Isolation, Identification and Optimization of Secondary Metabolite Compound from *Escherichia Fergusonii*.

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Abstract - *Antibiotics, primarily derived from microorganisms, are essential in both medicine and agriculture. However, extensive use has culminated in antibiotic resistance, thus rendering it essential to study a variety of microbiological sources for the purpose to identify additional bioactive compounds. This study focuses on the isolation, identification, and optimization of bacteria producing secondary metabolites with antibiotic potential. A Gram-negative bacterial colony with antibiotic activity against the test microorganism Escherichia coli was identified after soil samples collected from the university campus. Its identity has been confirmed through biochemical analysis; the bacteria were later identified as Escherichia fergusonii by 16S rRNA sequencing. Slight zones of inhibition against Staphylococcus aureus and E. coli have been identified during primary screening, indicating the potential of producing secondary metabolite. Optimization examinations were carried out, and analyzing parameters like pH, temperature, incubation time, light and dark conditions. Alteration in pH and incubation duration had no influence on antibacterial activity, while lower temperatures and incubation under dark conditions significantly improved it, based on the results. After fermentation, 0.071 g of brown crude extract enriched with secondary metabolites was extracted by butanol extraction. Antibacterial activity using the agar-well diffusion method indicate concentration-dependent activity against S. aureus. Zones of inhibition increased with higher metabolite concentrations; 0.2 mm for 20 µl, 0.25 mm for 30 µl, and 0.45 mm for 40 µl. This study shows that it is possible to isolate bacteria that produce antibiotics and maximize the production of their secondary metabolites; E. fergusonii exhibits an ability to produce metabolites.*

Keywords: Antimicrobial activity, *Escherichia fergusonii*, Optimization, Secondary metabolites, 16s rRNA sequencing.

1. INTRODUCTION

"Antibiotic," which comes from the Greek term antibiosis, meaning "against life," underlines its effectiveness. The drug works by preventing bacteria from growing. Antibiotics are primarily generated by fermenting microbial cells, whether they are naturally occurring or artificially developed compounds. These compounds have a crucial role in controlling microbial populations in a variety of natural circumstances, such as soil, water, wastewater, and composting. Only a small number of the numerous antibiotics that have been developed have been shown to be both safe and effective enough for application in medicine; most of them originate from microorganisms that belong to genera like *Bacillus, Streptomyces, Penicillium, Cephalosporium,* and *Micromonospora*. Remarkably, of the 5,000 known antibiotics that have been derived from bacteria, fungi, and plant cells, *Streptomyces* accounts for over 60% (Awais et al., 2007).

Despite their fundamental significance, the extensive and often excess of antibiotics in wastewater treatment, livestock, and aquaculture is resulting in an enormous increase in antibiotic residues in the environment. This contributes significantly to the formation of drug-resistant microbial populations (Nair et al., 2022). Modern antibiotics must be discovered and produced immediately to combat antibiotic resistant ailments.

During an attempt at finding new antibiotics, researchers are currently investigating a variety of microbiological sources due to the increasing prevalence of antibiotic resistance globally. They are among the most alluring alternatives because of their broad production of bioactive compounds. This study contributes to this field by identifying and characterizing bacterial strains that have the potential to produce antibiotics. (Kumari et al. 2013).

The potential for pathogenicity and distinctive biochemical characteristics, such as being sorbitol negative and adonitol positive, have brought attention to *Escherichia fergusonii*, a lesser known member of the *Escherichia genus* (Glover et al., 2017). As a recently identified pathogen with multidrug resistance, *E. fergusonii*, initially isolated from primates other than humans, poses a significant safety concern.

This research project proposes to characterize the antibiotic resistance characteristics of *E. fergusonii* by focusing on understanding its resistance mechanisms and their function within the broader framework of microbial diversity and antibiotic discovery. By analyzing the connections between factors affecting the environment, microbial behavior, and resistance characteristics, this research aims at improving current attempts to combat antibiotic resistance while discovering possible novel antimicrobial approaches.

2. MATERIALS AND METHODS

2.1 Collection of samples and isolation of micro-organisms

Collection of soil sample from university campus and to make it possible to process soil samples, 1 g of the test sample was dissolved in 10 ml of sterile distilled water, and then further serial dilutions were performed. Every dilution's 100 µl of supernatant was then spread across nutrient agar plates and kept overnight at 37°C. A test microorganism was introduced to each plate and incubated for 48 hours. A zone of inhibition was then detected on each plate, and colonies were selected to proceed with isolating testing. To isolate the bacteria, selected colonies were plated directly onto nutrient agar based on their morphology and zone of inhibition. Distinct bacterial colonies were chosen based on colony characteristics and streaked on new agar plates during an overnight incubation. After that, the plates were stored for 24 hours at 37°C. Purified bacterial cultures were obtained on nutrient agar plates using the multiple streak plate method. Following a 24-hour incubation period at 37°C, pure single colonies have been observed (Salim et al. 2017).

2.2 Morphological study of isolated bacterium

Following the isolation of microorganisms, identification tests were conducted according to Bergey's Manual regulations. The isolated strains were adequately identified and classified by using this technique (Buchanan et al. 1974).

