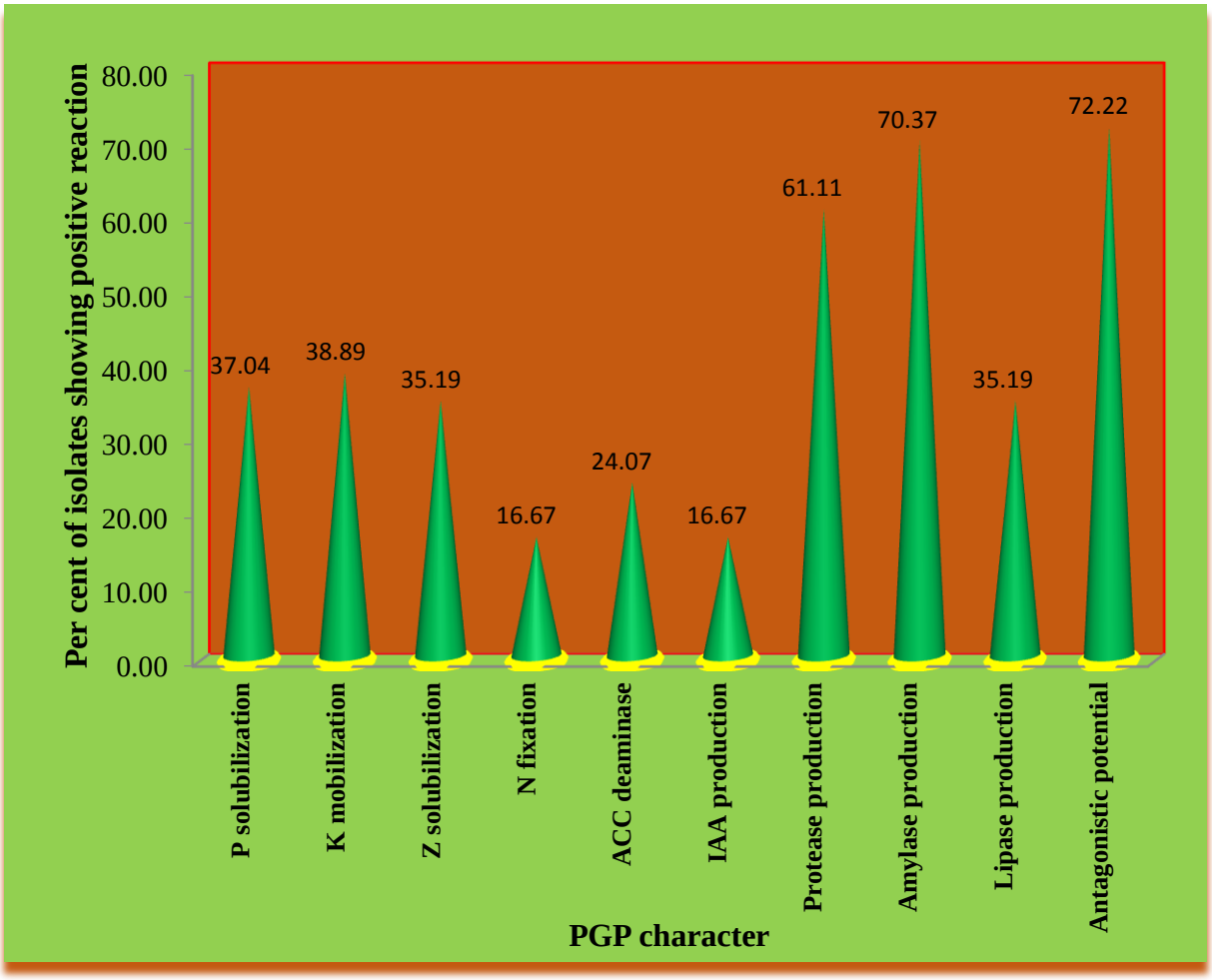
**Table 1: *In vitro* screening of different isolates**

