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Isolation and Characterization of a Silicate-Solubilizing Bacterial Strain Associated with the Roots of Groundnut (*Arachis hypogaea L.*)

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A B S T R A C T

The aim of this investigation was to isolate groundnut root-associated rhizobacteria and to study their efficiency to solubilize silicate, synthesize indole acetic acid (IAA), encourage plant growth, and promote silicon (Si) uptake and deposit in plants. Based on silica-solubilizing ability and IAA production, a bacterial strain was chosen in the present study for further studies. The bacterial isolate synthesized more quantities of IAA at pH 8. When the soil fertilized with silica was inoculated with this bacterial strain, there was an improvement in overall growth characteristics of groundnut. It was also found that more Si was deposited in plants fertilized with both insoluble silica and bacterial strain. Bacteria isolated in the present study can synthesize more IAA under alkaline conditions, solubilize silicate, and encourage the growth of groundnut.

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INTRODUCTION

The seed of groundnut (Arachis hypogaea L.) is a source of cooking oil. This crop is cultivated in many parts of the world. Hence, this crop is an economically important agricultural crop. According to Taurian et al., 2010, there is 22 to 30 % of proteins and 44 to 56 % of oil present in groundnut kernels on dry mass basis. Our nation, India produced 6.7 million tonnes of groundnut during 2021 and thus, it is a significant producer of groundnut. The major element, silicon (Si) helps the plants to recover from environmental stresses. Si confers the plants with disease resistance as well as drought resistance. It is also known that Si can rejuvenate the soils contaminated with poisonous heavy metals. It is possible for Si to neutralize the bad effects of Mn, Al and Fe. Si makes P available to plants. By helping the plants to form silicified tissues, Si can help the plants to exhibit more salt and drought tolerance (Sahebi et al., 2015). Si is the second most abundant element (28.2 %) on earth's crust, next to oxygen (46.1 %). However, as Si's solubility in soil is very low, plants cannot obtain it.

Solubility of minerals such as silicates and phosphates can be brought about by certain micro-organisms. The micro-organisms produce some organic acids that can dissolve the insoluble silicate and thus, the availability of silicate can be improved. Bacteria namely Enterobacter, Pseudomonas, Bacillus, Rhizobia, Proteus and Burkholderia are able to solubilize Si and hence, can help to enhance the growth of plants. So, the crops, soils and seeds can be inoculated with silicate-solubilizing bacteria (SSB). As this method appears to be eco-friendly and promising, this method may be suitable to increase agricultural productivity worldwide (Etesami and Jeong, 2022). The objective of the current study is to isolate rhizobacteria with a capacity (i) to produce more indole acetic acid (IAA) under alkaline conditions, (ii) to solubilize silicate, and (iii) to improve the growth parameters of groundnut.

THEORETICAL REVIEW

Molecular Identification

The silicate solubilizing bacteria with higher IAA production ability was identified using 16S rRNA sequencing. This method used the 27F (5′-AGAGTTTGATC(C/A)TGGCTCAG-3′) and 1492R (5′-CGG(T/C)TACCTTGTTACGACTT-3′) primers (Khan et al., 2014). The 16S rRNA sequence was then checked for species specificity using BLAST software in databases such as EzTaxon, NCBI (National Center for Biotechnology Information) and GenBank. Afterwards, a phylogenetic analysis was conducted using the neighbor-joining method and MEGA v. 6.0 (Tamura et al., 2007). A neighbor-joining tree was constructed with 16S rRNA gene sequences from AR 2019-4 and related strains. The bacteria were then identified as Bacillus paramycoides strain AR 2019-4. The 16S rRNA gene sequence was submitted to GenBank and allotted accession no MN150519.

Influence of pH on IAA Production by the Bacteria and Bacterial Inoculation

The influence of pH on the IAA production ability by the different bacterial isolates was studied. An additional experiment was conducted to find the role of IAA producing AR 2019-4 in promoting the growth of groundnut plants under alkaline conditions. The surface sterilized seeds were germinated with the help of moist filter papers kept in Petri dishes. Uniformly sized seedlings were then transplanted to 0.8 % agar media with different pH values (6, 7, 8) obtained using 6 N HCl or 2 N NaOH before sterilization. After 5 days, the roots of randomly selected plants were inoculated with AR 2019-4.