2.2.1 Gram's staining

A loop full of Culture was placed on a clean glass slide. To ensure adherence, the smear was heat-fixed. After adding crystal violet to the smear, it was let to sit for 30 to 60 seconds before being rinsed with water. The smear was then covered with Gram's iodine for a minute and then rinsed with water another time. The smear was then rinsed with water after being cleaned for 10–20 seconds with 95% alcohol or acetone. After adding safranin and allowing it remain for approximately a minute, the smear underwent another water rinse. After being blotted and allowed to air dry, the slide was examined under a microscope (Bailey and Scotts, 1966).

2.3 Biochemical test

2.3.1 Oxidase test

1% Kovács oxidase reagent was used to soak a small piece of filter paper, which was then let to dry. Using a fresh bacterial plate that had been cultured for 18 to 24 hours, a well-isolated colony was selected using a loop and smeared onto the whatmen filter paper. A careful examination for color changes was noted. When the microorganisms turned dark purple in 5–10 seconds, they were determined to be oxidase positive (Al-Rubaye et al. 2023).

2.3.2 Catalase test

A hydrogen peroxide bottle and a glass slide were obtained. A small number of bacteria were applied to the dried slide using a sterile inoculating loop. After that, a drop of hydrogen peroxide was added to the bacterium (Al-Rubaye et al. 2023).

2.3.3 Indole test

There was a tiny quantity of a pure culture added to the tryptone broth tube. After that, it was incubated for 24 to 48 hours at 37°C. 5 drops of Kovács reagent were added directly to the tube to examine for the production of indole (Al-Rubaye et al. 2023).

2.3.4 Methyl red test

Test tubes were used to prepare the MRVP broth. Two loopfuls of the respective bacterial cultures were added to the broth aseptically. The organism that was introduced appeared on the labels of the test tubes. After that, they were incubated for 48–72 hours at 37°C. A few drops of methyl red indicator have been added to the incubated tubes after incubation, and results were observed (Al-Rubaye et al. 2023).

2.3.5 Voges-Proskauer test

The broth was introduced with well-isolated colonies of sample bacteria from cultures that were 18 to 24 hours old using a sterile inoculating loop. After that, the tubes were incubated at 37°C for 18 to 24 hours. 2 ml of broth have been transferred to a sterile test tube after incubation. Reagent A (5% α-naphthol solution) was then added in 6-7 drops and thoroughly incorporated by shaking. 2-3 drops of Reagent B (40% KOH solution) were then added, and

everything was thoroughly mixed by shaking. Within 30 minutes, the tubes were examined to see whether a reddish-pink color developed at the medium's surface. The tubes were rapidly shaken continuously for the duration of the 30-minute waiting period (Al-Rubaye et al. 2023).

2.3.6 Starch hydrolysis test

A single streak inoculation of the organism to be investigated was made into the center of the selected plates using a sterile technique. Following inoculation, the plates were incubated for 48 hours at 37°C. After incubation, a dropper was used to saturate the surface of the plates with iodine solution for 30 seconds. They poured out the remaining iodine. The clear zone surrounding the bacterial growth line on the plates was observed (Al-Rubaye et al. 2023).

2.4 Molecular study of isolated bacterium

The process of 16S rRNA sequencing for bacterial identification began with the selection of a bacterial colony from the isolate. Polymerase chain reaction (PCR) with universal primers was used to amplify the 16S rRNA gene, and gel electrophoresis was used to verify the amplification efficacy. To provide high-quality input for sequencing, the PCR product was then filtered. Each primer's sequencing trace files were produced through Sanger sequencing, and the accuracy of the traces was confirmed by quality control (QC) checks. A consistent sequence record was generated from the sequencing traces, and subsequent quality control was performed to ensure its integrity. To determine the bacterial strain based on sequence similarity, the assembled sequence was subsequently submitted to BLAST database searches. Finally, the method of classification was verified by phylogenetic analysis.

2.5 Primary Screening of Antibacterial Activity

The agar well diffusion method was used to determine the antimicrobial activity of test pathogenic microorganisms, such as *Staphylococcus aureus* and *Escherichia coli*. After monitoring the inhibitory zones, only the isolated isolates showing broad-spectrum behavior have been selected for further examination (Kumari, et al., 2013).

2.6 Optimization of Growth and Antimicrobial Compounds Production

The isolates were inoculated into a 250 ml Erlenmeyer flask with 100 ml of Soybean Casein Digest Medium (Tryptone Soya Broth) to test growth and the production of antimicrobial compounds. The filtrate was then examined for antibacterial activity using the agar well diffusion method (Khattab et al., 2016).

2.6.1 Effect of pH

Using flasks containing 50 ml of Soybean Casein Digest Medium (Tryptone Soya Broth), the pH was varied to 6.5, 7, and 7.5 in order to determine the optimum pH value for the production of antimicrobial compounds. After inoculating the broth medium with isolated bacteria, the flasks were shaken and incubated for 7 days at 30°C. Following incubation, the antibacterial activity of the supernatant was examined (Khattab et al., 2016).

2.6.2 Effect of temperature

The bacterial isolates were inoculated in optimized medium and incubated at various temperatures (0, 20, 30, and 40°C) for 7 days. After incubation, the supernatant was tested for antimicrobial activity (Khattab et al., 2016).