| Soil sample No. | Isolate no. | P Solubilization* | K mobilization* | Zinc solubilization* | $N_2$ fixation | ACC deaminase activity | IAA production (µg/ml) | Protein degradation* | Starch degradation* | Lipid degradation* | Antagonistic potential (PGI) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| OF-E | OF-1 | 2.87 | 1.11 | - | - | + | - | 3.0 | 1.7 | - | 39.50% |
| | **OF-2** | **4.65** | **1.90** | **2.91** | - | - | **45.8** | **2.5** | **3.5** | **4.1** | **74.40%** |
| | OF-3 | 3.46 | 1.21 | - | - | - | - | - | 1.6 | - | - |
| OF-N | OF-4 | - | 1.12 | 2.12 | - | - | - | - | 1.4 | - | 34.80% |
| RHRS-E | RHRS-1 | - | - | - | - | + | - | 1.5 | 2.1 | - | 61.62% |
| | RHRS-2 | - | - | - | + | - | - | 1.4 | 1.5 | 2.4 | - |
| | RHRS-3 | - | - | - | + | - | - | 2.7 | 1.7 | 1.9 | 46.51% |
| | RHRS-4 | 2.56 | - | 2.63 | - | - | - | 3.2 | 1.6 | 3.2 | 68.60% |
| | RHRS-5 | - | 1.13 | - | - | + | - | 2.1 | 1.5 | - | - |
| | RHRS-6 | - | 1.12 | 1.14 | - | - | 15.4 | 1.5 | 2.1 | - | 17.23% |
| | RHRS-7 | - | - | - | + | - | 14.5 | - | - | - | - |
| | RHRS-8 | - | - | - | - | + | - | - | - | 3.2 | - |
| RHRS-N | RHRS-9 | - | - | - | - | - | - | - | 2.4 | 1.7 | 23.52% |
| | RHRS-10 | - | 2.10 | - | - | - | - | - | 2.2 | - | 80.23% |
| IF-E | IF-1 | - | - | 2.55 | - | - | - | 1.6 | 1.3 | - | 48.80% |
| | IF-2 | - | - | 1.84 | - | + | - | 2.5 | - | 3.2 | 36.00% |
| | IF-3 | 3.45 | - | - | - | + | - | - | 1.5 | - | - |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | IF-4 | 5.44 | - | 2.87 | - | + | - | 2.5 | - | - | 58.13% |
| | IF-5 | - | 1.29 | - | + | - | 10.9 | - | - | - | - |
| **IF-N** | IF-6 | 3.22 | 1.70 | - | - | - | - | - | 1.7 | - | - |
| | IF-7 | 2.44 | - | - | - | - | - | 1.9 | - | 2.1 | 38.37% |
| | IF-8 | - | - | 2.45 | - | - | - | - | - | 3.7 | - |
| | IF-9 | - | - | 3.10 | - | - | - | 3.5 | - | 2.4 | 31.41% |
| | IF-10 | - | 1.28 | - | - | - | 12.5 | 3.0 | - | - | 37.20% |
| | IF-11 | 4.23 | - | - | - | - | - | 1.9 | 1.2 | - | - |
