Секция «Микробиология»

Isolation and characterization bacteria from soil with potential to produce antibiotic compounds

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Abstract
Microorganisms represent valuable reservoirs of natural compounds, harboring immense potential for utilization in the realms of industrial applications in medicine, chemistry, and agriculture.(Strobel et al., 2004). It has been proposed that most of the novel antibiotics have been found by screening of isolates from soil (Baniya et al., 2019) This study focuses on isolating Actinomycetes from the rhizosphere of Alder trees. After isolation of actinomycetes, further extraction of antibiotics using the well diffusion method showed the antimicrobial activity against non multidrug-resistant-organism and MDR too based on Baniya, 2019. At present, there is a need to find out novel antimicrobial-producing strains as the pre-existing drugs have failed due to the development of resistance among the microorganisms. The isolates which showed broad-spectrum activity against the test microorganisms can be considered as candidates in regards of searching for potential antimicrobial compounds (Soumia el al., 2023).

Introduction

The emergence of antibiotic-resistant bacteria has cast a long shadow on global health, prompting an urgent search for novel antimicrobial agents. In this context, soil, a treasure trove of diverse microbial life, beckons as a promising frontier for the discovery of new antimicrobial compounds. (Danquah, C. A., et al., 2020) This study delves into the isolation and characterization of bacteria from soil, embarking on a quest to unlock their hidden potential in producing antimicrobial compounds.

Our journey begins with isolating and identifying bacteria from soil samples using established protocols (Kasarla, S., & Gattu, S., 2020). These isolates will then be subjected to rigorous screening for antimicrobial activity against a panel of pathogenic bacteria, including some notorious for their resistance to existing antibiotics (Kumar, S., et al., 2010). This screening process will employ well-established techniques such as the disk diffusion assay and minimum inhibitory concentration (MIC) determination (Eloff, J. N., 1998).

Objective

1.Identification of new antibiotic-producing bacteria
2.Identification of bacteria with emerging antibiotic resistance

Short-term goals

1.Isolate the bacteria from soil
2.Characterize them with different tests
3.Analyze their antibiotic producing potential
4.Confirm by genetic testing the identification of each antibiotic producing bacterium

Material and Methods

Isolation

The serial dilution method for isolating bacteria from soil involved mixing soil with purified water or LB broth (1g/10mL; w/v), diluted to concentrations of 10^-1 to 10^-5 by selecting 1/10 of the volume from the original solution. Initial culturing took place on plates containing the antifungal agent Nystatin. For antibiotic resistance evaluation, agar plates were supplemented with either tet50 or Amp25. Incubation occurred for 24-48 hours at 37°C. Pure colonies were subcultured onto new agar plates to confirm purity and progress to further identification.

Morphology Tests

At the initial stage, we described the appearance of colonies on NA, including their color, shape, edges, center, etc. Gram staining with Crystal Violet dye was conducted to characterize the isolated colonies, followed by microscopic examination.

Media Tests

Different types of media were employed for differentiation and selectivity tests. Selective media included EMB agar (Eosin-Methylene Blue), Cetrimide agar, KF streptococcus agar, and Pseudomonas agar. Differential media comprised Simmon Citrate media, Blood Agar, and MacConkey agar. All media were prepared according to the manufacturer's instructions.

Biochemical Tests

To analyze metabolic products and biochemical activity, the following tests were conducted: Gelatin Hydrolysis test, TSI (Triple Sugar Iron), Oxidase, Catalase, and the RapID one system - a one-step kit for the identification of medically important Enterobacteriaceae and other selected oxidase-negative, Gram-negative bacilli.

Antibiotic producing tests

Colonies were cultured on prepared NA plates with Tetracycline (3.75 mg/ml) or Ampicillin (50-100 mg/ml). The detection of antimicrobial activity was carried out using the Well Diffusion method. The analysis was performed on NA plates with cultivated pathogenic cultures (P. aeruginosa, E. coli, Salmonella, E. faecalis). The colonies under investigation were introduced into wells on the agar (100 μL) or with disks soaked in 100 μL of the diluted culture. For comparative analysis, colonies showing positive results in the initial screening were again tested against pathogenic cultures, along with disks impregnated with antibiotics (Streptomycin, Ampicillin, Penicillium).

Soil

Soil samples were collected from the forest-steppe zone of the park, under herbaceous cover, from two soil levels. The first sample was taken from the soil surface (2-3 cm), and the second from a deeper layer (8-10 cm). Multiple measurements of pH, as well as morphophysiological indicators, were conducted.(Fig.1)

Results and discussion

During the project eleven pure colonies were isolated (Fig. 3,4). Ten are Gram-negative, and one colony is Gram-positive. Nine exhibit a bacillus form, while two are cocci. The results of morphological, biochemical, and selective tests were compiled into a table (Fig. 5). Antibiotic resistance tests also revealed that the majority of bacteria possess resistance, particularly to ampicillin. During antimicrobial activity tests, it was identified that three colonies demonstrated positive efficacy. Samples N 2/4/L, T1/6, #2 showed nearly identical results in testing with various antibiotics and pathogenic cultures (Fig. 6).

In the attempt to identify cultures, one was successfully determined using the RapID One Kit - Salmonella Choleraesuis (T2/1/3 colony). However, to confirm and identify other colonies, samples need to be sent for 16S rRNA PCR.

Conclusion

The isolated and characterized bacteria from the soil exhibit a positive trend towards

antibiotic resistance and antimicrobial activity.

Future work

Secondary screening tests for the antimicrobial activity of bacteria need to be conducted, and the extracted compounds should be analyzed for their antibiotic nature. To fully understand the taxonomy of these colonies, their species characteristics must be confirmed through genetic analysis.

