A mangrove actinobacterium of Sundarbans: Taxonomical identification, exploration of metabolite production and biological activities.

Multiple drug resistance (MDR) is now a serious concern to the society and health system worldwide. The multidrug resistance may cause the death of ten million people as well as immense economic loss in the year in 2050(O'Neill, 2014). Several Gram-positive and Gram-negative organisms like *Escherichia coli, Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* cause serious infections to the people and cause death as there is no effective treatment due to the drug resistance or multidrug resistance (MDR). The important fact is that the discovery of new antibiotics has been declining for several years whereas antibiotic resistance is a rising phenomenon. This leads to the need for new antibiotics or secondary metabolites with the new mechanism of action. Several approaches are required to halt the anti-microbial resistance phenomena, among this one important event is the discovery of new antibiotics (Simpkin et al. 2017). Presently, the discovery of antibiotics invites the idea of the search of new microbes from the new ecology or habitats like the mangrove forest (Amrita et al. 2012), ocean (Thornburg et al 2010), desert (Bull andAsenjo, 2013) etc. Mangroves ecosystem are present in the tropical and sub-tropical region (Holguin et al. 2001), and are distinctive in nature due to variable factors like high salinity, high moisture content etc.(Wu and Jiang, 2012). Recent research has shown that the mangrove forest is the source of the numerous new microorganisms which produced several new or novel bioactive compounds including

antibiotics due to its unique ecological niche (Xu et al. 2014).World's largest tidal mangrove forest, Sundarbans, lies in the delta of Ganges, Brahmaputra and Meghna rivers and is an enormously rich biodiversity region. Till date the microbial diversity of the Sundarbans has not been studied in details, only a few groups endeavored to explore the cultureable and un-cultureable microorganisms. The researcher found the presence of the Actinomycetes in Sundarbans (Sengupta et al. 2015) and one novel species *Streptomyces sundarbansensis* sp. nov. discovered by Arumugam et al. (2011). The well-known fact is actinomycetes produces the maximum of the antibiotics and among this 80 % of antibiotics are produced by the genus *Streptomyces* (Pimentel-Elardo et al. 2010). The identification of the microbes is the important and primary task to explore the diversity or for the discovery of the antibiotics. Identification of the genus *Streptomyces* is difficult as this genus is heavily congested. Identification and characterization can be undertaken using different methods like morphological, physiological, biochemical, chemotaxonomical and molecular approach. The polyphasic taxonomic methods are currently used for the identification of the *Streptomyces* genus.

In this context, the proposed objectives- are as follows (1) Complete identification of an actinobacterium by polyphasic taxonomic approach isolated from the Sundarbans mangrove forest.

(2) Exploration of metabolites production of the bacterial strain MS 3/20 using analytical tools. (3) Investigation of antimicrobial property by considering minimum

inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), and probable mechanism of action of the extract.

We have fulfilled all the proposed objectives. (1) The strain MS 3/20^T is identified as *Streptomyces euryhalinus* sp. nov. by applying polyphasic taxonomic approach. The 16S rRNA gene sequence showed 100% similarity with four species and more than 99% similarity with the other seven strains which creates ambiguity to the identification. Strain MS 3/20^T distinguished from closely related species on the basis of low levels of DNA-DNA relatedness (27.24-53.79%), unique banding pattern in random amplified polymorphic DNA-PCR amplification (RAPD-PCR) and the distinctive MALDI-TOF/MS profile of whole-cell proteins. Strain MS 3/20^T shown growth at 0-20% NaCl whereas other reference strains failed to grow which is an important distinction for establishing the strain as a novel species. Strain was showed susceptibility to ampicillin, but other reference strains are resistant. The spore chain of MS 3/20^T was a retinaculum-apertum, which was distinct from the spore chains of phylogenetically close relatives (spiral to flexuous). The strain has exhibited a difference in spore surface property, the difference in aerial and myceliumcolor after growth at different International Streptomyces Project Medium (ISP). Other physiological, biochemical and chemotaxonomical properties of strain shown significant differences with the phylogenetically close relatives. Strain MS 3/20^T is a Gram-positive, aerobic, non-motile actinomycetes and named as *Streptomyces euryhalinus* sp. nov.