METHODOLOGY

Rhizospheric Bacteria Isolation and Culture

From a farmer's field in Pollachi, Coimbatore, India, twenty groundnut plants belonging to the variety Pollachi 2 (90 days after sowing) were selected randomly and uprooted in order to collect the rhizospheres. These rhizospheres were collected in sterile plastic bags and immediately transported to the laboratory. Ten grams of pooled soil of rhizosphere was transferred to sterile 100 mL Amies solution (Amies C A, 1967). An aliquot (0.1 mL) of a serially diluted suspension (1:104) was plated on Tryptic Soy Agar (TSA). The Petri dishes were observed daily to find if there is any bacterial growth. Finally, morphologically distinct bacterial colonies were observed. These colonies were then pure cultured in fresh TSA plates (Kang et al., 2017).

Screening Rhizosphere Isolates for Silicate-Solubilizing Ability and IAA Production

In order to perform silicate solubilizing test, silicate medium comprising of 2.5 g/litre magnesium trisilicate, 10 g/litre glucose and 20 g/litre agar was prepared. Morphologically distinct bacterial isolates were inoculated in silicate medium. Incubation was done at 28°C under dark for a week. When a bacterial colony solubilized the silicate, there was a development of clear zone in the nutrient medium. Such bacterial colonies were subjected to an IAA assay using Salkowski reagent (Khan et al., 2014).

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Studies on Plant Growth Promotion by AR 2019-4

To evaluate the Si-solubilizing capacity and plant growth-promoting activity of AR 2019-4, an experiment was conducted. Surface sterilization of the seeds was done by treating the seeds with NaOCl solution for 8 min, followed by cleansing the seeds using sterile double distilled water. Then, the seeds were kept in sterile water for 24 h and planted in a pot containing garden soil with a pH of 5 and moisture content of 20 %. The seeds germinated and were allowed to continue growing for 2 weeks. Morphologically similar seedlings were then planted in pots containing garden soil (500 g) enriched with 250 g of insoluble silicate. Plants were carefully kept in a green house with controlled environmental conditions (photoperiod – 13-hour light/11-hour dark; temperature – 30°C day/20°C night; light intensity during day – 1000 μmol μmol m–2 s –1. For the inoculation of groundnut roots, the AR 2019-4 isolate was cultured in TSB medium at pH 8. The experimented consisted of three treatments viz., control (non-treated plants), insoluble silica-fertilized plants, and insoluble silica-fertilized plants whose roots were treated with AR 2019-4. Plant growth parameters were studied after one month of time. The parameters studied include the lengths/fresh weights of shoot/root, as well as the chlorophyll content of fully expended leaves. Chlorophyll content was quantified using the conventional spectroscopic method.

RESULTS

Isolation of Rhizospheric Bacteria with Ability to Solubilize Silicate

Silicate solubilizing microorganisms were isolated from the groundnut rhizosphere using tryptic soy agar medium (TSA) which was meant to release Si element. Ten Morphologically distinct bacterial isolates were incubated in silicate medium for a week time to evaluate their silicate-solubilizing ability. Among them, four exhibited a clear zone and were selected for further analysis. Their colony morphology is tabulated (Table 1).

Table 1. Colony Morphology of Silicate Solubilizing Bacteria

Isolate	Shape	Margin	Elevation	Texture	Pigmentation	Optic
AR 2019-1	Irregular	Entire	Raised	Smooth	Creamy	Opaque
AR 2019-2	Round	Entire	Flat	Smooth	Creamy	Opaque
AR 2019-3	Irregular Spreading	Irregular	Flat	Smooth	Creamy	Opaque
AR 2019-4	Round	Undulated	Raised	Smooth & glossy	Milky white	Opaque

Pseudomonas stutzeri, *Bacillus globisporus*, *Bacillus* sp., *Rhizobium yantingense* and *Rhizobium tropici* were reported to exhibit mineral-weathering potential(Maheswari et al., 2010; Ribeiro et al., 2020; Wang et al., 2020). Besides, there are only few reports available in the literature on the isolation of bacteria with silicate-solubilizing potential from the rhizosphere of plants (Vasanthi and Rom; Calvaruso et al., 2006). Even among those few reports, forest soils were used for the isolation of bacteria with silicate-solubilizing potential. From the soil of paddy field, Vasanthi et al. (Vasanthi and Rom) could isolate *Bacillus* sp. that solubilized Si on agar media supplemented with magnesium trisilicate.

