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ABSTRACTS

IN-VITRO DEVELOPMENT AND CHARACTERISATION OF BIOFILMS USING MARINE MICROORGANISMS ISOLATED FROM BIOFOULING SITES

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Diverse marine microorganisms colonise both abiotic and biotic submerged surfaces to form biofilms. When biofilms develop on the submerged parts of ships, they form biofouling, which poses a significant challenge to the maritime industry. In the present study, biofilms were developed using marine microorganisms isolated from biofouling sites, to identify natural methods to disrupt these biofilms and characterise the biochemical composition of their extracellular polymeric substances (EPS) for potential ecological, industrial, and medicinal applications. Samples were collected from ship hulls affected by biofouling at the Mirissa Fisheries Harbour in Weligama, Sri Lanka. Six (06) marine bacteria were isolated using ZoBell marine agar (ZMA) under standardised conditions, designated as A, B, C, D, E, and F, and were further characterised by biochemical tests. The genomic DNA was extracted from marine bacterial samples using the Presto™ Mini gDNA bacteria kit (GBB100) for their molecular identification. Fungal-bacterial biofilms (FBBs) were developed by co-culturing each bacterium with *Aspergillus niger* in ZoBell marine broth (ZMB) and Yeast mannitol broth (YMB). Four (04) treatments were applied using the Crystal violet assay in a 96-well microtiter plate as follows: Treatment 1 and Treatment 2 involved simultaneous addition of marine bacteria and *A. niger* to YMB, and ZMB, respectively. Treatment 3 and Treatment 4: *A. niger* was introduced first, followed by marine bacteria after 24 hours to YMB, and ZMB, respectively. Each bacterium and *A. niger* were tested in triplicate to ensure the reliability of the data. Qualitative evaluations of biofilms were conducted using microscopy and the Congo red assay. The EPS of each FBB combination were extracted using a physicochemical method that involves heating, ultrasonication, and centrifugation. The four (04) treatments showed significant differences in microbial biomass based on a one-way ANOVA analysis (p = 0.008), with Treatment 1 displaying the highest optical density value. In Treatment 1, bacterium E exhibited the highest optical density value of 3.40, while the other isolates, A, B, C, D, and F exhibited optical density values of 3.06, 2.81, 2.57, 2.87, and 2.34, respectively (P = 0.582). Post-hoc Turkey's HSD test results revealed that the microbial biomass of Treatment 1 and Treatment 3 was significantly different (p = 0.009). This study demonstrated that the studied marine bacteria are capable of forming FBBs associated with *A. niger* under laboratory conditions. The identification of the individual microorganisms and the characterisation of their biofilm-EPS biochemicals are still ongoing processes.

Keywords: *Biofouling, EPS, Fungal-bacterial associations, Marine biofilms*