2.6.3 Effect of light and dark conditions

2 flasks containing the bacterial culture media were incubated for 7 days under light and dark conditions. After incubation, the supernatant was tested for antimicrobial activity (Khattab et al., 2016).

2.6.4 Optimum incubation period

The culture was incubated in growth media for 10 days in order to determine the effect of the incubation period. On days 4, 5, 6, 7, 8, 9, and 10, the broth was subjected to a series of tests for antibacterial activity using the disc diffusion method. After measuring the inhibition zones, the ideal incubation time was established for subsequent studies (Khattab et al., 2016).

2.7 Fermentation of isolated bacterium

The selected bacterial strain isolates were added to Tryptone Soy Broth and allowed to ferment for seven days at 30°C in a shaker. After fermentation, a Millipore filter (Millipore Millex-HV Hydrophilic PVDF 0.45 µm) was used to filter the filtrate, which was separated by centrifugation at 5000 rpm for 10 minutes. For subsequent tests, the filtrate was aseptically transferred into a conical flask and kept at 4°C (Khattab et al., 2016).

2.8 Solvent extraction and purification of secondary metabolite compound

After adding the solvent in a 1:1 (v/v) ratio to the filter, it was vigorously stirred for 20 minutes. A separating funnel

was used to separate the antibiotic-containing butanol phase from the aqueous phase. Methanol was used to purify the residue, which produced dark crude extract, after the butanol layer was concentrated by drying out at 110°C.

2.9 Secondary screening of antibacterial activity

After adding the solvent in a 1:1 (v/v) ratio to the filter, it was vigorously stirred for 20 minutes. A separating funnel was used to separate the antibiotic-containing butanol phase from the aqueous phase. Methanol was used to purify the residue, which produced dark crude extract, after the butanol layer was concentrated by drying out at 110°C. Using the agar well diffusion method, this compound's antibacterial effectiveness against test pathogenic bacteria was determined. After 12–16 hours at 4°C, the plate was incubated for the entire night at 37°C. The test microorganism's inhibition zones around the wells were measured (Khattab et al., 2016).

3. RESULT

Fig. 1 Collected soil sample from university campus.

Using the agar well diffusion method, this compound's antibacterial effectiveness against test pathogenic bacteria was determined (Khattab et al., 2016)

3.1 Collection of soil sample and Isolation of bacteria

Soil samples were collected from the roots of plants in a university campus (Fig. 1). For the isolation of microorganisms, 1g of soil was measured and added into 9 ml of sterile distilled water. Further serial dilutions were performed up to 7 test tubes. Then, the last four test tubes (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} diluted samples) in amounts of 100 µl were spread on nutrient agar plates and kept for incubation for 24 hours (Fig. 2). After the incubation period, the results obtained revealed the growth of different colonies on the agar plates. Subsequently, we checked whether the colonies were producing antibiotics or not by adding the test organism *(E. coli)*. Among the different isolated colonies, only one showed a clear zone of inhibition against the test organism (Fig. 3) and then they were streaked on agar plates for primary screening to obtain a pure culture.

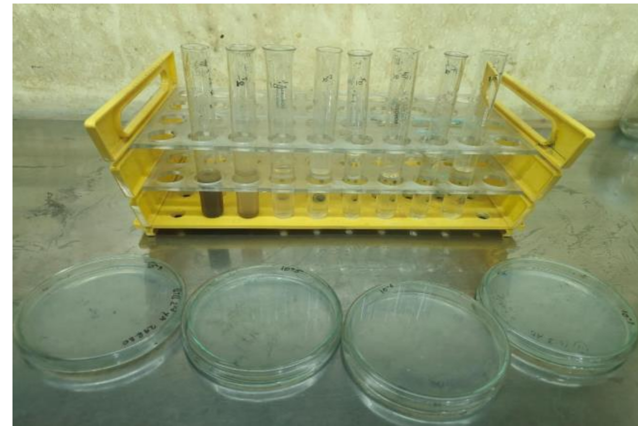

Fig. 2 Serial dilution of soil sample.

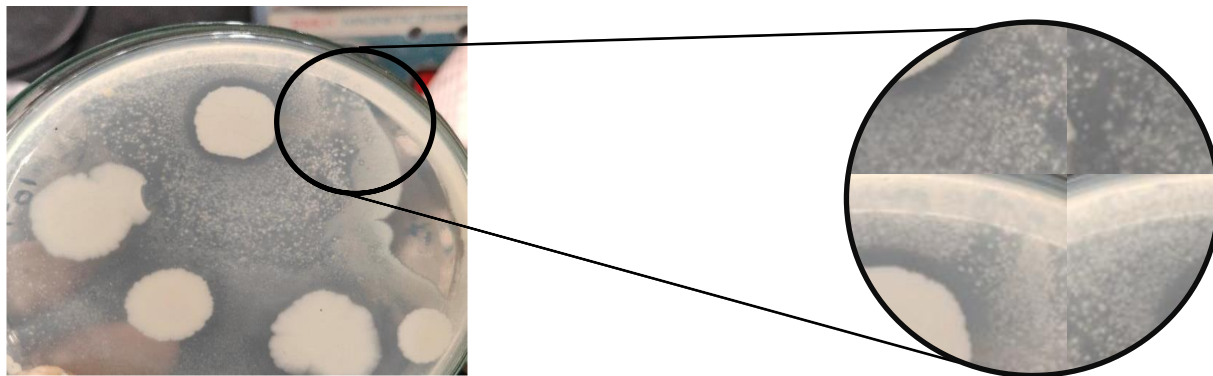

Fig. 3 Zone of inhibition after adding test organism (*E. coli*) on colonies.

