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Proceeding Paper

Characterization of Potential Chalky Soil Bacteria Isolated from Rhizosphere of *Acacia* spp. Growing in Abardae, Maekel Region of Eritrea †

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Abstract: The current study was carried out to characterize chalky soil bacteria obtained from the rhizosphere of *Acacia species* growing in Abardae, Maekel Region of Eritrea. This study collected three chalky soil samples from the rhizosphere of *Acacia ethibica*, *Acacia origena*, and non-rhizospheric soil. The samples contained 1.42×10^{10}, 5.35×10^{9}, and 5.68×10^{7} cfu/g of culturable bacteria, respectively. A total of 80 bacterial strains were isolated, with ten selected for further study based on their distinct morphology. The researchers examined the cell morphology and the antimicrobial and plant growth-promoting activity of the chosen bacterial isolates. The study's findings identified that the aerial mycelium of the strain EAE-1 displayed a unique and previously unreported arrangement of hyphae-bearing spores. The antimicrobial test results also showed that bacterial strains EAE-1, EAE-3, EAE-14, EAE-15, EAE-40, and EAO-24 displayed a wide range of antimicrobial activity against the examined phytopathogens. Furthermore, the seed germination result showed that the majority of bacterial strains had a positive effect on wheat growth, with strains EAE-40 and EAO-17 particularly enhancing maize growth. To sum up, the substantial capabilities of these strains position them as promising candidates for biotechnological applications. This study also represents the preliminary analysis of the microbial composition of Eritrean soil.

Keywords: *Acacia ethibica*; *Acacia origena*; antimicrobial activity; phytopathogenic microbes; plant growth-promoting activity; chalky soil

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1. Introduction

The rhizosphere is a preferred habitat for soil microorganisms because it has abundant nutrients [1]. Rhizobacteria are the predominant soil microorganisms, and their diversity can be influenced by the host plants and environmental factors, such as soil type [2]. Around 2–5% of Rhizobacteria exibit plant growth-promoting (PGP) characteristics [3]. Those bacteria possessing PGP characteristics are referred to as plant growth-promoting rhizobacteria (PGPR) [4]. Over the past few decades, multiple PGPRs have been documented for their capability to enhance growth and biocontrol potential [4–6]. The majority of PGPRs provide advantages to the host plants by different mechanisms, including nutrient mobilization, growth promotion, pathogen inhibition, bioremediation, and induction of disease resistance [3,7–9]. Lately, there has been a growing interest in soil microbiomes and their bioactive compounds due to their beneficial uses in agriculture, industry, the environment, and healthcare [10].

Using PGPRs in modern agriculture can minimize environmental and health risks caused by extensive use of agrochemicals [4]. The utilization of PGPR in such ways boosts the need for soil microorganisms. Hence, studying soil bacteria with promising biotechnological applications is highly crucial in today's society. In this study, we investigated chalky soil bacteria from non-rhizospheric soil and the rhizosphere of two *Acacia* sp. (*Acacia ethibica* and *Acacia origena*) in Abardae, Maekel region of Eritrea, where the soil microbial community had not been previously explored. Therefore, the aim of the current study was to quantify chalky soil rhizospheric bacteria, analyze cell and colony morphology, assess biocontrol potential against phytopathogens, and evaluate growth promotion capability.

2. Methods

2.1. Sample Collection and Bacteria Isolation

Chalky soil samples were collected from a grazing area in Abardae, Maekel Region of Eritrea (15°21′59.24″ N latitude and 38°82′48.25″ E longitude). The first two soil samples were obtained from the rhizosphere of *Acacia ethibica* and *Acacia origena* at the mature growth stage, while the third sample was taken from non-rhizospheric soil in October 2023. Each soil sample was transferred to a sterile polythene bag and taken to the laboratory after one week for analysis. Bacteria isolation and quantification were carried out using the method described in [6].

