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Article

Antimicrobial activity of edaphic microorganisms against clinically relevant bacteria

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ABSTRACT

In recent decades, the emergence of bacterial resistance to antimicrobial agents has raised a significant challenge in the medical field. The search for new therapeutic alternatives has become a challenging task. With an estimated diversity ranging from 100 million to 1 billion different bacterial types with unique functions and roles, it is critical to find antimicrobial solutions Recent studies have focused on bacterial species in soil as a source of antimicrobial compounds that can be used as potential therapeutics for the treatment of infectious diseases. This study focused on isolating, culturing, and characterizing the bacteria present in the soil of the *Universidad de Investigación de Tecnología Experimental Yachay* campus. To assess their potential therapeutic impact, antagonistic tests were carried out between the bacterial isolates and three strains of the most prevalent pathogens: *Escherichia coli*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*. The results showed that according to antimicrobial activity evaluations, the metabolites produced by two soil strains, UITEY-030 and UITEY-055, exhibited partially inhibitory effects on the growth of *S. aureus* and *E. coli*. These results highlight the capability of soil-derived compounds as candidates for developing novel antimicrobials.

.**Keywords:** Antimicrobial metabolites; antimicrobial resistance, soil microorganisms.

INTRODUCTION

In the era of modern medicine, where the ability to cure and treat diseases has been dramatically improved, the rise of antimicrobial-resistant (AMR) infections and multidrug-resistant organisms has become a global public health concern[1,2]. Recent studies suggest that AMR infections are the cause of 700,000 deaths per year worldwide[3], and this number is likely to rise to 10 million by 2050[4]. Therefore, it is essential to identify new antimicrobial substances that are clinically relevant5,6 promptly.

Natural products have historically stood out as the most important source of bioactive compounds[7]. In this context, soil bacteria have been extensively studied for their ability to produce a variety of metabolites[8,9]. Within soil ecosystems, these bacteria routinely synthesize secondary metabolites that are necessary for communication, interspecific interaction, and competition with other microorganisms[10]. Consequently, antimicrobial compounds and bioactive products synthesized by these bacteria emerge as highly promising resources in the biotechnological industry. These biologically active molecules exhibit the potential to either partially or completely inhibit the growth of bacteria and fungi making them invaluable in industrial applications. [11].

The discovery of new bioactive metabolites with antimicrobial properties is a challenging field of research. The screening of these compounds would contribute to the development of effective drugs against pathogenic bacteria. Thus, this study aimed to investigate the antimicrobial activity exhibited by soil strains isolated from the *Universidad de Investigación de Tecnología Experimental Yachay* (UITEY) campus against the standard strain *Escherichia coli* ATCC 25927 and clinical isolates *of E. coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa*.

MATERIAL AND METHODS

Sampling was conducted at the UITEY campus in Urcuquí-Ecuador (0°24.4430'N, 78°10.2790'W). The selected area was characterized by low precipitation levels, high solar radiation, and limited vegetation diversity, with a predominance of plants from the Poaceae and Rosaceae families. Two plots of 100 m^2 were randomly selected and divided into three subplots. In each subplot, 5 subsamples were taken from the top 20 cm of soil with a metal auger and stored in plastic bags at 4°C.

A tenfold dilution was performed for bacterial isolation, 100 µl of the 10^{-4} dilution was plated on nutrient agar. The plates were incubated overnight at 30°C and the bacterial colonies were counted manually. Bacterial colonies were characterized by color, size, elevation, shape, and border. In addition, Gram staining and a catalase test were performed to differentiate the morphotypes. The data obtained were grouped to create a heat map in which each variable was assigned a value from 0 to 1.

To assess the antimicrobial effect, the test strains were cultivated in nutrient broth until an absorption of 0.3 was reached. Subsequently, the test and soil strains were co-inoculated in nutrient agar and incubated overnight at 30°C. After further incubation, the presence or absence of an inhibition zone was observed.

For the filtrate inhibition test, 1.5 ml of the filtrate produced by the soil bacteria was added to 25 ml of nutrient agar. For the control, 1.5 ml of filtered nutrient broth was combined with 25 ml of nutrient agar. Both solutions were dispensed into Petri dishes. Once the agar solidified, the pathogenic bacteria were inoculated and incubated overnight at 30°C. The following day, the number of colonies was determined through manual counting.

For the statistical analysis, a Python program employing the Mann-Whitney U test was used. Statistical significance was defined as $P > 0.05$, assuming equivalent means between the tested and control groups.

