ACTINOMYCETES AS CANDIDATES FOR BIOFERTILIZER FORMULATION

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Application of actinomycetes to improve plant growth, nutrient availability and soil properties is an eco-friendly alternative strategy in sustainable agriculture. This study aimed to evaluate the potential of previously characterized plant growth promoting (PGP) actinomycetes in the soil as biofertilizers. For the development of consortia, 27 strains of PGP actinomycetes were screened for their ability to produce iron-chelating siderophores using the liquid Chrome Azurol S assay. Two strains, ACM25 and ACM31 showed positive results and ACM25 strain was the most efficient. Therefore, ACM25 was selected as an essential member of each consortium. Considering the overall PGP properties, four consortia were prepared with ACM25, ACM28, ACM35, ACM37, ACM42 and ACM45 in different combinations. Their compatibility to stay together in a consortium was tested in co-culture plate assay. Each consortium contained a combination of two or three strains and they were separately inoculated into the soil and determined the availability of N, P, K, organic C and total bacterial count and total microbial activity compared to the non-inoculated soil after 30 days. There was a significant enhancement of NH_4^+ -N and NO_3^- -N in soil treated with all four consortia. Among all, C1 (ACM25+ACM45+ACM3) treatment showed the highest level of available N in the soil. Orthophosphate and exchangeable potassium contents in all treatments found significantly increased and C3 (ACM25+ACM42+ACM37) gave the highest. Organic matter content in the soil appeared to have no significant effect when treated with any of the consortia. This may be due to the accelerated decomposition rate by the significantly enhanced bacterial count and total microbial activity in actinomycetes-treated soil and their involvement in the cycling of nutrients in the soil. Therefore, the enhanced nutrient availability in actinomycetes-treated soil may be due to the cumulative effect of introduced actinomycetes and enhanced growth of microbial community and their activities in the soil. Further research is needed to overcome challenges related to production efficiency, stability in soil, and extensive field trials. Despite these challenges, actinomycetes hold great promise as biofertilizers for promoting sustainable agriculture, improving soil health, increasing crop yield and reducing reliance on chemical fertilizers.

Key words: actinomycetes, biofertilizer, consortia, nutrient availability, plant growth promotion

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INTRODUCTION

Biofertilizers can be defined as products containing beneficial microorganisms, either a single or multiple strains with the potential to improve soil fertility and crop productivity. Globally, applications of biofertilizers are becoming popularly enhanced along with the concept of the sustainable agriculture. Among the diverse types of plant growth promoting microorganisms, actinomycetes are considered as more potential candidates due to the possession of a range of plant growth promoting and other bioactivities (Rani et al., 2018). Actinomycetes are a group of Gram-positive bacteria, widely spread in both aquatic and terrestrial habitats including rhizosphere. Their bioactivities include, atmospheric nitrogen fixation, solubilizing phosphate and potassium, producing plant-growth promoting hormone such as Indole-3-acetic-acid, antibiotics, antifungals, siderophores and a wide array of lytic and hydrolytic enzymes (Shanthi, 2021). Hence, actinomycetes are potent contenders as biofertilizers. Therefore, this study aimed to evaluate the potential of previously characterized plant growth promoting (PGP) actinomycetes in the soil as biofertilizers.

METHODOLOGY

Screening for siderophore production

Twenty-seven strains of actinomycetes which were previously characterized for having plant-growth promoting (PGP) properties such as free-living nitrogen fixation, phosphate solubilization, indole-3-acetic acid (IAA) production, antibacterial activity and antagonism against plant pathogenic *Sclerotium rolfsii* were selected for this study (Abhayathunga et al. 2022). They were screened for an additional PGP property; iron chelating-siderophore production following the liquid Chrome Azurol S (CAS) assay using the culture broth grown for 7 days at room temperature (approximately 30 ºC) with constant shaking at 100 rpm. Siderophore production efficiency was estimated as percentage of siderophore units using following formula where, Ar is absorbance of reference at and As is absorbance of sample (Ghosh et al., 2017). Absorbance was measured at 630 nm.

$$\text{\% of siderophore units} = \frac{Ar - As}{Ar} \times 100$$

Preparation of plant-growth promoting consortia of actinomycetes

Considering overall PGP properties of strains, 6 strains of actinomycetes, ACM25, ACM28, ACM35, ACM37, ACM42 and ACM45 were selected and 4 consortia were prepared. Those consortia included, C1 (ACM25+ACM45+ACM37), C2 (ACM25+ACM45), C3 (ACM25+ACM42+ACM37) and C4 (ACM25 + ACM37). Initially, compatibility of strains within each consortium was confirmed by a plate assay.

Soil application

Soil was air-dried for 7 days at room temperature and sterilized by autoclaving in 250 mL conical flasks containing 200g of soil in each. Soil was then supplemented with $CaCO_3$ (0.1% w/w) and inoculated with 60 mL of consortia in 3 replicates. Briefly, consortia inoculants were prepared by mixing 20 mL of culture broth of members in the relevant consortium. If a consortium contains two strains, 20 mL of two strains and 20 mL of sterile distilled water were used (AbdElgawad et al., 2020). For control, soil was inoculated with 60 mL of nutrient broth and sterilized distilled water and it was incubated in dark at room temperature for 30 days.