3.2 Morphological study of isolated bacterium

3.2.1 Gram's staining

A loopful of bacterial culture was placed to a cleaned glass slide, allowed to heat-fix, and then successively treated with safranin, alcohol, Gram's iodine, and crystal violet. After being allowed to air dry, the slide was examined under a microscope. The bacterium appeared pink in color, indicating a Gram-negative bacterium, with rod-shaped structures (Fig. 4).

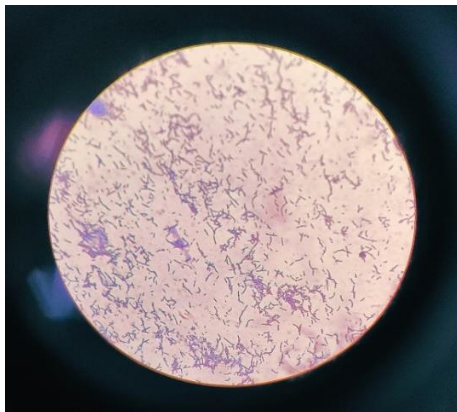

Fig. 4 Bacterium showing pink, rod-shaped structure under the microscope.

3.3 Biochemical tests (Table 1)

3.3.1 Oxidase test

After being immersed in 1% Kovács oxidase reagent, a small piece of filter paper was allowed to dry. Using a new bacterial plate that had been cultured for 18 to 24 hours, a well-isolated colony was selected using a loop and rubbed onto the whatmen filter paper. A slight dark purple coloration appeared within 5-10 seconds, indicating a positive oxidase test result.

3.3.2 Catalase test

A hydrogen peroxide bottle and a glass slide were acquired. A small number of bacteria were applied to the dried slide using a sterile inoculating loop. The bacteria were then covered with a drop of hydrogen peroxide. A positive catalase reaction was shown by the immediate bubble formation that was observed (Fig. 5).

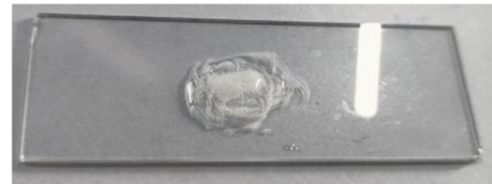

Fig. 5 Immediate bubble formation was observed.

3.3.3 Indole test

A small quantity of a pure culture has been added to the tryptone broth tube as the inoculation. After that, it was incubated for 24 to 48 hours at 37°C. 5 drops of Kovács reagent were added directly to the tube to examine for the production of indole. A dark brown-colored ring formed on the broth surface, indicating a positive indole production result (Fig. 6).

Fig. 6 Dark brown-colored ring formed on the broth surface.

3.3.4 Methyl red test

Test tubes were used to prepare the MRVP broth. 2 loopfuls of the respective bacterial cultures have been introduced to the broth aseptically. The organism that was introduced was listed on the labels of the test tubes. After that, they were incubated for 48–72 hours at 37°C. A few drops of methyl red indicator were applied to the incubated tubes after incubation. The appearance of the color red indicated a positive methyl red test result (Fig. 7).

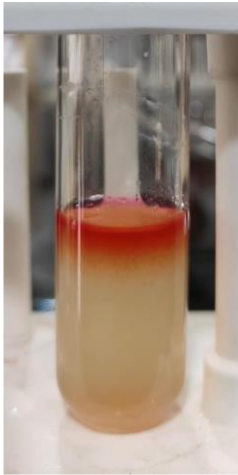

Fig. 7 A positive methyl red test result was indicated by the appearance of a red color.

3.3.5 Voges-Proskauer Test

The broth was introduced with well-isolated colonies of sample bacteria from cultures that were 18 to 24 hours old using a sterile inoculating loop. After that, the tubes were incubated at 37°C in the presence of oxygen for 18 to 24 hours. 2 ml of broth were moved to a sterile (ideally) test tube after incubation. Reagent A (5% α-naphthol solution) was then added in 6-7 drops and thoroughly mixed by shaking. 2-3 drops of Reagent B (40% KOH solution) were then added, and everything was thoroughly mixed by shaking. Within 30 minutes, the tubes were examined to determine whether a reddish-pink color developed at the medium's surface. Throughout the 30-minute waiting period, the tubes were continuously shaken vigorously. A brownish-red coloration appeared on the surface of the broth within 30 minutes, confirming a positive Voges-Proskauer test result (Fig. 8).

Fig. 8 A brownish-red coloration appeared on the surface of the broth.

3.3.6 Starch hydrolysis test

The organism to be studied was inoculated into the center of the labeled plates in a single streak using a sterile technique. After that, the bacterially introduced plates were incubated at 37°C for 48 hours. After incubation, a dropper was used to saturate the plates' surface with iodine solution for 30 seconds. The extra iodine had been eliminated out. A clear zone around the bacterial growth was observed, indicating starch degradation, while the remaining starch reacted with iodine to produce a dark brown coloration (Fig. 9).