RESULTS

Isolation of soil bacteria

The bacterial isolation process showed interesting patterns in terms of soil strain richness between the first and second areas of the study. In the first plot (P-1) the mean values of CFU/g obtained from the 10^{-4} dilution were 16.5×10^6, indicating a moderate bacterial population (Table 1). Meanwhile, in the second plot (P-2), the CFU/g mean was 18.8×10^6 (Table 1).

Area	CFU/g soil
P1-1	20.1×10^6
P1-2	9.5×10^6
P1-3	19.9×10^6
P2-1	33.6×10^6
P2-2	219.1×10^6
P2-3	3.8×10^6

Table 1. CFU/g value of the 3 subareas of plot 1 and plot 2.

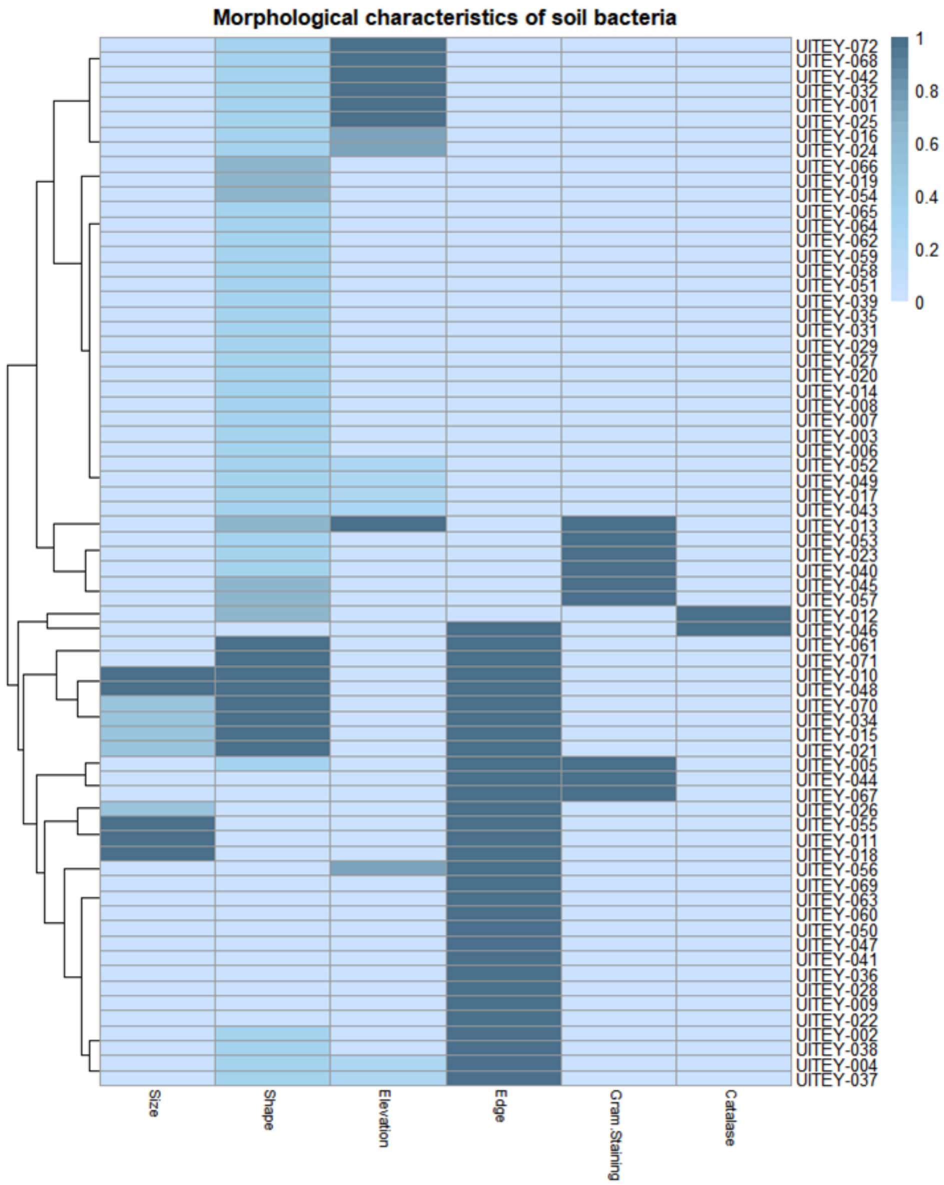

Figure 1. A heat map with a dendrogram showing the grouping of 71 soil bacteria according to their morphological characteristics. The color scale represents the value of each variable (size, shape, elevation, edge, Gram staining, and catalase type), which were grouped in a range from 0 to 1.

