Soil Microorganisms with Activity Against Mycobacteria

Results 1: Spectrum of action of Candidate UD

Method: The candidate strain was inoculated as a stripe across the center of a B. subtilis 461 plate; 461 plates were uniformly scored for inhibition of growth of two dozen common lab bacteria (Gram negative and positive) by agar streaking with double endosmosis 

Results: In Table 1 are shown the growth-inhibitory effects of candidate UD against 24 bacterial strains. The strain was very effective against a variety of bacterial strains, including both Gram-negative and Gram-positive bacteria. The results demonstrated the potential of candidate UD as a new antibiotic with broad-spectrum activity against a wide range of bacterial species.

Results 2: Partial Purification of active compound from Strain UD

Method: Preparation of conditioned medium: Cultures of Candidate UD were grown in 25 cm plate of Middlebrook 7H9 broth for 7 days. The conditioned medium was collected after 7 days and sterilized by autoclaving.

Results: The conditioned medium was dialyzed against 0.15 M NaCl and subjected to DEAE-cellulose chromatography. The active compound was eluted at 0.2 M NaCl, indicating a molecular weight of less than 10,000 Da.

Results 3: Characterization of active compound from Strain UD

Method: A. Estimating molecular weight of active compound: The conditioned medium was subjected to size-exclusion chromatography on a Sephadex G-50 column, and the active compound was eluted at 1500 Da. The results suggested a molecular weight of less than 1500 Da.

Method: B. Temperature Sensitivity of active compound: The conditioned medium was incubated for 30 min at 37°C, 55°C, and 80°C, and the inhibitory activity was measured against B. subtilis 461. The inhibitory activity was lost at 80°C, indicating a high temperature sensitivity.

Results: C. Protease Sensitivity of active compound

Method: The conditioned medium was treated with three different proteases: pronase, proteinase K, and bacterial elastase. The results showed that the active compound was sensitive to bacterial elastase but resistant to pronase and proteinase K.

Conclusion: Our lab collection of soil bacteria which secrete broad spectrum antimicrobial compounds (eg, polyketides) with the possibility that candidate UD is secreting cerexin A or a variant thereof.

Acknowledgements

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Poster A-127

Isolation and Characterization of Antibacterial Compounds from Soil Microorganisms with Activity Against Mycobacteria

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Abstract

Full-length proteases, such as trypsin and chymotrypsin, can be used as tools for the study of molecular mechanisms of protein degradation. These enzymes are non-specific in their digestion patterns, meaning they can degrade almost any protein present in the cell. By examining the inhibitory activity of the candidate UD strain against a variety of proteases, we can determine its specificity and potential selectivity for certain substrates.

Introduction

Soil microorganisms are known to produce antibiotic compounds with high potential for the treatment of various diseases. Antibiotics are used extensively to combat bacterial infections, and their use can lead to the development of antibiotic-resistant strains. Our lab seeks new antibiotics from natural sources (soil bacteria) to target a variety of soil-borne diseases. We have discovered several candidate soil bacteria with activity against mycobacteria.

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