Fig. 9 A clear zone around the bacterial growth was observed, indicating starch degradation, while the remaining starch reacted with iodine to produce a dark brown coloration.

Table 1. Results of Various Biochemical Tests

No.	Biochemical Test	Positive result	Negative result
1.	Indole Test	✓	✗
2.	Oxidase test	✓	✗
3.	Catalase test	✓	✗
4.	Methyl red	✓	✗
5.	Voges-Proskauer test	✓	✗
6.	Starch hydrolysis	✓	✗

Footnote: ✓ indicates a positive test result, confirming the presence of the respective biochemical reaction. ✗ indicates a negative test result, showing the absence of the reaction.

3.4 Molecular study of isolated bacterium

The bacterial strain was identified as *Escherichia fergusonii* through 16S rRNA sequencing (Table 2). PCR amplification of the 16S rRNA gene was verified by gel electrophoresis, and Sanger sequencing produced high-quality trace files (Fig. 10 & 11). The assembled sequence showed high similarity in BLAST analysis, and the identification was confirmed by phylogenetic analysis (Fig. 12).

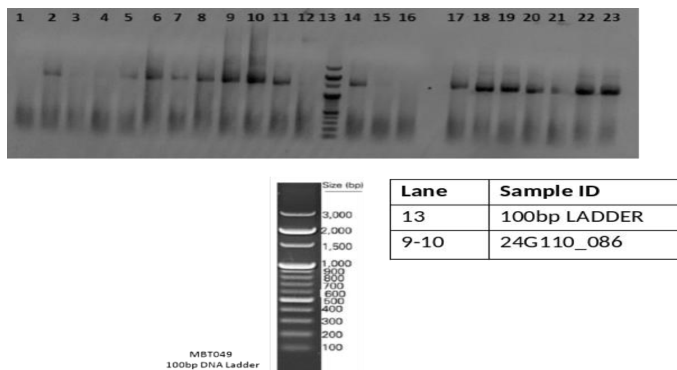

Fig. 10 Gel Image (Raw PCR Product)

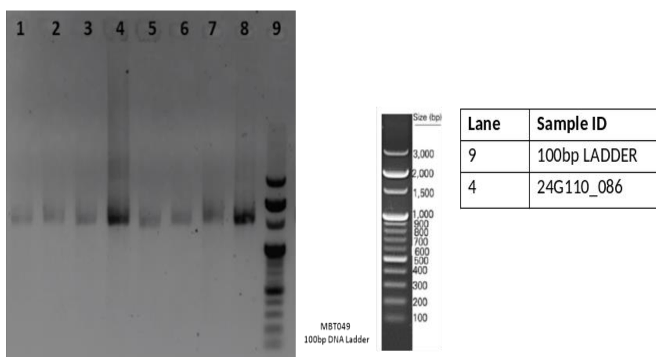

Fig. 11 Gel Image (Purified PCR Product)

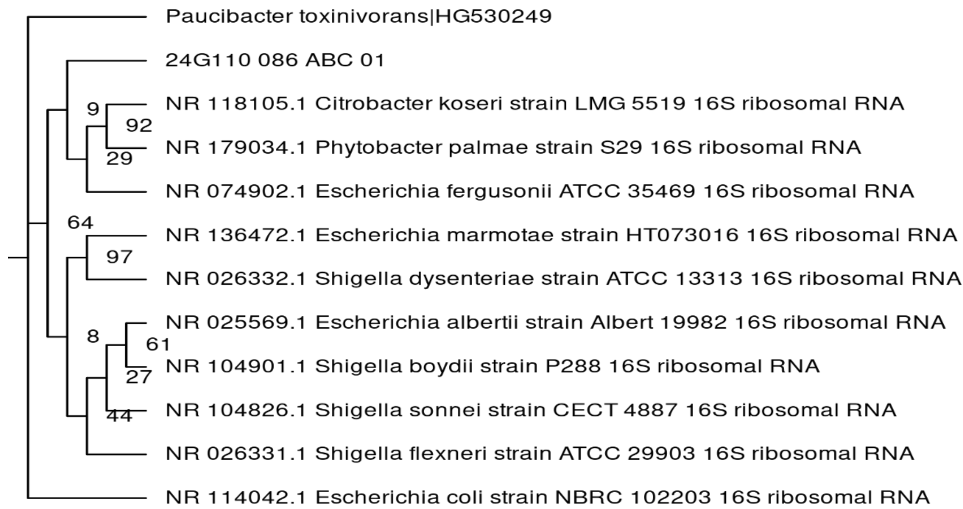

Fig. 12 Phylogenetic analysis tree

Table 2. TaxonomicClassification of *Escherichia fergusonii.*

Classification	Kingdom	Bacteria
	Phylum	Pseudomonadota
	Class	Gammaproteobacteria
	Order	Enterobacterales
	Family	Enterobacteriaceae
	Genus	Escherichia
	Genus Species	*Escherichia fergusonii*

3.5 Primary screening of antibacterial activity

In the initial primary screening for antibacterial activity, *Escherichia fergusonii* showed a slightly clear zone against two significant pathogens that is, *Escherichia coli* and *Staphylococcus aureus* (Fig. 13 &14). Although the zones of inhibition were relatively small, they indicated that *E. fergusonii* produces microbial compound with antibacterial properties. Based on this result, the bacterial strain may not exhibit strong broad-spectrum antibacterial effects, it does have the potential to slow the growth of certain harmful bacteria.