2.2. Morphological Characteristics of Bacterial Isolates

To examine bacterial cell structure, phase contrast microscopy was used with a Nikon Eclipse Ci microscope equipped with a ProgRes SpeedXT camera (Jenoptic, Jena, Germany) [6]. To characterize colony morphology, bacterial isolates were inoculated on LB agar plates and kept in an incubator at 29 °C for 24 h. Characteristics including margin, elevation, surface, color, and pigmentation were observed and recorded [11]. Gram staining was performed following the standard staining procedure [12].

2.3. Antimicrobial Activity Against Phytopathogens

Antimicrobial activity of ten bacterial isolates (EAE 1, EAE-3, EAE-14, EAE-15, EAE-40, EAO-9, EAO-17, EAO-18, EAO-24, and EAO-26) was examined against *Erwinia herbicola* ATCC 27155, *Pectobacterium carotovorum* B15, *Micrococcus roseus* B1236, *Fusarium avenaceum* F-132, *Rhizoctonia solani* F-895, *Pythium vexans* F-1193, *Aspergillus unguis* F-1754, *Alternaria brassicicola* F-1864, *Bipolaris sorokiniana* F-4006, and *Pythium ultimum* F-4782, using the method described in [6].

2.4. Growth-Promotion Ability of Bacterial Isolates

In order to assess the growth promotion capabilities of bacterial isolates, a seed germination experiment was carried out with four types of seeds (wheat, maize, oats, and lentils) using the method outlined in [6,9]. On a total of 44 plates, 50 surface-sterilized seeds were placed on each plate and inoculated with 15 mL of bacterial solution (OD 600 = 0.1). The experiment was performed in triplicate. Following the germination process, the seedling's germination rate, shoot length, root length, and fresh weight were measured. The data were analyzed using a t-test with a significance level of $p = 0.05$.

3. Results and Discussion

3.1. Bacteria Quantification and Isolation

The number of bacteria in the three soil samples was compared. The highest colony forming unit (cfu) was found to be 1.42×10^{10} cfu/g from the rhizosphere of *A. ethibica.* The rhizosphere of *A. origena* had a bacteria count of 5.35×10^{9} cfu/g. Nevertheless, the non-rhizospheric soil sample had a bacteria count of 5.68×10^{7} cfu/g, suggesting a relatively low quantity. The current result revealed that the Eritrean chalky soil had a higher average bacterial count (1.96×10^{10} cfu/g) than Belgorod chalky soil (2.95×10^{9} cfu/g) [6]. After several purification steps, a total of 80 chalky soil bacteria were isolated based on their morphological differences.

3.2. Cell and Colony Morphology of Bacterial Isolates

The findings from the microscopy analysis presented in Figure 1 revealed that most of the bacterial isolates exhibited a filamentous structure. Particularly, the aerial mycelium of strain EAE-1 is distinguished by an original, previously undescribed organization of spore-bearing hyphae. According to morphological criteria, this strain can be tentatively classified as a monosporous actinomycete. The unique aspect of the morphology is the inclusion of short, convoluted hyphae, ranging from 10 to 20 µm in length, with one refractory spore located at the tip, alongside the densely packed aerial mycelium formed by highly branched hyphae. The colony morphology of the bacterial isolates was diverse, as described in Table 1. Moreover, the gram staining result revealed that all the representative bacteria were Gram-positive, with the exception of bacterial strains EAO-9 and EAO-26.