Characterization of soil bacteria

After the 71 soil bacterial isolates were characterized, several clusters based on morphological characteristics were observed. However, four main clades included bacteria of both plots (Figure 1). One clade is composed of bacteria with irregular colony borders, while bacteria with entire colony borders dominate another clade. The same occurs with the shape variable, where one clade is dominated by bacteria with a circular shape, while the other clade is dominated by bacteria with an irregular shape.

Assessment of antimicrobial activity

In the dual culture test, the soil strain UITEY-030 (*Bacillus circulans*) produces a clear zone of inhibition against *S. aureus*. In contrast, UITEY-055 (*B. mycoides*) showed a slight inhibitory activity against *E. coli* (Figure 2).

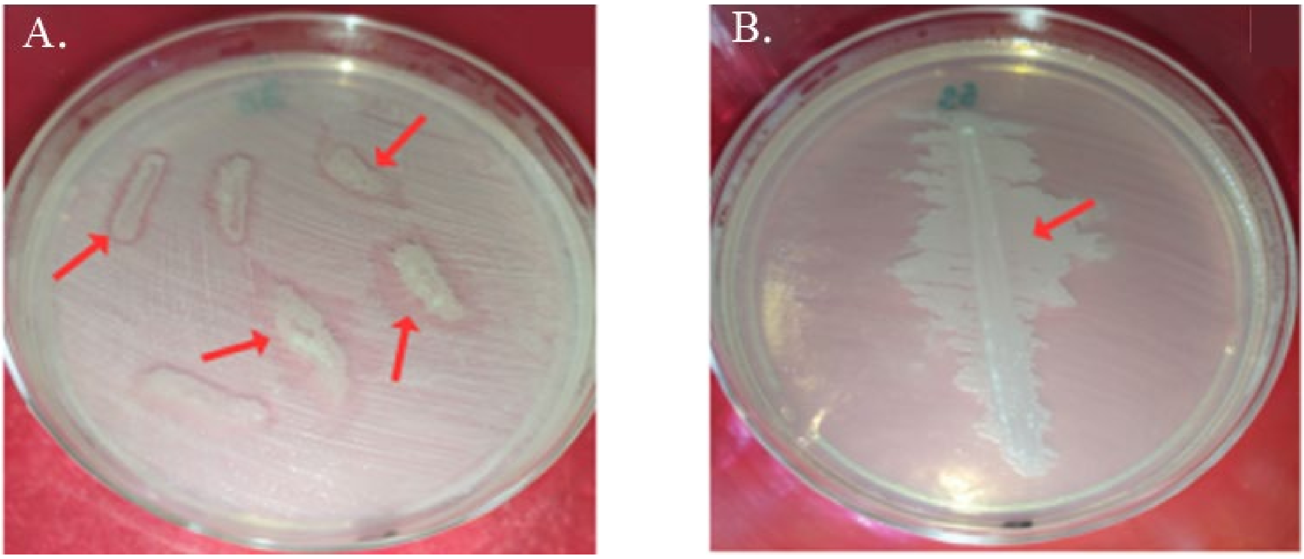

Figure 2. Dual culture test for the detection of antimicrobial activity. (a) *S. aureus* against soil strain UITEY-030. (b) *E. coli* against soil strain UITEY-055. Red arrows mark the inhibitory zone.

After incubation of the test strains with the filtrate of UITEY-030 and UITEY-055, it was suggested that the metabolic products of the soil bacteria have antimicrobial activity against *S. aureus* and *E. coli*, respectively.

The number of *S. aureus* colonies was significantly reduced when exposed to the metabolites of the soil strain UITEY-030 ($P > 0.5$). On the other hand, the filtrate of strain UITEY-055 only showed an effect against *E. coli*, with the number of colonies decreasing in contrast to the control ($P > 0.5$) (Figure 3).

