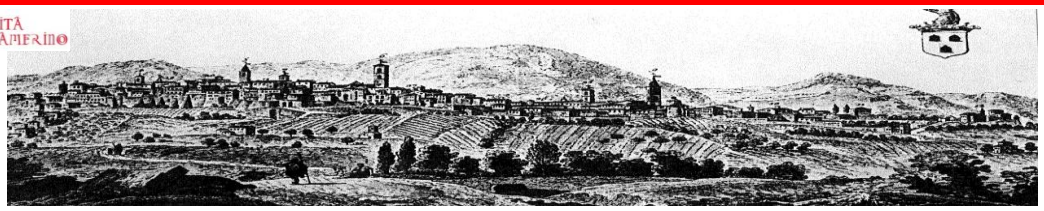

Screening an Archetypal Collection of Microorganisms for the Presence of Unexplored Antimicrobial Compounds

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State of the Art

According to WHO estimates, more than 700.000 people die every year due to drug-resistant infections. For this reason, the development and characterization of new drugs that can bypass the resistance mechanisms of pathogenic microorganisms are highly required. The design of advanced strategies for drug discovery and the promotion of early-stage research activities are the critical starting points to overcome the problem of antimicrobial resistance.

Aim of the Study

The objectives of this work are defined by three strategic points:

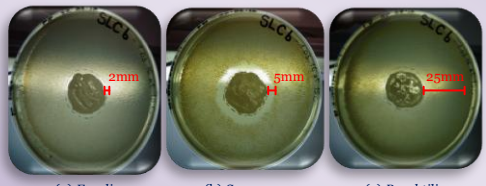
a) Set-up of a library of microorganisms collected from different environmental settings;
b) Screening of the collection of microorganisms for growth inhibition of three bacteria (tester species);
c) Characterization of newly discovered secondary metabolites with antibacterial activity.

Materials and Methods

In this study, more than 900 microorganisms were isolated from different environmental samples. They are maintained in an archetypal culture collection at the University of Camerino (Unicam).
A Gram- (*Escherichia coli*) and two Gram+ microorganisms (*Bacillus subtilis* and *Staphylococcus aureus*) were used as tester species for the antibacterial activity investigation on approximately 300 strains of the culture collection. Each microorganism was streaked in a circle at the center of three plates of solid medium. Once the growth was markedly detectable, the three tester species were transferred on these plates by replica plating and the size of the inhibition zone was regularly monitored and measured. Subsequently, to identify the compound(s) responsible for the inhibitory activity, supernatants obtained from the fermentation media were taken at different time points over a period of about two weeks. These samples were subjected to fractionation by reverse-phase chromatography and all the eluted compounds were evaluated by disk-diffusion assay.

Antibacterial Activity Results

Using this straightforward approach, we identified several microorganisms able to produce secondary metabolites with antimicrobial activity. Here, we present the results of one of the active strains, MES18, isolated from a soil sample next to the Lake of Cingoli in Central Italy. Based on 16S rRNA sequence analysis, MES18 has been placed in the *Bacillus spp*. group.



(a) *E. coli* (b) *S. aureus* (c) *B. subtilis*

Fig 1. Halo inhibition assay. LB agar plates with the active microorganism, MES18, tested on three tester species. The inhibition zone is shown by a red line.

After five days of incubation, the inhibition displayed by MES18 reached 2mm in the case of *Escherichia coli* (Fig 1a), 5mm in the case of *Staphylococcus aureus* (Fig 1b) and a notably reduced growth of *Bacillus subtilis*, which appears fuzzy over a zone of at least 25mm (Fig 1c).

To investigate further this activity against *B. subtilis*, 20 ml of supernatant of MES18 cell culture were fractionated on reversed-phase column. This procedure allowed us to concentrate the metabolites, whose antibacterial activity displayed by fractions 3-5 is shown in Fig 2.

Fig 2. Antimicrobial susceptibility test. Zones of inhibition of the tester strain *B. subtilis* were observed in three out of five disks containing fractions of reversed-phase column.

Research Perspectives

Further analysis aimed at identifying the physical-chemical properties of the compound(s) produced by MES18 (as well as of all the active microorganisms present in the culture collection) are currently ongoing. A combination of HPLC and mass-spectrometry techniques will be used to separate the bioactive compounds and to characterize their mass with high accuracy.

Conclusions

In this work, we have presented an investigation carried out on our collection of microorganisms (bacteria, fungi, algae) as part of an ongoing project on the identification of metabolites for bio-industrial use.
The effect of metabolites produced by MES18 on three tester species is presented as a proof-of-concept study to highlight the potential repertoire of molecules synthesized by microorganisms.
The isolation and characterization of these active molecules is a key step to advance the research on new lead compounds that could be used as template for the development of new drugs.
In light of the global challenge of the antibiotic-resistant infections, this research activity should be regarded as a 'categorical imperative' (in Kant's words).

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