| **EF-E** | EF-1 | 3.43 | - | 1.20 | - | - | - | 1.5 | 1.6 | - | 70.93% |
| | EF-2 | 1.43 | - | - | + | - | - | - | - | - | 56.97% |
| | EF-3 | - | - | - | - | - | - | 3.5 | 2.5 | - | - |
| | EF-4 | - | - | - | - | - | 23.8 | 2.5 | - | 1.4 | 39.50% |
| | EF-5 | - | - | 2.00 | - | - | 11.6 | - | - | - | - |
| | EF-6 | - | 1.90 | - | - | - | - | 2.0 | 1.8 | - | - |
| | EF-7 | 2.54 | - | 1.20 | - | - | - | 1.5 | 1.6 | - | 70.93% |
| **EF-N** | EF-8 | - | - | 1.80 | + | - | - | 3.3 | 1.3 | - | 31.39% |
| | EF-9 | - | - | - | - | + | - | 2.9 | 1.4 | - | 67.44% |
| **SWM-E** | SWM-1 | 3.33 | - | 2.10 | - | + | - | 3.0 | 1.1 | 3.0 | 61.60% |
| **SWM-N** | SWM-2 | - | 1.75 | - | - | - | - | 2.1 | 1.4 | - | 61.60% |
| | SWM-3 | - | - | - | - | - | - | - | - | 4.2 | - |
| **BPF-E** | BPF-1 | 2.11 | 1.12 | - | - | - | - | 2.6 | 1.9 | 1.4 | 40.60% |
| | BPF-2 | - | 1.80 | 2.10 | - | - | - | 1.2 | 1.8 | - | 46.51% |
| | BPF-3 | - | - | - | + | - | - | 1.1 | - | 2.1 | 24.52% |
| | BPF-4 | - | 2.50 | - | - | + | 11.2 | - | 1.9 | - | 27.05% |
| **BPF-N** | BPF-5 | - | 2.40 | - | - | - | - | - | 1.4 | - | 33.70% |
| | BPF-6 | - | 1.45 | - | - | - | - | - | 1.1 | - | - |
| | BPF-7 | 1.98 | - | - | + | - | - | 1.3 | 2.2 | - | 48.80% |
| | BPF-8 | - | 2.20 | - | - | - | - | - | 1.5 | - | 38.37% |
| | BPF-9 | 3.22 | - | 3.84 | - | - | - | 2.0 | 1.4 | 1.1 | 45.34% |
| | BPF-10 | - | - | - | - | - | - | 1.5 | 1.8 | - | 47.67% |
| | BPF-11 | - | - | - | - | - | - | 1.3 | - | 3.2 | 38.37% |
| | **BPF-12** | **5.23** | **3.20** | **3.66** | **-** | **+** | **16.5** | **3.2** | **2.9** | **2.4** | **54.65%** |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **CFA-E** | CFA-1 | - | - | - | - | + | - | 3.0 | 1.5 | - | 59.30% |
| | CFA-2 | - | 1.60 | 2.30 | + | - | - | - | - | 1.5 | 24.65% |
| **CFA-N** | CFA-3 | 1.87 | - | 2.20 | - | - | - | - | 1.8 | - | 47.67% |
| | CFA-4 | 1.89 | - | - | - | + | - | - | 2.3 | - | 59.30% |
| | CFA-5 | 2.65 | 1.42 | - | - | - | - | - | 1.1 | - | 66.82% |