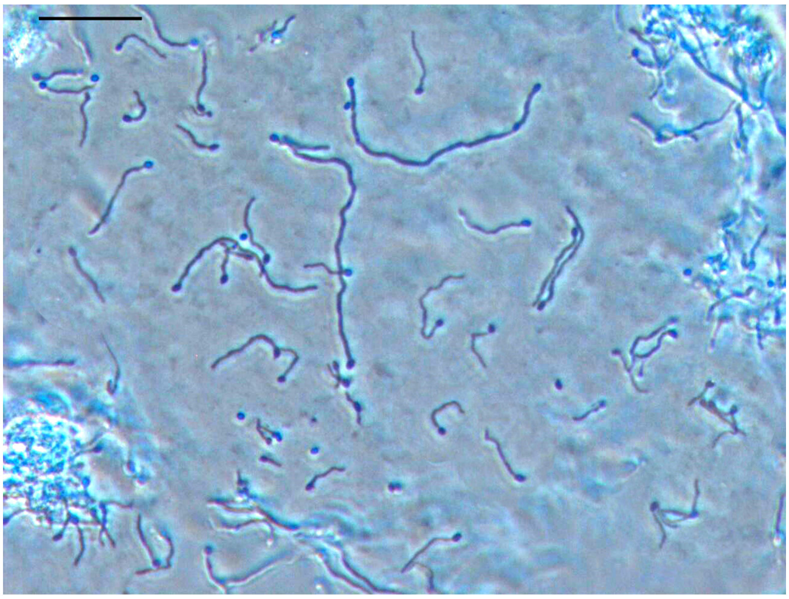

Figure 1. The microscopy cell morphology of bacterial isolate EAE-1. Scale bar is 10 µm.

Table 1. Morphological characteristics of bacterial colonies isolated from the rhizosphere of *Acacia* sp. in the Maekel Region of Eritrea. Gram-positive bacteria (+), Gram-negative bacteria (-).

Bacterial Isolates	Gram Type	Morphological Parameters					
		Shape	Margin	Elevation	Surface	Color	Diffusible Pigment
EAE-1	+	Circular	Lobate	Raised	Rough	White	Grayish yellow
EAE-3	+	Circular	Lobate	Raised	Rough	White	Grayish yellow
EAE-14	+	Irregular	Entire	Raised	Rough	White	Light brown
EAE-15	+	Irregular	Undulate	Raised	Smooth	Yellowish white	No
EAE-40	+	Circular	Entire	Flat	Smooth	Dark gray	No
EAO-9	-	Circular	Entire	Raised	Smooth	Dark orange yellow	No
EAO-17	+	Circular	Entire	Raised	Smooth	Brilliant yellow green	No
EAO-18	+	Circular	Entire	Flat	Rough	Strong brown	Moderate purplish red
EAO-24	+	Circular	Entire	Flat	Rough	Yellowish white	No
EAO-26	-	Circular	Entire	Flat	Smooth	Strong brown	No

3.3. Biocontrol Potential Against Phytopathogens

The antimicrobial activity presented in Table 2 demonstrated that the bacterial isolates EAE-1, EAE-3, EAE-14, EAE-15, EAE-40, and EAE-24 exhibited a broad spectrum of antimicrobial activity against the examined phytopathogens. Moreover, bacterial isolates EAE-1, EAE-3, and EAE-14 exhibited strong growth inhibition against *P. vexans* F-1193, *P. ultimum* F-4782, and *A. brassicola* F-1864, respectively.

Table 2. Antimicrobial activity of representative chalky soil rhizobacteria against phytopathogens.

		Phytopathogenic Bacteria			Phytopathogenic Fungi						
Bacterial Strains	**Host Plant**	***M. roseus***	***E. herbicola***	***P. caratovora***	**F-132**	**F-895**	**F-1193**	**F-1754**	**F-1864**	**F-4006**	**F-4782**
EAE-1	*A. etibica*	+	-	-	+	+	++	-	+	+	+
EAE-3	*A. etibica*	+	-	-	+	+	+	+	+	+	++
EAE-14	*A. etibica*	+	+	+	-	+	+	+	+++	+	+
EAE-15	*A. etibica*	+	+	-	+	+	+	+	+	+	-
EAE-40	*A. etibica*	+	-	-	-	+	+	+	-	+	+
EAO-9	*A. origena*	-	-	-	-	-	-	+	+	-	-
EAO-17	*A. origena*	-	-	-	-	-	+	+	-	+	-
EAO-18	*A. origena*	-	-	-	-	+	-	-	-	-	-
EAO-24	*A. origena*	-	-	-	-	+	+	+	+	++	+
EAO-26	*A. origena*	-	-	-	-	-	+	+	+	+	+

No inhibition zone (-), inhibition zone of 10–20 mm (+), inhibition zone of 20–30 mm (++), and inhibition zone of 30–40 mm (+++).

