SUMMARY

Ten isolates of actinomycete were obtained from the marine sediment collected from Coromandel Coast region of the Bay of Bengal, Tamilnadu, grown on starch yeast extract peptone –sea water agar plates. The selected isolates were screened for the production of melanin pigment using tyrosine agar. Out of 10 isolates, only five strains engendered melanin, which was designated as BI 241 to BI 245. The comparison of the biochemical and morphological characteristics of the isolates with those reported in Bergey’s Manual of Systematic Bacteriology revealed its identity as Streptomyces sp. In the physiological characterization, all isolates were competent to get good growth at pH 7, temperatures viz. 30-40°C and 4% NaCl. All the isolates utilised different carbon and nitrogen sources. An isolate BI 244 was selected as physiologically efficient and potential isolates for the maximum synthesis of melanin. The combination of morphological, biochemical and molecular approach was found to be advantageous for the authentic identification of new strain of marine Streptomyces sp. BI 244.

The significantly highest level of pigment formation by Streptomyces sp. BI 244 was detected with starch as carbon source, L-tyrosine at 2.7 g/L as nitrogen source, NaCl at 4% concentration, pH 7.0, temperature 30°C and for 6th day of incubation. In the Plackett- Burman design, four factors such as pH, starch, sea water and L-tyrosine are significant process variables and media components and therefore these four factors were taken up for optimization of media components using response surface methodology. The optimum values of the variables in actual unit was predicted as pH (7.50), starch (26.59g/L), sea water (601 ml/L), and L-tyrosine (2.50g/L) with the predicted maximum pigment production of 14.14µg/ml of fermented media. The organism produced 14.22 µg/ml confirming the validity of model.

Extracted melanin pigments gave one band with Rf value of 0.90 using using solvent system of petroleum ether, ethyl acetate, (95%) ethanol, ammonia (4: 4: 6: 1, v/v) After the preparative TLC, the quantity of partially purified compound was found to be 28 mg/100ml of the statistically optimized media. Mostly all the physical and chemical characters were similar with standard melanin.
The pigment showed more inhibitory activity against Gram-positive organisms than Gram-negative organisms. The IC₅₀ value of DPPH, scavenging of Hydrogen peroxide, Nitric oxide, and superoxide radical of melanin were found to be 100.70µg/mL, 129.91 µg/mL, 103.54µg/mL and 91.77µg/mL respectively. Total reducing power showed 0.3 absorbance units at concentrations of 100µg/ml.

The IC50 value of pigment on HeLa cells under MTT assay was 1962.8 ppm after treatment for 65h incubation. The melanin concentration of 30,000 and 15,000 ppm showed 94.46 and 90.88 mU/ml respectively for maximum lactate dehydrogenase leakage. When stained with a nuclear fluorochrome Hoechst 33342, HeLa cells treated with melanin pigments showed nuclear fragmentation, shrinkage of cell nucleus, with condensation of the chromatin (karyopyknosis) which were the characteristic features of apoptotic morphology.

The results confirm that marine sediments are a good source of melanin producing actinomycetes. Thus, pigmented *Streptomyces* sp. BI 244 reveals tremendous potential as an organism producing melanin for antimicrobials, anti-oxidants, and anti-tumour compounds. To exploit these findings for human welfare, it is necessary to carry out clinical trials and strategies for optimization of large-scale production of novel bioactive compounds.

Clinically prospecting of the marine environment for microorganisms is a promising inspiration of novel metabolites with yet unexploited potential. Coromandel Coast Region of the Bay of Bengal, Tamilnadu, India is blessed with a tropical climate and vast coastline that can be utilized to isolate useful microorganisms that will be of immense benefit to human kind. Thus, the screening of bioactive marine products from marine actinomycetes for human welfare is highly auspicious and couturier rewardable.