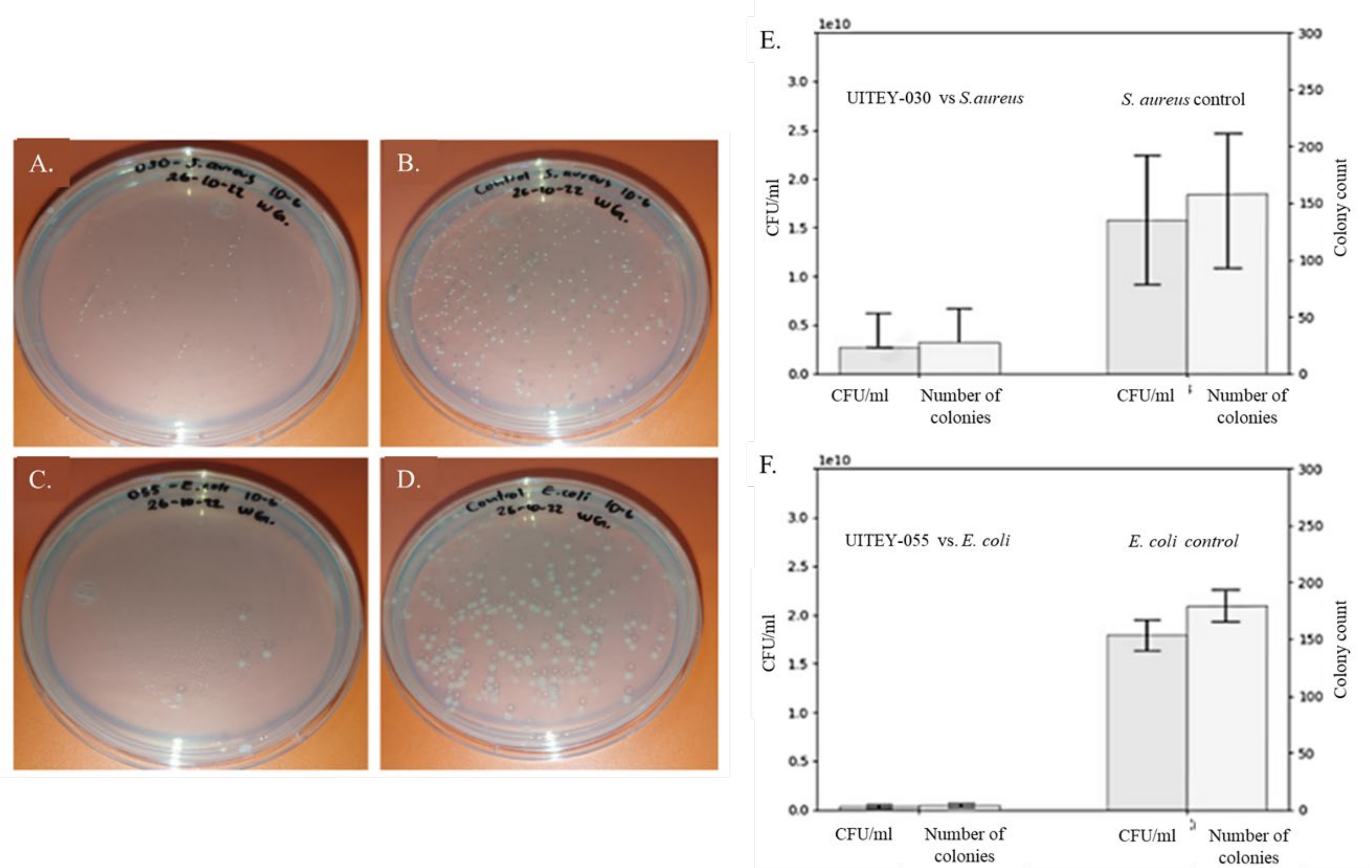

Figure 3. Soil strain filtrate inhibition assay. (A) *S. aureus* inoculated with UITEY-030 filtrate. (B) Growth control of *S. aureus*. (C) *E.coli* inoculated with UITEY-055 filtrate. (D) Growth control of *E.coli.*(E) y (F) CFU/ml values and number of colonies in the presence and absence of the soil strain filtrate.

DISCUSSION

Antibiotic resistance has become a public health problem that complicates the treatment of patients and can even lead to their death. *S. aureus*, *E. coli,* and *P. aeruginosa* are some of the bacteria responsible for these infections. *S. aureus* is a major public health problem worldwide[12]. It can cause many infections, from mild skin problems to serious illnesses such as pneumonia, sepsis, and endocarditis[13–15]. *S. aureus* can acquire resistance to several antibiotics of the penicillin family, including methicillin, which is the most common form of resistance[16].

