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Isolation and characterization of dynamic plant growth promoting microorganisms from rhizosphere

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Abstract

Bio-inoculant (Consortium of Rhizospheric Microorganisms) serves as an energy source for improving the growth and productivity of various agricultural crops in dry areas. Because Bhokar is located in a hilly area, the soil lacks the necessary microbiota for phosphate solubilization, symbiotic or asymbiotic nitrogen fixation, manufacture of plant growth hormones, siderophore, and other biogeochemical processes. As a result of the overuse of pesticides in agriculture, the remaining microflora population is reduced. The presence of Rhizospheric microbiome from the Bhokar agricultural area has been discovered in the current experiment. Three separate agricultural soil samples (Sample A, B, and C) were collected and examined at the VNMK University's Soil Testing Department in Parbhani, MS, India. The pH of Samples A and B is somewhat alkaline, at 8.08 and 8.33, respectively. Sample A had a high concentration of organic carbon at 1.0 percent, while sample B had an average organic carbon concentration of 0.58 percent. Electric conductivity, $CaCO_3$ concentration, nitrogen, phosphorus, potassium, copper, iron, magnesium, and zinc concentrations were all measured in both samples. From the soil samples collected, 43 morphologically distinct species were isolated. Phosphate Solubilizing Microorganisms (PSM), *Rhizobium* sp., *Pseudomonas* sp., *Azotobacter* sp., *Trichoderma* sp., *Aspergillus* sp., and *Penicillium* sp. were isolated using selective media. In the Bhokar agricultural areas, all of these isolated microorganisms will play an important role in improving growth, crop productivity, and controlling plant diseases.

Keywords: Bio-inoculant, Rhizospheric microorganisms, biochemical study, soil samples, PSM.

1. Introduction

Chemical inputs (fertilizers, pesticides, herbicides, and so on) are widely utilized in current agricultural practices, which have a detrimental influence on the nutritional content of farm goods as well as the health of farm workers and customers, all things being equal. Excessive and indiscriminate use of these chemicals has been related to food contamination, weed and disease resistance, and negative environmental consequences, all of which have a significant impact on human health [1, 2]. Hazardous chemicals are deposited in soils as a result of the usage of these chemical inputs. Chemicals in the soil are absorbed by most crops. Acid radicals are found in some synthetic fertilizers, including hydrochloride and sulfuric radicals, which increase soil acidity and impair soil and plant health [3]. Some plants can also absorb substances that are extremely difficult to ingest. People might develop major health problems if they consume such crops on a regular basis [4]. Certain insecticides and herbicides have been linked to the development of cancer. The increased awareness of the health dangers associated with ingesting low-quality crops has motivated a search for new and improved technologies that can increase agricultural output and quality while minimizing human health concerns [5]. Microbial inoculants can be used as biofertilizers, bioherbicides, biopesticides, and biocontrol agents. Microorganisms can help with plant growth stimulation, pest and disease management, and weed control [6, 7]. Microbial inoculants are helpful microorganisms that are injected into the soil or plants to increase agricultural yield and health. Microbial inoculants are natural substances that are used to control pests, enhance soil and crop quality, and improve human health [8, 9].

2. Experimentation

Soil Sampling and chemical analysis of soil:
Three soil samples (A, B, and C) were obtained from 5-7cm depth using a sterile spatula and transported to pre-autoclaved sterile glass vials with rubber stoppers. The samples were taken to the lab and stored at a low temperature [10, 11]. Three agricultural soil samples (Samples A, B, and C) were obtained, with two of them being tested at the VNMK University's Soil Testing Department in Parbhani, (MS), India.

Serial Dilution method:
To isolate various microorganisms from three soil samples, one g of each sample was mixed in 9 ml of sterile Ringer's solution placed in test tubes, and the suspension was serially diluted up to 10^{-7}. The diluted suspensions (0.1 ml) of dilutions 3 onwards were inoculated on used selective media and incubated at 35°C for 3 days [10, 11].

Morphological Characterization: Shape, size, elevation, color, opacity, grams nature, and other morphological properties of isolates were studied for characterization [10, 11].

Isolation of Bacteria:
Pseudomonas (Pseudomonas Agar Base supplemented with Pseudomonas Selective Supplement; 35°C for 48–72 h), Rhizobium isolation medium (Rhizobium sp.), Azotobacter medium, and Trichoderma isolation medium were used to isolate Rhizospheric bacterial species from the obtained soil samples [10, 11, 12].

Isolation of Fungi:
For the isolation of soil fungus, the soil culture was serially diluted and disseminated on several selective media such as Potato dextrose agar medium, Sabaurdor's Agar, Czapek-Dox agar, *Trichoderma harziarum* (TH) selective agar base, and so on. The spread plates were incubated for 72 hours at 35°C [10, 11, 12, 13, 14].