*** is zone ratio (colony diameter/zone diameter); + and – sign indicates positive and negative test respectively**




**Graph-1: Per cent of isolates showing positive reaction for PGP characters**

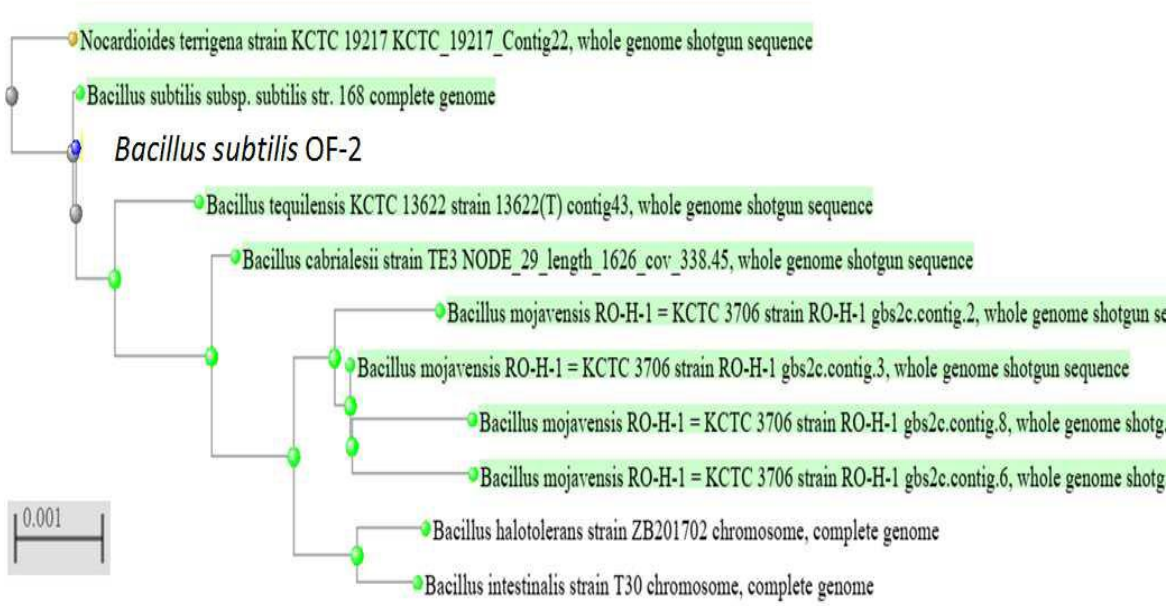



**Figure-1: Phylogenetic tree of *Bacillus subtilis* (OF-2)**

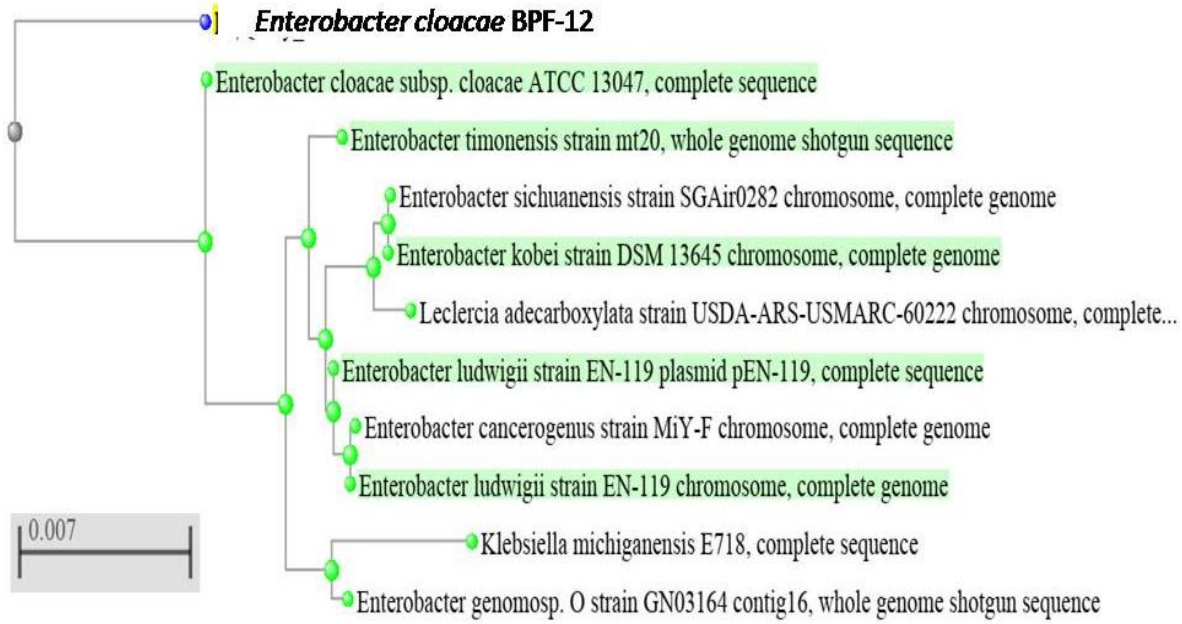



**Figure-2: Phylogenetic tree of *Enterobacter cloacae* (BPF-12)**

## CONCLUSION

Plant productivity compromised due to different biotic and abiotic stresses pertain during growth of plant. In the conventional agricultural practices, use of different agrochemicals are increasing to cope with devastating effect of different stresses and to increase productivity. However, repeated and injudicious use of synthetic agrochemicals is not only costly but also causes plant, soil and human health related issues. Use of PGPR in the eco-friendly and sustainable agriculture may be the ideal solution to cope up with harmful effect of synthetic chemicals. Further

efforts are required to explore multifaceted PGPR in the agricultural practices to preserve environment and to maintain sustainability of soil.

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