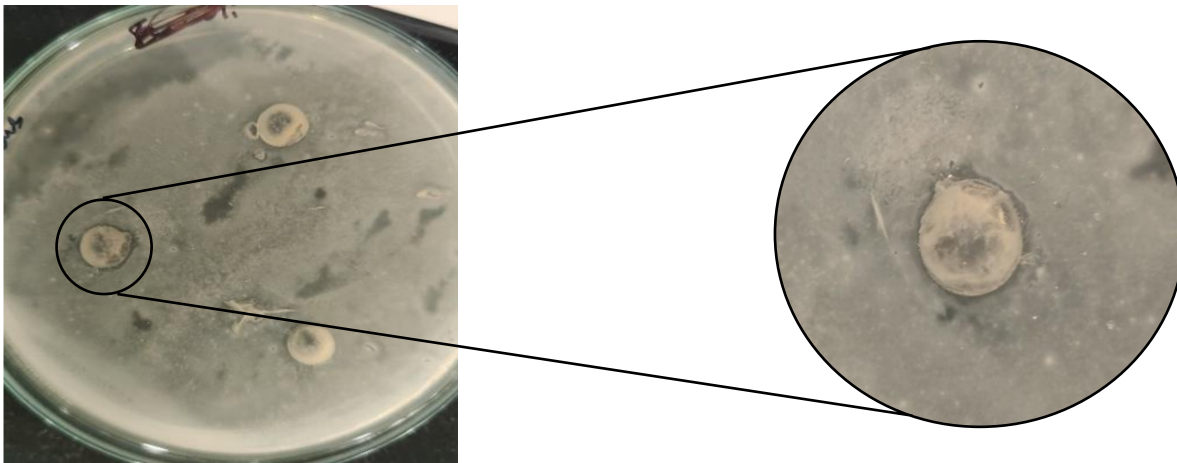

Fig. 13 Primary screening of antibacterial activity against *Staphylococcus aureus.*

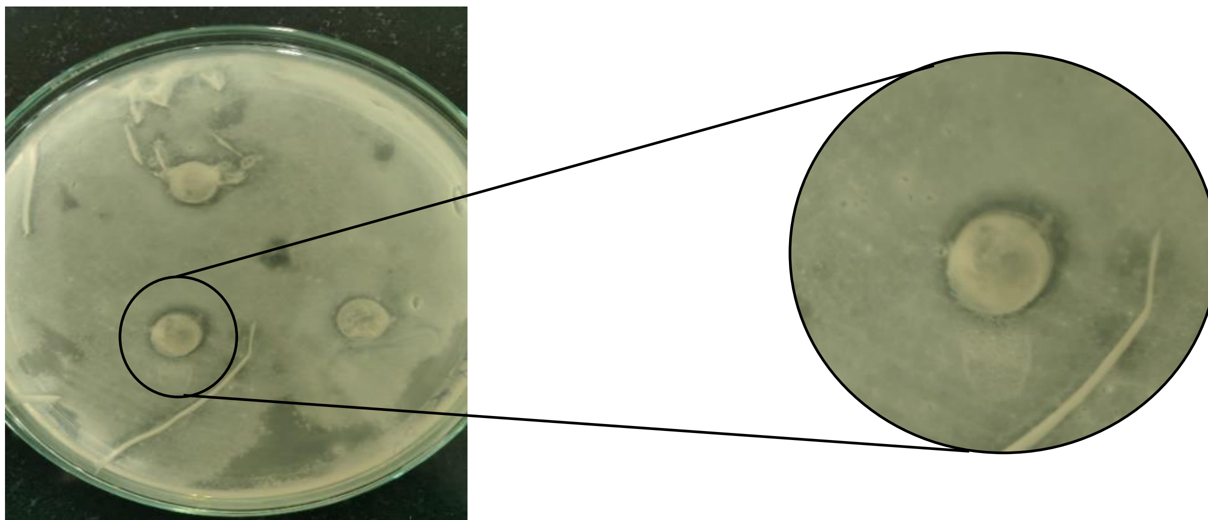

Fig. 14 Primary screening of antibacterial activity against *Escherichia coli.*

3.6 Optimization of growth and antimicrobial compounds production

3.6.1 Effect of pH

The pH levels of the Tryptone Soya Broth (Soybean Casein Digest Medium) have been adjusted to 6.5, 7.5, and 7. *Escherichia fergusonii* was introduced to the broth medium, and the flasks were shaken and cultured for 7 days at 30°C. Following an evaluation of the supernatant's antibacterial activity from cultures cultivated at 6.5, 7 and 7.5, no clear zones of inhibition were observed against *Staphylococcus aureus*. That shows the different pH values not enhance the production of antimicrobial compounds capable of inhibiting *S. aureus* under the tested conditions.

3.6.2 Effect of temperature

After testing the antimicrobial activity of the supernatant broth from cultures grown at different temperatures (0^0C, 20^0C, 30^0C & 40^0C), slight zones of inhibition were observed against *Staphylococcus aureus* at 0°C (Fig. 15 a) and 20°C (Fig. 15 b). However, no clear zones of inhibition were seen at 30°C and 40°C. This indicates that lower temperatures may facilitate some level of antibacterial activity against *S. aureus*, while higher temperatures appear to reduce this effect.

