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# ISOLATION, PURIFICATION, STRUCTURAL ELUCIDATION AND ANTIMICROBIAL ACTIVITIES OF KOCUMARIN, A NOVEL ANTIBIOTIC ISOLATED FROM ACTINOBACTERIUM KOCURIA MARINA CMG S2 ASSOCIATED WITH THE BROWN SEAWEED PELVETIA CANALICULATA

**Farid Menaa**

Stem Cells Fluorotronics, Inc USA

**Aim:** Screening of seaweed-associated bacteria capable of producing antimicrobials.

**Methods & Results:** Fifteen microbial strains, associated to the brown seaweed *Pelvetia canaliculata* (Linnaeus) attached to the rocks of Sonmiani Beach (Karachi, Pakistan), were screened. Crude extract filtrates of CMG S2 strain grew on Zobell marine agar (ZMA) had the most remarkable antimicrobial activity. Based on its phenotypic aspects (e.g. Gram-positive, microcoid form), biochemical characteristics (e.g. halotolerance) and genetic analyses, CMG S2 is identified as a putatively new *Kocuria marina* type strain belonging to the actinobacteria's class and micrococcaceae family. Thereby, the nucleotide sequence analysis of its full-length 16S ribosomal ribonucleic acid (rRNA) gene (GenBank accession number EU073966.1) displayed highest identity (i.e. 99%) and score (2630) with *K. marina* KMM 3905. Phylogenetic trees analysis using the neighbor-joining method showed closest evolutionary distance of CMG S2 with KMM 3905 strain and *K. carniphila* (DC2201) species. Interestingly, a unique ultraviolet (UV)-bioactive compound was purified from CMG S2 crude extracts by flash silica gel column and thin-layer chromatography (TLC) techniques. Its chemical structure was unravelled as 4-[(Z)-2 phenyl ethenyl] benzoic acid (PEBA, later named kocumarin) by nuclear magnetic resonance (NMR) spectroscopy techniques. Importantly, kocumarin demonstrated prominent and rapid growth inhibition against all tested fungi and pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), with a minimal fungal inhibitory concentration (MFC) of 15-25 µg/mL and a minimal (bacterial) inhibitory concentration (MIC) of 10-15 µg/mL.

dr.fmenaa@gmail.com