Table 2 provides details on the silicon solubilizing activity of various bacterial isolates in terms of diameter of clear zone formed due to consumption of silicon from the media by the bacterial isolates. The bacteria that consumed more silicon developed more-clear zone. Thus, the isolate, AR 2019-4 was noticed to have high silicon solubilizing activity as it developed large clear zone than any other bacterial isolates.

Table 2. Silicate Solubilising Activity of Different Bacterial Isolates

Isolates	Diameter of Clear Zone (mm)
AR 2019-1	9.±0.1
AR 2019-2	8±1.2
AR 2019-3	4.5±2
AR 2019-4	16.3±0.6

The growth hormone, IAA is produced by the different isolates at different levels (Table 3). The pH influenced the bacterial synthesis of IAA. AR 2019-1 and AR 2019-4 produced maximum IAA at an alkaline pH value of 8 while AR 2019-2 and AR 2019-3 produced maximum IAA at neutral pH values. The maximum IAA production noticed at pH 8 was exhibited by AR 2019-4 and hence, this strain was used for further studies on the effect of silicate solubilizing bacteria with IAA activity on the growth parameters of groundnut under alkaline conditions (pH 8).

Table 3. IAA Production by the Bacterial Isolates in Different pH Range

Isolate	**IAA Production**		
	pH 6 (ppm/mg biomass)	**pH 7 (ppm/mg biomass)**	**pH 8 (ppm/mg biomass)**
AR 2019-1	0.56	0.51	0.63
AR 2019-2	0.30	0.73	0.27
AR 2019-3	0.06	0.14	0.11
AR 2019-4	0.61	0.92	1.36

When the isolate, AR 2019-4 was inoculated on to the silica-fertilized groundnut plants, tremendous increase was observed in terms of shoot length, root length, shoot fresh length, root fresh length and chlorophyll content as given in Table 4. The reason for the improvement in growth parameters in groundnut can be attributed to the solubilization of silicate and IAA production by AR 2019-4. Thus, this study proves that inoculation of silicate fertilized agricultural fields with bacteria possessing silicate-solubilizing activity and IAA production can help in boosting agricultural productivity(Jian et al., 2006).

Table 4. Influence of the Isolate, AR 2019-4 on the Growth Parameters of Groundnut

Treatment	**Growth Parameters**				
	Shoot length (cm)	**Root length (cm)**	**Shoot Fresh length (mg)**	**Root fresh length (mg)**	**Chlorophyll mg/g^{-1} fresh weight**
Control	15.3 ± 1.2	10.5 ± 1.0	10±1.5	9.4±0.7	2.9
Insoluble silica-fertilized plants	16 ± 0.6	11.2 ± 1.5	12.6 ±0.3	9.8±0.9	3.6
Insoluble silicate-fertilized plants treated with AR 2019-4	20.3 ± 0.2	14.5±0 .33	14.6 ±0.5	12.8±0.3	4.6

CONCLUSIONS AND RECOMMENDATIONS

This research work has resulted in the successful isolation of a bacterial strain AR 2019-4 which solubilises Si, thus promoted the growth of groundnut. The growth promotion was also due to the production of IAA by the bacterial strain. Thus this bacterial strain is a promising one to increase agricultural productivity of the country. Further studies using this strain with other agricultural crops are necessary in the future.

FURTHER STUDY

This research still has limitations, so it is necessary to carry out further research related to the topic of Isolation and Characterization of a Silicate-Solubilizing Bacterial Strain Associated. Future researchers can choose other variables, such as plants that are different from this study.

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