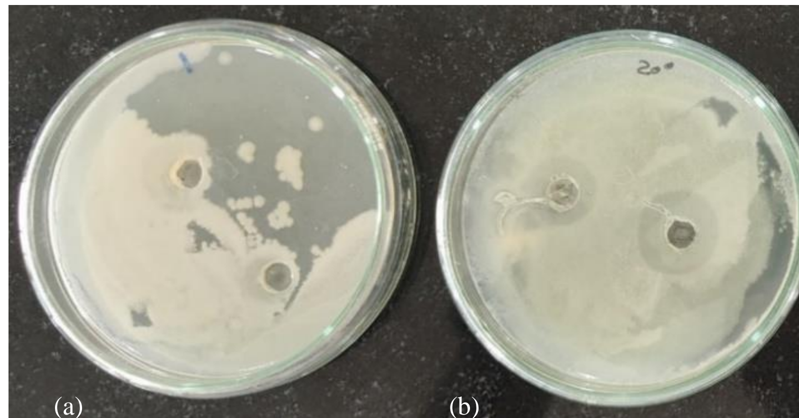

Fig. 15 Effect of temperature 0^0C (a) & 20^0C (b).

3.6.3 Effect of Light and Dark Conditions

The antimicrobial activity was tested by incubating 2 flasks encompassing culture media for 7 days under light and dark conditions. In the dark condition, a slight zone of inhibition was observed (Fig. 16 a), indicating some level of antimicrobial activity. In the light condition, zones of inhibition not observed (Fig. 16 b).

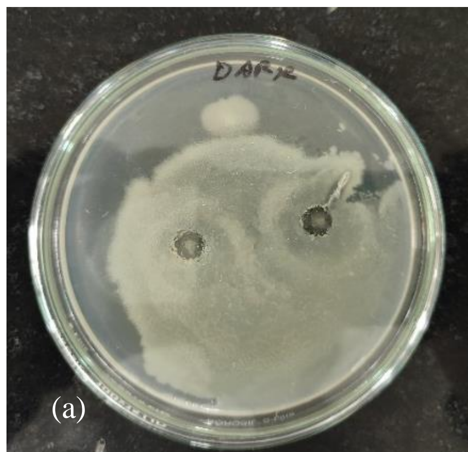

Fig. 16 (a) Effect of dark condition.

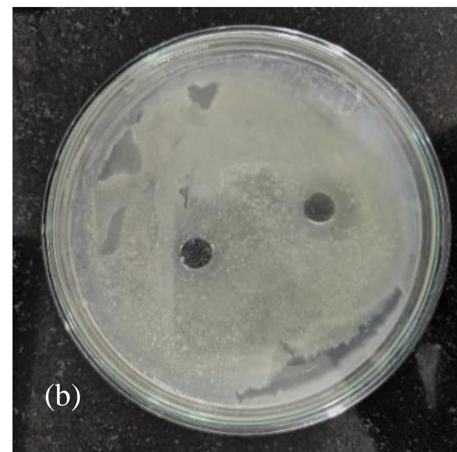

Fig. 16 (b) Effect of dark condition.

3.6.4 Optimum Incubation Period

The disc diffusion method was performed to test for antimicrobial activity on days 4, 5, 6, 7, 8, 9, and 10 after the culture was inoculated in growth medium for 10 days as part of the study on the effect of the incubation time. However, none of these days showed any zones of inhibition, suggesting that the incubation period was not successful in the production of antimicrobial compounds against the pathogens under examination.

3.7 Fermentation of isolated bacterium

Tryptone Soy Broth was successfully inoculated with the *E. fergusonii*, and the mixture fermented for 7 days at 30°C in a shaker. After fermentation, the solid biomass was separated from the liquid by centrifuging the culture for 10 minutes at 5000 rpm. After that, a Millipore filter was used to make sure the supernatant was clear of any remaining cells or debris.

3.8 Solvent extraction and purification of secondary metabolite compound

To recover any remaining antibiotic compounds, the 200 ml culture filtrate was extracted three times using butanol. Butanol was added to the filtrate in a 1:1 (v/v) ratio, and the mixture was agitated for 20 minutes. The aqueous phase was separated from the butanol phase, which most likely included the antibiotic (Fig. 17 & 18). This layer was then evaporated to dryness at 110°C, and the resulting residue had been purified with methanol, yielding 0.071g of brown crude extract in powder form (Fig. 19). This extract is rich in secondary metabolites, potentially containing antimicrobial compounds for further study.

Fig. 17 Extraction by separating funnel after addition of butanol solution in fermented sample.

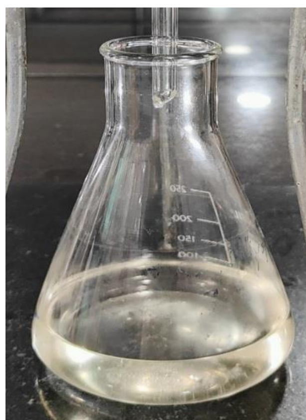

Fig. 18 Butanol layer containing secondary metabolites compound.

Fig. 19 Brown crude extract (0.071 g) secondary metabolites compound.