3. Results and Discussion

Chemical analysis of the gathered soil samples was performed at the VNMKV, Parbhani. There were 11 parameters examined, with chosen soil samples having a pH that was somewhat higher than the usual range, indicating that they were alkaline. Although the bacterial species' growth occurs in the ideal pH range,

entomopathogenic fungi, are also present in the soil sample (EPF). When soil alkalinity rises, the number of entomopathogenic fungus falls dramatically, and the insect's attack is no longer naturally regulated. The carbon level in sample B was found to be lower than in sample A, whereas the quantity of $CaCO_3$ in both samples was nearly doubled. So much calcium in the soil can result in a high pH, indicating that the soil is too alkaline, affecting the absorption of macronutrients in other fertilizers Table 1. Similarly, the soil nitrogen levels in samples A and B were lower, at 225 and 130.50 kg/Hec, respectively [15, 16].

Table 1: Characterization of soil by Chemical analysis of sample A & B

Sr. no	Parameters	Optimal Range	Sample-A	Sample-B
1	pH	6.5 to 7.5	**8.08**	**8.33**
2	Electric Conductivity	Below 0.4 siemens	0.31	0.22
3	Carbon	Above 0.75 %	1.00	0.58
4	$CaCO_3$	Below 5.0 %	9.40	9.00
5	Nitrogen	Above 250 kg/Hec	225.00	130.50
6	Phosphorus	Above 22.5 Kg/Hec	24.62	12.68
7	Potassium	Above150 kg/Hec	953.21	935.31
8	Copper	Below 0.2 PPM	2.09	2.30
9	Iron	Above 4.5 PPM	10.656	6.48
10	Magnesium	Above 2.0 ppm	23.52	14.88
11	Zinc	Above 0.6 ppm	1.18	3.45

Table 2: Used various media and growth of microorganisms on plate

Sr. No.	Used Media	Sample A	Sample B	Sample C
1	Pikovaskaya's Media	Green, Orange, White, Black mold	Green, White, Black mold	Green, White, Black mold
2	Rhizobioum agar medium	Yellow, white, orange	Cream & waterish colored colony	Waterish pink colored colony
3	Pseudomonas agar	Large cream color colony	Large cream color & Pale white color colony	Large cream color colony
4	Azotobacter Medium	Shiny small colonies	Small pale white color	pale white color
5	Kings-B medium	White and pale white colored colonies	White and pale white colored colonies	pale white colored colonies
6	Czapekdox agar medium	Green, black & white colored mold	Black cottonish type mold, green mold	Green, black & white colored mold
7	Sabaurdor's Agar	Green & white colored mold	Black and green mold	Black cottonish type mold
8	TH medium	Black & white mold	Black & white mold	Black & white mold

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Table 3: Morphological characteristics of isolated bacteria from soil.

Sr. No	Isolate	Soil Sample	Media	Size (mm)	Shape	Surface	Color	Opacity	Elevation	Consistency	Grams Nature
1.	S-1	C	Kings-B	1	Punctiform	Smooth	Cream	Opaque	Flat	Non sticky	Gram Negative
2.	S-2	C	Kings-B	2	Circular	Smooth	Pale white	Opaque	Convex	Sticky	Gram negative
3.	S-3	C	Kings-B	1	Circular	Smooth	Pale white	Opaque	Flat	Non sticky	Gram negative
4.	S-4	A	Azotobacter agar Medium	1	Small circular	Smooth and shiny	Waterish type	Opaque	Convex	Sticky	Gram negative
5.	S-5	C	Azotobacter agar Medium	0.5	Small and circular	Smooth and shiny	Dull cream	Opaque	Convex	Non-Sticky	Gram negative
6.	S-6	B	Kings B	5	Irregular	Smooth	Dull	Opaque	Flat	Non Sticky	Gram negative
7.	S-7	C	Kings B	0.5	Punctiform	Smooth	Pale white	Opaque	Flat	Non Sticky	Gram Negative
8.	S-8	B	Pseudomonas agar	0.5	Small and Circular	Smooth	Pale yellow	Slight Opaque	Convex	Non sticky	Gram Negative
9.	S-9	A	Pseudomonas agar	3	Circular	Shiny	Pale white	Translucent	Flat	Sticky	Gram negative
10.	S-10	B	Pseudomonas agar	5	Large & Circular	Shiny	Cream	Translucent	Flat	Non Sticky	Gram negative
11.	S-11	A	Rhizobium agar	1	Punctiform	Shiny	Light Red	Opaque	Convex	Slight sticky	Gram negative
12.	S-12	A	Rhizobium agar	2	Circular	Shining	Waterish type & Faint Red	Opaque	Convex	Sticky	Gram negative
13.	S-13	B	Rhizobium agar	3	Circular	Smooth	Light red	Opaque	Raised	Sticky	Gram negative
14.	S-14	B	Rhizobium agar	3	Circular	Rough	Light red	Opaque	Pulvinate	Slight sticky	Gram negative

Table 3 : Continued...