3.4. Plant Growth-Promotion Ability

The growth-promotion capability of chalky soil rhizobacteria on the germinated seeds is presented in Figure 2. Figure 2a demonstrated that most of the bacterial isolates exhibited a significant effect on the germination rate and root length of wheat ($p = 0.05$). In Figure 2b, bacterial isolates EAE-40 and EAO-17 exhibited significant effects on the overall growth of maize ($p = 0.05$). However, no bacterial isolates had an effect on the growth of oats and lentils.

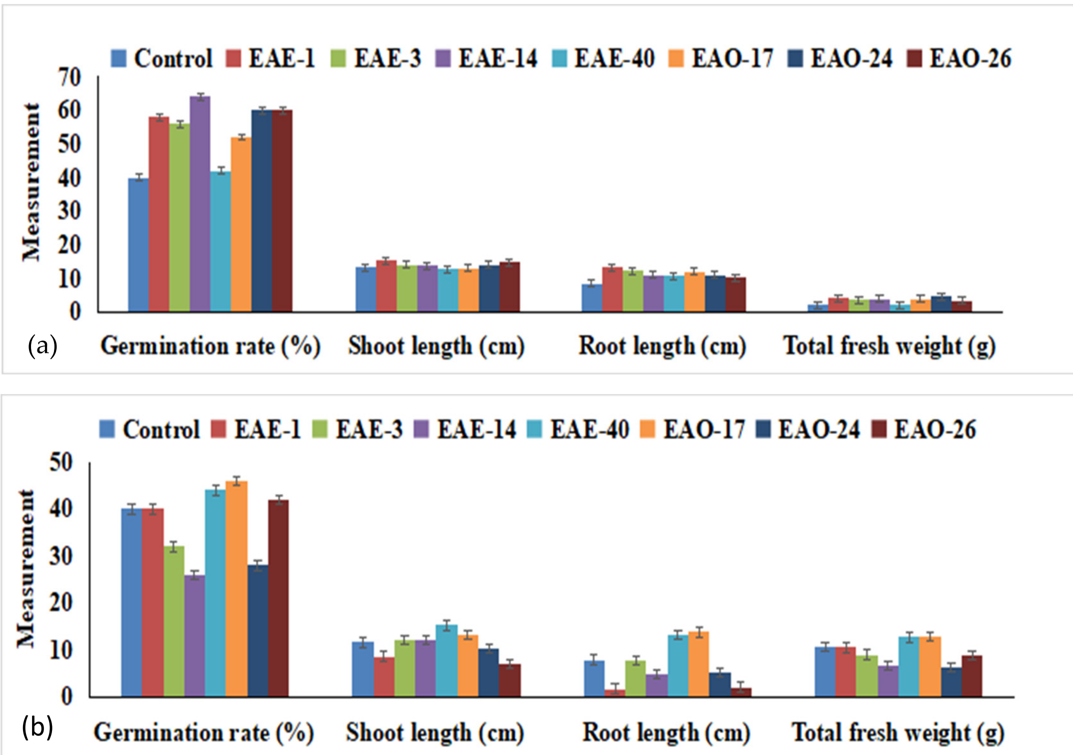

Figure 2. Growth promotion effect of bacterial isolates on the germination rate, shoot length, root length, and fresh weight of wheat (**a**) and maize (**b**). The error bars represent the least significant difference among. treatments at $p < 0.05$.

4. Conclusions

The present research investigated the rhizospheric bacteria found in the chalky soil of Eritrea. Most of the bacterial isolates possessed a diverse morphological feature. Yet, the morphology of EAE-1 had a unique and previously undescribed organization of spore-bearing hyphae. Bacterial isolates such as EAE-1, EAE-3, EAE-14, EAE-40, and EAO-17 showed effective growth-promotion and biocontrol potential compared to other bacterial isolates examined. This impressive potential of bacterial isolates makes them strong candidates for biotechnological use. Finally, the representative chalky soil rhizospheric bacteria will be the subject of more research in the future, with a particular focus on strain EAE-1. Moreover, this study also represents a preliminary analysis of Eritrea's soil microbial composition.

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