Determination of nutrient availability and microbial activity

After 30 days of incubation, soil pH, conductivity, NH_4^+ -N and NO_3^- -N content, organic matter content, orthophosphate content and exchangeable potassium content were measured following standard protocols. Total bacterial count was determined by standard plate count method and total microbial activity by fluorescein diacetate (FDA) assays (Patle et al. 2018).

RESULTS AND DISCUSSION

Two strains, ACM25 and ACM31 showed positive results for the CAS assay with 93.2% and 82.4% percentage siderophore units. Therefore, ACM25 was included as a member of each consortium. Soil pH, conductivity, N, P, K nutrient parameters, total bacterial count and microbial activity were compared with control (Table 1).

The pH in soil inoculated with C1 (ACM25+ACM45+ACM37) and C2 (ACM25+ACM45) significantly decreased to became moderately acidic. This reduction of pH may indicate organic acids production of actinomycetes strains involved in the solubilization of mineral phosphate and potassium.

Table 1. The effect of actinomycetes consortia on nutrient availability and soil microbial activity after 30 days of treatment

	Control	Consortium 1	Consortium 2	Consortium 3	Consortium 4
pH	6.47±0.24^{a}	5.73±0.01^{b}	5.75±0.04^{b}	5.77±0.03^{ab}	5.81±0.02^{ab}
Conductivity (dS/m)	0.41±0.02^{d}	1.02±0.02^{a}	0.75±0.02^{c}	0.92±0.01^{b}	0.77±0.02^{c}
NH_4^+ -N×10^3 (mg/L)	1.08±1.50^{b}	1.10±1.00^{a}	1.07±1.00^{d}	1.07±0.58^{c}	1.07±2.5^{bc}
NO_3^- -N×10^3 (mg/L)	0.94±4.62^{b}	1.03±11.00^{a}	0.92±23.35^{b}	0.87±14.85^{b}	1.01±12.13^{a}
Organic matter content (%)	5.8±0.00^{b}	6.7±0.10^{ab}	7.05±0.01^{a}	6.83±0.04^{ab}	7.02±0.20^{a}
Orthophosphate (mg/L)	14.79±0.67^{d}	50.42±0.24^{b}	46.61±1.21^{c}	63.85±0.52^{a}	47.85±0.09^{c}
Exchangeable potassium (mg/L)	4.85±0.25^{d}	7.85±0.18^{b}	6.25±0.17^{c}	9.13±0.00^{a}	8.60±0.26^{ab}
Bacterial count (CFU×10^6/mL)	0.30±0.01^{d}	1.09±0.05^{a}	0.57±0.01^{c}	0.73±0.01^{b}	0.66±0.01^{bc}
Total microbial activity (mg/L)	50.61±0.87^{b}	55.08±3.67^{a} ^{b}	54.63±2.74^{a} ^{b}	55.78±2.15^{a} ^{b}	64.98±0.24^{a}

Values are the mean ± SE; mean values sharing similar letter(s) within a row are non-significant at 0.05 significance level.C1: ACM25+ACM45+ACM3, C2: ACM25+ACM45, C3: ACM25+ACM42+ACM37, C4: ACM25 + ACM37

According to the NH_4^+ -N andNO_3^- -N contents in soil, actinomycetes treatments may have contributed to the increment of nitrogen availability in soil. There was a significant increase in the NH_4^+ -N and NO_3^- -N availability in soil treated with C1 over control and other treatments.

The effect of actinomycetes consortia on the soil organic matter content was not significant but, C2 and C4 showed slight enhancement than C1 and C3 consortia. It may be due to the enhanced decomposition of organic matter by actinomycetes and other microorganisms in the soil.

Orthophosphate and exchangeable potassium contents in all actinomycetes treated soil were found significantly increased. The C3 treated soil gave the highest orthophosphate and potassium contents. These results indicate the ability of actinomycetes consortia to enhance phosphorus and potassium availability in soil by the solubilization of insoluble phosphates and potassium sources in the soil.

There was a significantly enhanced bacterial count and total microbial activity in soil treated with actinomycetes consortia. Therefore, it appears that actinomycetes not only improve nutrient availability but promote growth of other microorganisms in the soil. Microorganisms in soil involve in organic matter decomposition and cycling of nutrients in the soil. As a result, they contribute toward enhancing the nutrient availability of soil. Therefore, the observed nutrient availability in soil treated with actinomycetes may be the cumulative effect of introduced actinomycetes and enhanced microbial community and their activities in the soil.

CONCLUSION

The overall results of the study clearly showed that the selected actinomycetes consortia have the potential to enhance the availability of key nutrients, N, P, K and promote total microbial activity in the soil. Hence, all four consortia can be considered as ideal candidates for the development of biofertilizer formulations to promote sustainable agriculture practices. However, further research is needed to overcome challenges related to production efficiency, stability in soil, and extensive field trials. Therefore, these findings suggest that actinomycetes can be valuable tools in sustainable agriculture, contributing to soil health improvement, increased crop yields, and reduced reliance on chemical fertilizers. Despite these challenges, actinomycetes hold great promise as biofertilizers for promoting sustainable agriculture practices.

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