P. aeruginosa is normally found in the environment and can cause human infections, especially in immunocompromised patients[17]. *E. coli*, on the other hand, is a bacterium that inhabits the intestines of humans and animals and can cause life-threatening conditions such as sepsis, gastroenteritis, and urinary tract infections[18]. Although many strains are harmless, multidrug-resistant *E. coli* has become a worrying problem in human and veterinary medicine[19].

Due to the increasing prevalence of antibiotic-resistant bacteria in recent years, one of the leading research priorities involves the identification of novel compounds capable of combating these microorganisms[20,21]. Therefore, our study comprehensively screened soil bacteria to assess their antimicrobial activity. Out of the 71 strains analyzed, there might be a small sample size to definitively evaluate the antimicrobial potential of soil bacteria in the studied area. However, it was found that two strains, UITEY-030 and UITEY-055, secreted metabolites that only exhibited partial inhibition against the growth of *S. aureus* and *E. coli*, respectively.

While the observed number of isolates with the ability to inhibit the growth of other bacteria seems to be low, in previous studies involving 263 isolates, only three of them exhibited antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa,* and *K. pneumoniae*. This behavior could be explained by the fact that the interaction between soil bacteria and the test strains is not normal, so it is unusual for them to produce metabolites that inhibit their growth.
Additionally, it was observed that the antagonistic activity of these strains presented variations during the initial screening. Notably, strain UITEY-030 generated an inhibition zone, while strain UITEY-055 produced a type of growth monopolization in the first assay. This difference in behavior could be attributed to the presence of distinct secondary metabolites that each strain releases when confronted with the other. The antagonistic activity of the strains presented variations during the initial screening. This highlights the complexity of interactions between soil bacteria and pathogens. A study that showed interspecific interactions significantly affect soil bacteria's antibacterial activity lends credence to this. According to that research, some bacterial isolates exhibited antibiotic activity solely in monoculture, whereas other isolates only showed activity when tested in interactions[22]. It is also important to note that the clinical isolate *E. coli* was nearly defeated by the soil strain whereas *E. coli* ATCC 25927 did not flinch. Despite belonging to the same species, this behavior may be attributed to each strain's different adaptations and virulence factors, enabling them to compete more effectively in various environments.

By expanding the soil screening area in Urcuquí, it is possible that more strains secreting compounds with antimicrobial activity capacity could be found. In addition, it is interesting to note that strains UITEY-030 and UITEY-055 both belong to the genus *Bacillus* sp. This genus is widely known for antibiotic production with antagonistic activity against various bacterial and fungal infections[23]. Moreover, *Bacillus* sp. is also characterized by the release of a variety of secondary metabolites, including siderophores, antibiotics, and antifungals[23]. These metabolites play a crucial role in the rhizosphere microbiota by creating a hostile environment for pathogens or by activating host defense mechanisms[24]. In a previous study, bacterial species belonging to the genus *Bacillus* were found to have the ability to inhibit the growth of *E. coli* and *S. aureus*, which led to the finding that the antibiotic produced by these strains is a broad-spectrum antibiotic[25].

The discovery of these soil strains underlines their promising potential for the search for new antimicrobial agents. This study did not identify or characterize the bioactive substances responsible for the antimicrobial activity as the crucial step to understanding the mode of action of the microbial metabolites for drug development. Therefore, it becomes imperative to identify and characterize the bioactive substance responsible for the partial or complete inhibition of bacterial growth. It is also crucial to clarify the mode of action of the secreted compounds and investigate their potential application in antimicrobial therapy and other relevant areas. In addition, it is recommended to take more composite soil samples from other environments with greater vegetation cover to explore a broader spectrum of microbial diversity. The study was conducted in a specific area with low precipitation and limited vegetation diversity. Investigating soil from environments with different characteristics might reveal a wider range of antimicrobial properties. Besides, this study only evaluated the antimicrobial activity against three bacterial strains (*S. aureus, E. coli, and P. aeruginosa*) further testing against a broader range of pathogens is necessary. Overall, these study findings emphasize the need for further exploration of soil bacteria's potential as a source of novel compounds. This exploration is essential to develop effective strategies to combat antibiotic resistance.

CONCLUSIONS

Since soil bacteria coexist with other microorganisms, they secrete secondary metabolites as part of communication and defense mechanisms. Hence, investigating soils from diverse ecosystems is crucial since it is possible to discover bacteria capable of secreting compounds with antimicrobial properties.

Identifying and thoroughly characterizing these molecules are crucial steps towards understanding their mode of action. This knowledge will facilitate the synthesis of new drugs to treat infections caused by microorganisms resistant to current antimicrobial agents.

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