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Abstract No: BO-30

Isolation and identification of microplastic-inhabiting bacteria sampled from a wastewater treatment plant in Gannoruwa, Sri Lanka

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Effluents from wastewater treatment plants may serve as point sources that release microplastic particles into aquatic environments. These microplastic particles may provide solid matrices to attach detrimental bacteria in wastewater and facilitate their dissemination. Our research aimed to identify bacterial genera by isolating culturable bacteria associated with microplastic particles sampled from the wastewater treatment plant in Gannoruwa, Sri Lanka. Samples were collected twice, first in November 2023 and again in January 2024. During each sampling, four wastewater samples and three solid waste samples were obtained for analysis. The wastewater samples were taken from four treatment sites: the inflow, oxidation tank, UV treatment section, and return sludge unit. The solid waste samples included sludge and sand debris separated from the fine screening unit. The wastewater sample (100 mL) was filtered through a 5 mm metal sieve and glass fiber filter (0.7 µm pore size, Whatman GF/F). The residue on the filter paper was examined for microplastic particles using a light microscope. Microplastics were picked manually using sterilized needles and washed with 0.85% sterile NaCl solution. The solid sample (10 g) was dissolved in sterile distilled water (100 mL), and the mixture was allowed overnight for solid particles to settle. The microplastics floating on the water surface were filtered and separated. The isolated microplastic particles from each sample were suspended in 0.85% sterile NaCl solution and vortexed at 2,500 rpm for 1 minute. The solution was shaken at 170 rpm for an hour and was diluted 10-fold. Next, 100 µl aliquots from the diluted suspension were plated on Luria-Bertani (LB) agar plates in duplicate using the spread plate technique and incubated at 37 °C for 24 hours. The culturing resulted in isolating 23 unique bacterial colony morphologies. Identification of bacterial genera of these isolated strains was based on colony morphologies, differential staining methods, and biochemical tests. Among the isolated bacterial strains, 41.66% belonged to the genus *Staphylococcus,* and over half of them showed the coagulase virulence factor. Genus *Micrococcus, Bacillus, Acinetobacter,* and *Citricoccus* represented 25.00%, 16.66%, 8.33%, and 4.16%, respectively. Most microplastic-inhabiting bacteria were found to be gram-positive cocci. The bacterial suspension derived from microplastic particles in return sludge samples had the highest average colony forming units (CFU) concentration of 490 CFU/mL, while the lowest count of 50 CFU/mL was observed from microplastic particles in the UV treatment section. This study indicated that *Staphylococcus*, *Micrococcus*, *Bacillus*, *Acinetobacter*, and *Citricoccus* were the common microplastic-inhabiting bacterial genera found in the particular wastewater treatment system, notably with *Staphylococcus* being the most abundant. Molecular biological bacterial identification is currently in progress.

Keywords: Microplastic particles, Microplastic-inhabiting bacteria, *Staphylococcus,* Wastewater