References

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6) Kumar, S., et al. (2010). Isolation, characterization and antimicrobial activity of Bacillus sp. strain SJ-101 from soil. Brazilian Journal of Microbiology, 41(1), 90-97.
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Illustrations

Description	Both soil samples have the same structure. When you add a little bit of water it looks like clay. Pretty smooth and flexible. But not stable if you add too much water.					
			pH			
date	Water	LB+nys	Soil #1 water	soil #2 water	Soil #1 LB	Soil #2 LB
6/10/23		7.1	7.16	07.07	07.05	07.05
9/10/23	6.7					
10/10/23			7.010	7.060	6.16	7.17
18/10/23			7.17	6.85	6.33	6.96

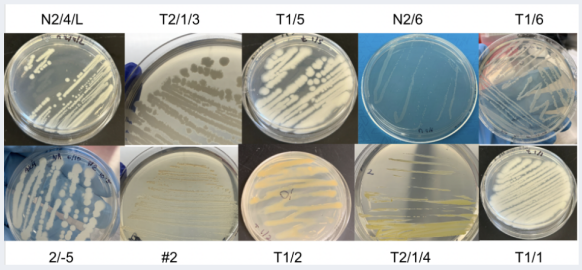
Figure 1. pH measurements and soil description

Рис. : figure 1

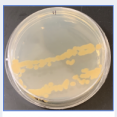
Colonies	Media of growth	Tet. growth 3,75mg/ml	Amp 100mg/ml	Shapes of bacteria	Nutritient Agar Colony color	Description	Gram stain	EMB agar	Centramide Agar	pigment	Oxidase test	Catalase test	Triple sugar/Iron Slant	interpret TSI result	MacConkey	MacConkey interpratation
N 2/4/L 1	NA/Nys 0.1mg/m	-	+	diplobacilli	whitish	Circular, entire, smooth, convex	negative	G	NG		-	+	yellow/yellow	Glucose and lactose and/or sucrose fermentation	NG	
T 2/1/3 2	NA/Tet 3,75 mg/ml	-	+	Streptobacilli	dusty white	Citcular, scalloped, smooth, convex	negative	G	NG		-	+	yellow/yellow	Glucose and lactose and/or sucrose fermentation	NG	
T 1/5 3	NA/Tet 3,75 mg/ml	-	+	Streptobacilli	whitish	circular, entire, smooth, flat	negative	G pink	NG		+	+	red/yellow	Glucose fermentation only; Peptone catabolized	NG	
N 2/6 4	NA/ Nys 0.1mg/m	-	+	Streptobacilli	transperent - white	circular, entire, smooth, flat	negative	G	NG		+	-	red/red	No fermentation ,Peptone catabolized	NG	
T 1/6 5	NA/Tet 3,75 mg/ml	+	-	streptococci	white	circular, round, smooth, flat	negative	G pink	positive		+	+	yellow/yellow	Glucose and lactose and/or sucrose fermentation	G	Lactose non-fermenting
2/-5 6	NA / Nys 0.1mg/m	-	+	Streptobacilli	dusty white	circular, entire, smooth, flat	negative	G pink	NG		+	+	red(black)/yellow	Glucose fermentation only; H2S produced	G	Lactose non-fermenting
#2 7	NA/centramide	-	+	bacilli	white, produce dark yellowish secret	circular, entire, smooth, flat	negative	G	positive	dark yellowish	+	+	yellow/yellow	Glucose and lactose and/or sucrose fermentation	G pinkish	Lactose fermentation will produce acidic byproducts that lower the pH, and this turns the pH indicator to pink.
T1/2 8	NA/Tet 3,75 mg/ml	+	+	streptobacilli	yellow	Circular, entire, smooth, convex	negative	NG	NG		-	+	red/red	No fermentation ,Peptone catabolized	G	Lactose non-fermenting
T 2/1/4 9	NA/Tet 3,75 mg/ml	-	+	tetrococci	bright yellow	circular, entire, smooth, flat	negative	G	NG		-	+	red/red	No fermentation ,Peptone catabolized	G	Lactose non-fermenting
T 1/1 10	NA/Tet 3,75 mg/ml	+	+	streptobacilli	dusty white	circular, entire, smooth, flat	negative	G pinkish	NG		+	+	red/yellow, black head, gas	Glucose fermentation only; Gas produced; H2S produced	NG	
Colonies	**Media of growth**	**Tet. growth**	**Amp**	**Shapes of bacteria**	**Nutritient Agar Colony color**	**Description**	**Gram stain**	**EMB agar**	**Centramide Agar**		**Oxidase test**	**Catalase test**	**Triple sugar/Iron**	**interpret TSI result**		
T 1/4 11	NA/Tet 3,75 mg/ml	+	+	bacillus	yellow	Circular, entire, smooth, convex	positive	NG	NG		-	+	red/red	No fermentation ,Peptone catabolized		

Figure 5. Tests results for 11 colonies

Рис. : figure 5



Figures 3. G-Ve colonies



T1/4

Figures 4. G+Ve colony

Рис. : figure 3 and 4

	antibiotics disk method					
	Streptomycin	Ampicillin	Peniccilium	1	5	7
P.Aureginosa	15	-	-	9	9	10
E. Coli	23	12	-	-	9	9
Salmonella	contaminated					
E.Faecalis	17	7	7	9	10	10

	primary disk screening		
	1	5	7
P.Aureginosa	7 mm	8	8
E. Coli	-	11	10
Salmonella	8	10	8

Figure 6. Antimicrobial results

Рис. : figure 6