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Identification and Analysis of Antimicrobial Activity in Filamentous Fungi from Soil Samples

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INTRODUCTION & AIM

Soil is a dynamic biological system, being considered the main system of biological diversity. In this system there is an average abundant life of approximately 10 thousand different species per gram of soil. Quantifying and isolating microbial species from soil samples represents a significant challenge due to the high density and microbial diversity in this environment. The discovery of penicillin by Alexander Fleming in 1929 was a milestone that boosted the use of microorganisms to combat infections [1]. The increasing resistance of microorganisms to antibiotics has highlighted the urgent need for new antimicrobial agents. Filamentous fungi, commonly found in soil, are known producers of bioactive compounds, including antimicrobial agents [2].

This study aimed to isolate and evaluate the antimicrobial activity of filamentous fungi found in soil samples, with the objective of identifying potential new sources of antimicrobial compounds that may offer alternatives to conventional antibiotics.

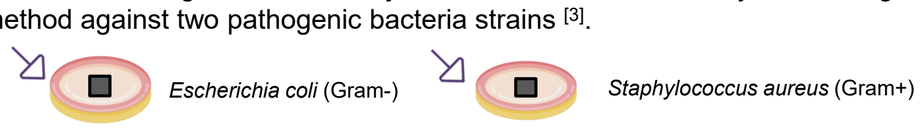
METHOD

1.Sample collection and isolation

Soil samples were collected from a biological garden on the Lusófona University Campus to isolate filamentous fungi using selective media Sabouraud Dextrose Agar with Chloramphenicol for 7 days at 37°C.

2.Diffusion test (method 1)

The isolated fungi were then subjected to antimicrobial activity tests using the agar well diffusion method against two pathogenic bacteria strains [3].



3. Extraction of bioactive compounds (method 2)

The isolated fungi were grown in Sabouraud Dextrose Agar liquid medium under 200 rpm, with an incubation time of 7 days at 25°C, for the subsequent extraction of bioactive compounds.

3.1.After the incubation period, centrifugation was performed to concentrate the compounds produced by the fungi.

3.2. To confirm the effectiveness of concentrated bioactive compounds the diffusion method was reused [3].

3.3. The antimicrobial activity of the active compounds was assessed by adding 50 µl of the active compounds to each well, followed by an incubation period of 24 hours at 37°C.

4. Fungal identification

4.1. The fungi that showed greater effectiveness in method 2 were selected for identification using DNA analyses.

4.2. To accurately identify the fungal species, DNA extraction and polymerase chain reaction (PCR) amplification were carried out.

4.3. The PCR products were purified and identified by Sanger Sequencing

4.4. The sequencing data was analyzed using the Nucleotide BLAST[4] algorithm to confirm the identities of the fungal isolates.

RESULTS & DISCUSSION

With the isolated fungi of interest, a plate diffusion test was carried out according to the official method 1, where it was possible to see the inhibition zone against the bacteria under study. The isolation of the target species was successful, as evidenced by the absence of contamination by other species in all the samples analyzed. Figure 1 clearly shows the inhibition zone, demonstrating that samples S1 and S2 have antimicrobial activity against *E. coli*. The same test was also carried out on a plate containing *S. aureus*, where inhibition zone were also shown, indicating antimicrobial activity. Each test was carried out in duplicate.

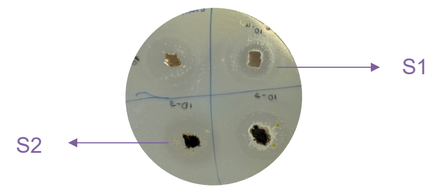


Figure 1: image of the diffusion test with the inhibition zone using *E. coli*

As shown in figure 2, the inhibition zone generated by the active compounds samples h a uniform diameter. The uniformity in the size of the inhibition zone indicates that, regardless of the fungal sample and microorganism tested, the active compounds show a similar pattern of antimicrobial efficacy. The figure shows the inhibition zone tested on *S. aureus and E. coli*. The compounds produced by growth in liquid media by the selected fungi were subsequently concentrated by centrifugation and their antibacterial efficacy confirmed, using the diffusion method by introducing 50ul of active compound into 4 wells produced by microorganism S1 and S2.

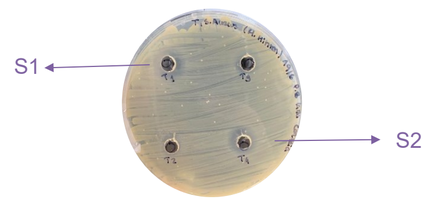


Figure 2: inhibition zone of fungi S1 and S2 using active compounds for *S. aureus*

After the tests were carried out with both *E. coli* and *S. aureus* and the sizes of the inhibition zone were determined, the results are shown in Table 1.

Table 1: diameter of inhibition zone using *E. coli* and *S. aureus*

Inhibition zone	Method 1		Method 2	
Microorganism	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
S1	2,4 (± 0,2cm)	1,8 (± 0,2cm)	1,5 (± 0,1cm)	
S2	2,3 (± 0,4cm)	1,1 (± 0,1cm)		

After isolating and growing fungi S1 and S2, they were observed by optical microscope (figure 3).

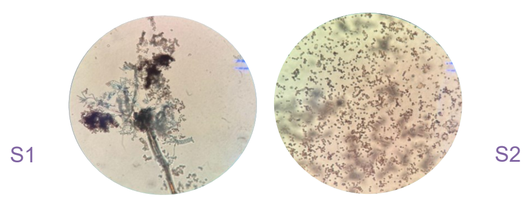


Figure 3: microscopic observation of fungi S1 and S2 under the microscope. Amp: 400x

After the PCR assay, sequencing was carried out, where the data was analyzed using the BLAST algorithm to confirm the identities of the isolated fungi (Table 2).

Table 2: Percent identity determine by Blast algorithm[4]

Microorganism	Percent identity
Penicillium pimiteouiense	*99,08%*
Aspergillus niger	99,65%

CONCLUSION

This study supports the potential of soil microbiota, particularly filamentous fungi, as a rich resource for discovering new antimicrobial compounds. The findings highlight the importance of further research to explore the mechanisms of action of these compounds and to develop them for clinical applications. The isolated fungi, namely *P. pimiteouiense* and *A. niger*, show promise as sources of new antimicrobial agents that could help combat antibiotic resistance and pathogenic bacteria.

REFERENCES

[1] Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, *6*(2), 71-79.

[2] WANDA F. CANAS FERREIRA JOAO CARLOS F. DE SOUSA. (1998). MICROBIOLOGIA. Lidel, edições técnicas.

[3] Clinical and Laboratory Standards Institute (2006). Methods for dilution antimicrobial susceptibility tests for bacteria. Approved Standard. CLSI publication nº M100-S16: Wayne, PA.

[4] http://blast.ncbi.nlm.nih.gov/Blast.cgi accessed on 10 September 2024.