CHARACTERIZATION OF ANTIFUNGAL COMPOUNDS PRODUCED BY *BACILLUS MACERANS* ANTAGONISTIC TO FUNGAL INVADERS OF COCONUT

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This is a continuation of a study on the feasibility of using microorganisms isolated from food sources to control fungal invaders of coconut kernels. In this study, the antifungal compounds produced by *B. macerans* were analysed. The bacterium was cultured in potato dextrose broth at room temperature (48 h), cells were removed by centrifuging (x 1000 g, for 20 min.) and the supernatents were and freeze dried. The antifungal compounds were extracted into dichloromethane and the solvent was evaporated in a rotary evaporator followed by drying in a vacuum oven. Fractions were further purified by medium pressure liquid chromatography (MPLC). The following solvent combination was used in succession for extraction through a packed silica gel (Merck 1.09385.1000, particle size 0.040-0.063 mm, 230-400 mesh ASTM silicagel 60) column; methanol: dichloromethane 4:96 (v/v), 8:92, 10:90, 20:80, 40:60, 50:50.

Aliquots (approx. 20 □l) of each fraction collected were spotted on TLC plates (8 x 5 cm^2). The following fractions, in the test tube numbers specified, were developed in the solvent systems as given below; test tubes 1-17, methanol:dichloromethane (4:96,v/v), test tubes 17-32, methanol:dichloromethane (10:90,v/v) and test tubes 32-49, methanol:dichloromethane (40:60,v/v). The TLC plates were allowed to dry and observed under UV light (314 nm). The Rf values of UV active compounds were noted. They were then sprayed with anisaldehyde reagent and heated on a hot plate (approx. 110 °C for 60 seconds). The plates were observed for colouration and the corresponding Rf values were noted. Contents of test tubes corresponding to fractions having similar Rf value, similar colour to anisaldehyde, and similar UV activity were combined and subjected to TLC *Cladosporium* bioassay. Two active compounds were observed. These were further purified by another MPLC and then purified using thin layer chromatography (TLC). Chemical analysis was by gas chromatography mass spectroscopy (GC-MS) and H'NMR spectroscopy (Varian mercury 300). The two fractions were analysed using Varian Saturn 2000 Mass Spectrometer in electronic ionization (EI) mode which is coupled with Varian 3800 Gas Chromatography.

The major antifungal compound detected may be a derivative of 1,2-Benzenedicarboxylic acid and diphenyl ether. However this information was obtained NIST Mass Spectra library. The identity of these compounds can only be ascertained when other spectral data such as NMR, UV and IR are available.

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