Drug Discovery From Soil Bacteria

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Introduction

- Soil bacteria, particularly *Streptomyces,* are prolific producers of antibiotics.
- Our aim is to discover bacterial-derived new antifungal agents against plant pathogens.
- Strains used: MBRL-10 and MBRL-574, from Manipur, India.
- The strains were collected from The Hundung Cement Factory, abandoned as of 2003 due to the swampy location.

Examples of Plant Fungal Infections

Rhizoctonia solani

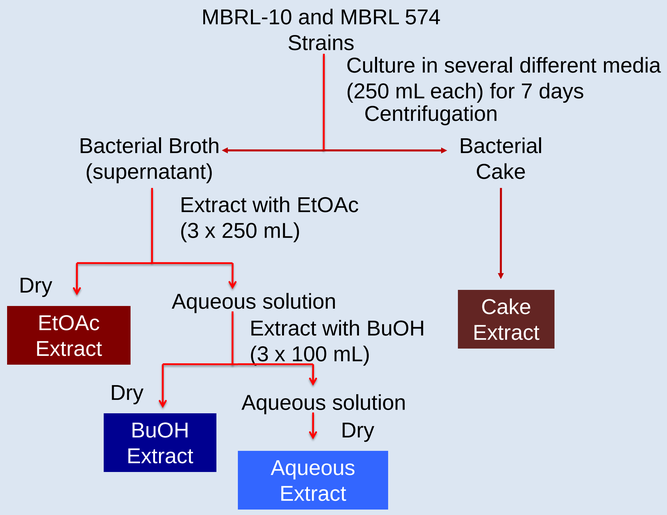
Bipolaris oryzae

Fusarium oxysporum

***Curvularia* sp.**

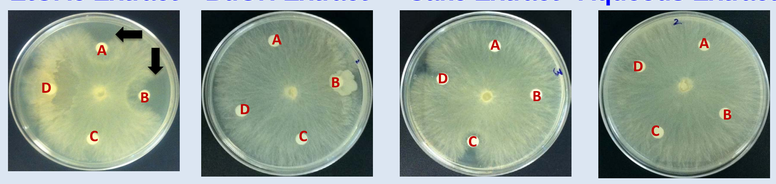
Preliminary Antifungal Activity Assays

Extraction Scheme



Antifungal Activity Assay Against *R. solani*

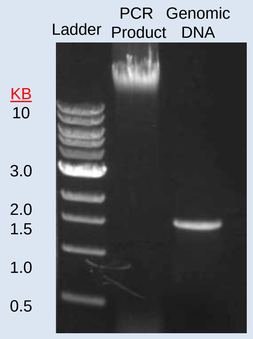
EtOAc Extract **BuOH Extract** **Cake Extract** **Aqueous Extract**



A: MBRL-10 (Gauze); **B**: MBRL-10 (YMG); **C**: MBRL-574 (YMG); **D**: MBRL-574 (SCN)

Classification of Bacterial Strain

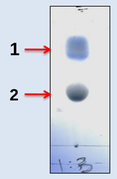
- **DNA Extraction**
- **PCR amplification**
- **Electrophoresis**
- **Purification using a gel extraction kit**
- **Concentration measurement**
- **Sent to be sequenced**
- ***Streptomyces* genus identified**



Further Analysis of MBRL-10

- ***Scaling Up of Liquid Culture:***
  -YMG medium (4 L) was inoculated with the MBRL-10 strain
- ***Supernatant Extraction:***
  -EtOAc extraction
  -Crude extract yield: 40 mg/L
- ***Crude Extract Analysis:***
  -Thin Layer Chromatography, two spots observed
  -The two "compounds" were isolated using preparative TLC
- ***Antifungal Activity Test of Compound 1 & 2***
  -Used plant pathogenic fungus *Rhizoctonia solani* as test organism
  -Inhibition noted by compound 2
- ***Purification of Compound 2 from EtOAc Extract***
  -Silica Gel Column Chromatography; $CHCl_3$-MeOH (1:5)
  -Analyzed fractions using TLC
    -Observed TLC under UV light
    -Combined appropriate fractions
    -Yield: 2 mg/L

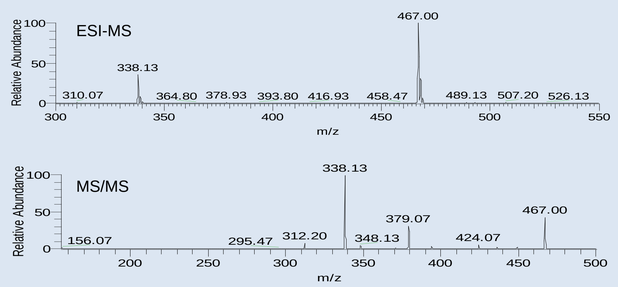
TLC
$CHCl_3$-MeOH (1:3)



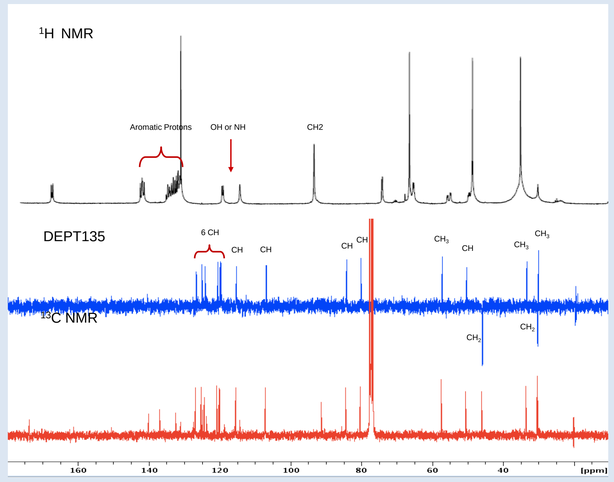
Antifungal Assay

Structure Elucidation

Mass Spectrometry Analysis of Compound 2



Nuclear Magnetic Resonance

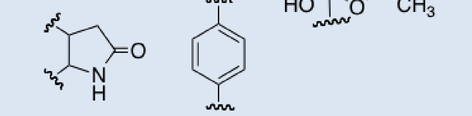


Proposed Partial Structure of Compound 2

- Based on 1D and 2D NMR (^{1}H-^{1}H COSY, HSQC, HMBC)

Known Number of Groups:

- CH_3: 3
- CH_2: 2
- CH: 11
- 4˚ C: 9



Conclusion

- Strain MBRL-10 was found to produce compounds with strong antifungal activity against *Rhizoctonia solani*.
- Among the media tested, YMG was the best medium for the production of the antifungal compound.
- Within the crude EtOAc extract two spots were observed on TLC plates, and spot #2 was active against *R. solani*.
- Compound 2 was not active however, against pathogenic bacteria.
- At this stage, we propose only partial structures of compound 2, as further analysis is necessary to determine the complete structure of the compound.

Acknowledgments

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Subsurface Biosphere Initiative
Research and education focused on life below Earth's surface.