3.9 Secondary screening of antibacterial activity

The antibacterial activity of the crude extract has been assessed by using the agar-well diffusion method. On Muller-Hinton agar, 20 µl of S. aureus was spread out. Using a sterile 6 mm cork-borer, wells were made and filled with 20 µl, 30 µl, and 40 µl of the crude metabolite. After 12–16 hours at 4°C, the plate was incubated for the entire night at 37°C. When the crude metabolites concentration increased, it also increased the zone of inhibition. In particular, a zone of inhibition of about 0.2 mm was produced by 20 µl of the crude metabolite, while a zone of inhibition of about 0.25 mm was produced by 30 µl. The broadest inhibiting zone, measuring around 0.45 mm, was produced by the highest concentration, 40 µl (Table 3). These results show that the crude extract has antibacterial activity against S. aureus (Fig. 20).

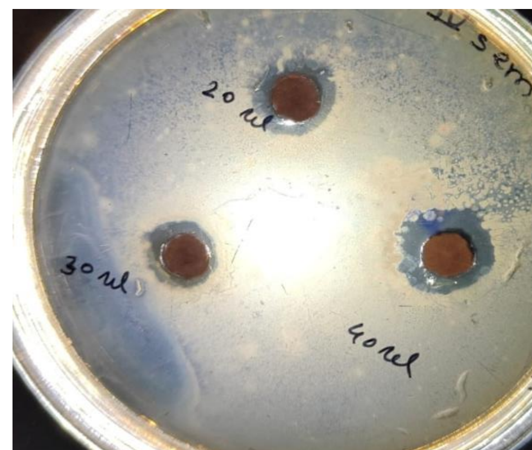

Fig. 20 Antibacterial activity showed zones of inhibition for 20 µl, 30 µl, and 40 µl of crude metabolite against *Staphylococcus aureus.*

Table 3. Effect of Crude Metabolite Concentration on Antimicrobial Activity

Crude metabolites concentration (µl)	Zone of inhibition (mm)
20 µl	0.2mm
30 µl	0.25mm
40 µl	0.45mm

Footnote: µL (microliter) represents the volume of crude metabolites used, and mm (millimeter) denotes the diameter of the zone of inhibition.

4. DISCUSSION

The significance of researching unconventional bacterial strains for their ability to produce antibiotics has been demonstrated by the study we conducted of *Escherichia fergusonii*. The methodologies applied, which include collecting soil samples, isolating bacterial colonies, and executing 16S rRNA sequencing for molecular identification, adhere to standard protocols, ensuring the consistency of the observations. The identification of *E. fergusonii* as a rod-shaped, Gram-negative bacterium with moderate antibacterial activity against Staphylococcus aureus and *E. coli* is consistent with previous studies on its modest but substantial antimicrobial properties (Awais et al., 2007; Ram, 2014).

However, their inhibitory properties are not as effective as those produced by *Streptomyces* or comparable standard antibiotic-producing bacteria, the small zones of inhibition seen in primary and secondary screening indicates the potential of antibiotic a synthesis process. These results emphasize the importance of optimization, which was demonstrated through studies (Salim et al. 2017) and Singh et al. 2012). Lower temperatures and dark conditions significantly improved inhibitory zones, indicating that external variables play an essential part in metabolite growth, even though variations in pH and incubation times had no effect on antibacterial activity.

Utilizing butanol for extraction of secondary metabolites produced a crude extract having concentration-dependent antibacterial activity (Al-Reubay et al., 2023). The broadest zone of inhibition was produced by the highest amount (40 µl), supporting the effectiveness of butanol extraction in isolating compounds that are bioactive. The crude extract yield (0.071 g) emphasizes the necessity for further advancement in growing circumstances and methods of extraction in order to optimize yield.

All things considered, these results add to the comprehension of bacterial strains isolated from soil and their ability to produce antibiotics. Although *E. fergusonii* exhibits modest behavior, the study provides potential for additional investigation, especially into improving metabolite yield, maximizing growth conditions, and determining the precise bioactive compounds that provide it antibiotic properties. These findings correspond to alignment with international initiatives to address antibiotic resistance through the identification of novel antimicrobial medicines (Khattab et al., 2016; Nair et al., 2022).

5. CONCLUSION

Escherichia fergusonii was successfully isolated, identified, and its ability to produce antibiotics assessed from soil samples obtained from a university campus. The bacterium classification as a Gram-negative rod has been confirmed through biochemical analysis and 16S rRNA sequencing. The crude extract from butanol extraction showed concentration-dependent inhibition, signifying the existence of bioactive compounds, even though primary and secondary screenings showed moderate antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

Based on optimization studies, the production of secondary metabolites is significantly affected by environmental parameters including temperature and light phases, with dark incubation and lower temperatures increasing antibacterial activity. The results emphasize the potential of *E. fergusonii* as a source for novel antibacterial compounds, despite the crude extract's constrained yield.

This research enhances our understanding of the soil microbiology as an important source of bacteria that produce antibiotics and emphasizes the necessity of additional research and improvement of less commonly recognized bacterial strains. In order to address the pressing global issue of antibiotic resistance, more research focusing on the structural clarification of bioactive compounds and their modes of action could reveal up the possibilities to novel therapeutic applications.

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