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47

Study of antimicrobial derivative from marine *Streptomyces* species isolated from west coast of India

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Objectives Marine microorganisms have become an important point of study in the search for novel microbial natural products. Actinomycetes are a group of bacteria, which possess many important and interesting features.

Method Twenty marine sediment samples were collected from different sites of Alibag and Janjira coast of Maharashtra and Goa at the time of low tide. The surface layers of sediments were removed and about 100 g of central portions of sediments were aseptically transferred into sterile plastic bags. These samples were kept in a cold box containing ice (5 °C) and transferred to the laboratory. Marine samples were preheated (41 °C for 30, 60 days) and, by using selective media, marine actinomycetes were isolated.

Results Out of 80 strains, 35 showed antimicrobial activity determined by cylinder plate method and cup plate method. *Streptomyces* species was selected for further studies based on spectrum of species. This species was identified based on morphological, cultural, physiological and biochemical characteristics, presence of LL-diaminopimelic acid (DAP) in the cell wall and spore characteristics using scanning electron microscopy (SEM). The antibiotic was produced in maltose yeast extract medium prepared in artificial seawater and showed its maximum activity after 7 days fermentation at 28 °C, 150 rpm and pH 7. After 7 days, broth was centrifuged at 10 000 rpm and 40 °C for 15 min to separate the mycelial biomass. After centrifugation the supernatant was extracted three times with half quantity of ethyl acetate. The organic phase was collected and ethyl acetate was removed by subjecting the sample to rotating evaporator at 40 °C and 50 rpm. The concentrated residue was collected and tested for antimicrobial activity against sensitive bacteria and fungi by agar well method. Crude antibiotic residue (1.62 g) was tested for number of components by using thin layer chromatography plates (Merck, 60 gel) using benzene:methanol (90:10) system. Purification of antibiotic was carried out by column chromatography using silica gel of column chromatography grade. The pure antibiotic sample was dissolved (3 mg for ^{1}H NMR and 10 mg for ^{13}C NMR) in 2–3 mL of $CDCl_3$ and analysed by NMR (500 MH_2, Varion, USA) and peaks were identified for antibiotic. The pure antibiotic sample was subjected to ^{1}H NMR, ^{13}C NMR and D_2O exchange, and DEPT spectra. The GCMS spectrum was obtained from HP CHEM instrument (GC-LC/MS). The antibiotic has a molecular formula of $C_{10}H_{13}NO_3$. By using UV, ^{1}H NMR, ^{13}C NMR and mass spectra, the structure of the compound was identified as N-[2-(4-hydroxyphenyl)ethyl] acetamide (Figure 1). The acetamide antibiotic exhibited broad antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi. The minimum inhibitory concentration (MIC) of acetamide antibiotic against different microorganisms ranged from 30 to 105 μg/mL determined by cup plate method. This is the first report of N-[2-(4-hydroxyphenyl) ethyl] acetamide antibiotic production from marine *Streptomyces* species.

Conclusion This study clearly indicates that marine sediment samples from Alibag, Janjira and Goa are potent sources for the isolation of bioactive actinomycetes.

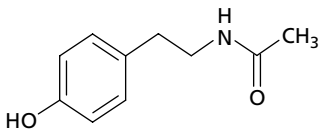

Figure 1 Structure of antibiotic.

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48

Liposomal delivery of antimicrobial natural products

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Objectives In addition to antioxidant and anti-inflammatory properties, many essential plant oils have antimicrobial activity against bacteria and fungi. One of the most popular essential oils is tea tree oil (TTO), which contains more than one hundred compounds, but mainly monoterpenes, sesquiterpenes and their alcohol derivatives (Cox et al 2000). Monoterpenes can partition into the cell membrane resulting in bilayer expansion and increased fluidity, as well as inhibition of membrane-embedded enzymes. This study aims to investigate: 1) the stability of oil-in-water (o/w) TTO-poly(vinyl alcohol) [PVA] emulsions; 2) the preparation of liposomes encapsulating TTO-PVA emulsions; and 3) the antimicrobial efficacy of liposomal TTO-PVA emulsions against three different microorganisms: gram negative (*Pseudomonas aeruginosa*) and gram positive bacteria (*Staphylococcus aureus*) as well as a yeast (*Candida albicans*).

Methods Emulsions of 5%v/v TTO (Holland & Barrett, UK) in PVA (Sigma, UK) were prepared from three molecular weights of PVA (13–23 kDa, 30–70 kDa and 70–100 kDa) at three different concentrations (1, 5 and 10%w/v) by vigorous stirring. After preparation, emulsions were transferred to clear glass measuring cylinders and maintained at room temperature; the time taken for the oil and aqueous phases to separate was determined for each formulation. Liposomes composed of Phosphatidylcholine (Lipoid, Germany) and Cholesterol (Sigma, UK) [2:1 molar ratio] were prepared by the Reverse-Phase Evaporation Vesicle method (Szoka & Papahadjopoulos 1978) using the most stable TTO-PVA emulsion as the aqueous phase. Antimicrobial efficacy was assessed with microorganisms cultured routinely at 37 °C for 24 h using 2% MEA for *C. albicans* and TSB for *P. aeruginosa* and *S. aureus*. Culture medium was inoculated using 1 mL of overnight cultures with the following volumes of liposomal TTO-PVA emulsions: *C. albicans* – 2 mL; *P. aeruginosa* – 16 mL and *S. aureus* – 4 mL. All experiments were conducted in triplicate and the Minimum Inhibitory Concentration (MIC) of TTO required was determined for each microorganism.

Results PVA 10%w/v, TTO emulsions remained stable with no cracking after four days incubation. With 5%w/v PVA, the 30–70 kDa and 70–100 kDa emulsions cracked after 11 and 13 min, respectively; however the 13–23 kDa emulsion remained stable for four days. At 1%w/v PVA, stability decreased concurrently with cracking occurring after 4 (30–70 kDa) and 7 (70–100 kDa) mins, respectively; again the 13–23 kDa emulsion remained stable for the duration of the experiment. Antifungal activity of liposomal TTO-PVA emulsions showed that *C. albicans* is the most susceptible organism with an MIC level of 0.1 after 5 h. *P. aeruginosa* showed median susceptibility to encapsulated TTO with an MIC level of 0.4 between 1 and 3 h. *S. aureus* was the most resistant organism with an MIC level of 0.5 after 5 h.

Conclusions Microencapsulating TTO within liposomes in an o/w emulsion, resulted in significant decreases in MIC counts within 5 h of administration against three different micro-organisms. These results indicate the potential application of liposomally entrapped TTO emulsions for treatment and prolonged control of bacterial or yeast infection.

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