Sr. No	Isolate	Soil Sample	Media	Size (mm)	Shape	Surface	Color	Opacity	Elevation	Consistency	Grams Nature
15.	S-15	B	Rhizobium agar	4	Irregular	Rough	Faint red	Opaque	Umbonate	Non Sticky	Gram negative
16.	S-16	B	Rhizobium agar	1	Circular	Dull	Faint red	Opaque	Convex	Non Sticky	Gram negative
17.	S-17	B	YEMA	5	Irregular	Smooth and shiny	Faint red	Opaque	Flat	Slight sticky	Gram negative
18.	S-18	C	YEMA	3	Circular	Smooth	Waterish	Opaque	Convex	Sticky	Gram negative
19.	S-19	A	YEMA	1	Circular	Irregular	Red	Opaque	Raised	Waxy	Gram negative
20.	P-1	A	Pikovaskayas agar	3	Filamentous	Irregular	Pale White	Opaque	Umbonate	Waxy	Gram Positive
21.	P-2	B	Pikovaskayas agar	4	Circular	Smooth	Cream	Opaque	Flat	Sticky	Gram Positive
22.	P-3	C	Pikovaskayas agar	5	Circular	Wrinkle	Faint yellow	Translucent	Flat	Sticky	Gram Positive

The presence of nitrogen surrounding the roots or in soil samples, according to rhizospheric microorganisms, can lead to symbiotic or non-symbiotic phenomena. Sample A had a greater phosphorus content than sample B, with 24.62 kg/Hec and 12.68 kg/Hec, respectively. According to soil chemical analysis, potassium ions are comparably highest in both samples, with values of 953.21 and 935.31 kg/Hec, respectively. High K concentrations in the soil solution restrict Mg absorption and may cause Mg deficiency in plants. The ideal copper ion concentration in soil is less than 0.2 PPM, while soil samples A and B had extremely high copper concentrations of 2.09 and 2.30 ppm, respectively. According to studies, excessive copper concentrations in the soil resulted in reduced plant survival, total plant biomass, blooming and fruiting delays, and low seed development. The consequences, however, varied depending on the species (2, 4). The iron concentration in soil samples A and B was greater than the acceptable amount, and as a result Iron toxicity in plants can develop when there is an excess of ferrous iron, however this is very dependent on the plant type. Iron deficiency or chlorosis may arise if ferrous iron is not present in soils leading to precipitation of ferric iron compounds. Mg ions were found in significant concentrations in both soil samples. The appropriate level of zinc in soil is greater than 0.6 ppm, however the collected soil samples had high levels of zinc, with 1.18 and 3.45 ppm in sample A and B, respectively. At high zinc concentrations, severe root damage can result in overall yellowing and wilting. The intake of iron is inhibited by high amounts of zinc, and symptoms of acute iron insufficiency caused by zinc poisoning are prevalent [7, 8, 10, 11].

To isolate the various types of microbe from the soil sample, soil samples A, B, and C were serially diluted and a 0.1 ml sample was collected. In the current study, eight different bacterial and fungal mediums were studied. The goal of this research is to extract Rhizospheric microorganisms for culture and to create an efficient bio inoculant for plants to boost their fruit quality and development. On the used media, diverse microbiological growth was found (Table: 2).

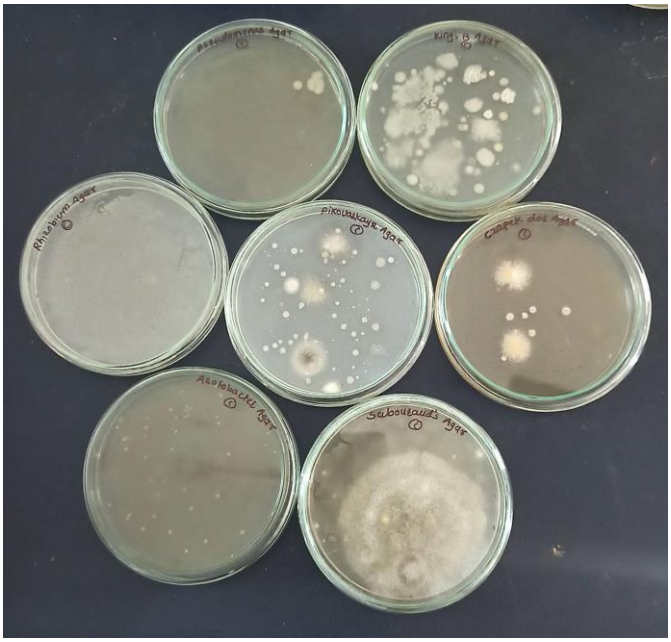

Fig 1: The isolated various microorganisms on media.

4. Conclusion

Based on the results of screening, it seems that the rhizosphere in certain chosen agricultural areas in the Bhokar region has a metabolically diversified and robust population of microorganisms. The isolated rhizospheric bacteria may have a great potential for biogeochemical cycle involvement. This research is still ongoing in order to isolate the most effective Rhizospheric organisms for plant growth enhancement.

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