(2) The ethyl acetate extract of the strain MS 3/20^{T} showed the presence of 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Dibutyl phthalate, Diisooctyl phthalate, Phenanthrene etc which are known antimicrobial, antifungal, antibiofilm and antioxidant compounds.

(3) The extract was evaluated for its antimicrobial activity using *S. aureus* MTCC 2940 and *E. coli* MTCC-1195 as organisms which showed minimum inhibitory concentration (MIC) of 1µg/mL and 2 µg/mL, respectively. The MIC of the extract against *C. albicans* MTCC 227 is 2µg/mL. The minimum bactericidal concentration (MBC) evaluated is 4 µg/ml and 8 µg/ml against *S. aureus* MTCC 2940 and *E. Coli* MTCC-1195, respectively. The minimum fungicidal concentration determined (MFC) is 6 µg/ml against *C. albicans* MTCC 227. The extract was exhibited the cell membrane damage property and also causes an alteration in inner-membrane permeability. The bacterial membrane damage could be measured by the release of UV absorbing materials (260 nm) (Li et al. 2014) like intracellular components. At 2 MIC concentration extract was displayed highest membrane damage ability against both *S. aureus* MTCC 2940 *and E. Coli* MTCC-1195. The extract displayed an increase of crystal violet uptake from 18.59 % (in control) to 74.49 % at 2 MIC concentrations in the case of *E. coli* whereas in the case of *S. aureus* the uptake of crystal violet increased from 19.09 % (in control) to 79.63 % after treatment with extract which indicates the membrane damage and alteration in the membrane permeability. This alteration or damage in membrane permeability against Gram-positive and Gram-negative bacteria evidenced after the treatment with extract may be

corroborated with its probable mechanism of action. The extract at 2 MIC dose exhibited 78. 49 % (±0.037) inhibition in the biofilm formation and at MIC concentration extract causes 62.99% (±0.049) inhibition in biofilm formation against *S. aureus*. The extract showed inhibition of biofilm formation of 53.64% (±0.60) and 67.90% (± 0.027) at MIC and 2 MIC dose, respectively, against *E. coli*. The extract significantly reduced the hemolysis of erythrocytes at 2 MIC dose against *S.aureus* [61.29% (±0.0009) to 18.28% (±0.0002)] and *E. coli* [68.88% (±0.002) to 19.6% (±0.001)] which could be attributed to the inhibition of virulence factor. The extract also inhibited the secretions of virulence factor secreted aspartyl proteinase (Sap) against *C. albicans* MTCC 227. The extract showed potent antioxidant activity (DPPH) by inhibiting free radical (82.22% ±0.68) at 100 µg/mL compared to standard ascorbic acid (91.85% ±0.30%). The extract was exhibited significant anti-inflammatory property and demonstrated 73.19% (±2.08) inhibition of inflammation at concentration 200 µg/ml which is significant though less than 88.73% (±0.89) inhibition observed in standard Diclofenac sodium. The isolation of the compound of antimicrobial property from the crude extract was attempted but EI mass spectra and NMR (^{1}H NMR, ^{13}C NMR) data showed the presence of impurities. The structural elucidation of the compound was not completed which remains as the future task to be fulfilled.

The present synopsis demonstrating the identification of a novel actinobacterium strain MS 3/20^{T} *Streptomyces euryhalinus* sp. nov isolated from the Lothian Island of the Indian Sundarbans. Metabolite production of the strain was explored by using GCMS

which have shown the presence of numerous bioactive compounds of different biological activities. The extract was displayed significant antibacterial and antifungal activity, and cell membrane damage property.