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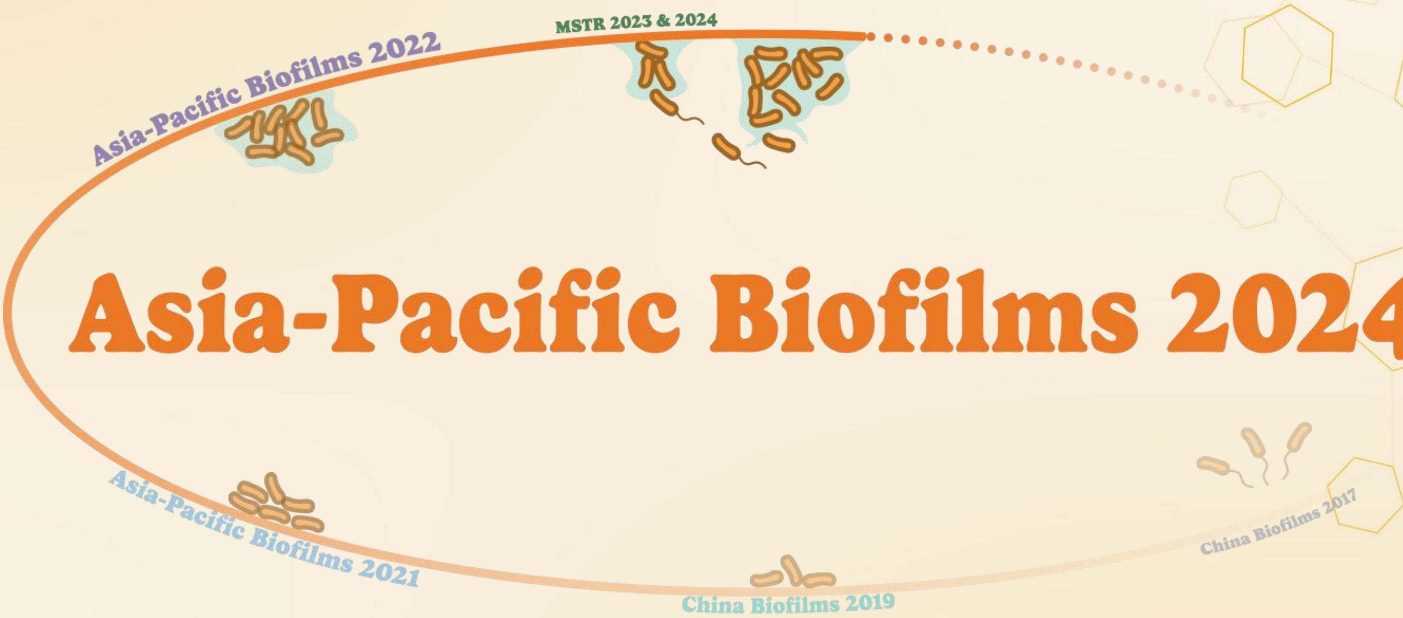

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Biofilm formation and production of extracellular polymeric substances by perchlorate reducing microorganisms isolated from serpentine soils in Sri Lanka

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Abstract: In this study, pure cultures of a perchlorate-reducing bacteria (PRB, n=1) and perchlorate-tolerant fungi (n=3) isolated from serpentine soils of Ussangoda National Park (UNP), Sri Lanka, (6°05'55"N 80°59'12"E), were screened for biofilm formation and their bioremediation potential for perchlorate ions. Given the elemental similarity between UNP's soils and Martian regolith, overarching aim of this study is to evaluate the perchlorate reduction capabilities of microbial isolates under Martian environmental conditions. The bacterial (A) and three fungal species (W, Y, P), were first prepared as monocultures in perchlorate reducing bacteria selective media (0.02 mol dm^{-3} $NaClO_4$). Then mixed fungi-bacteria cultures (AP, AW, AY) were prepared and screened for their ability for perchlorate reduction efficiency by FTIR analysis. AW demonstrated the highest perchlorate reduction efficiency (60.9%) followed by AY (48.8%) while AW indicated low efficiency (1.6%) after three weeks of incubation. Building on these findings, biofilm formation in AP, AY and AW were investigated qualitatively using Congo Red agar method which indicates the production of extracellular polymeric substances (EPS). Further, a UV spectrophotometry-based Congo Red (0.1 and 0.2 mg/L) broths were used as a quantitative approach for assessment of the biofilms formed. The biofilm structures were confirmed by microscopic observations. Results showed that microbial combinations AP and AW formed biofilms after 72 hrs, while AY has taken 14 days for positive indication of the biofilm. At 0.1 mg/l concentration of the stain, compared to the average absorbance of the control, (1.516) AP biofilm reported a significantly higher average absorbance (2.587) and a similar pattern was found at the higher concentration of the stain. These findings underscore the potential of the biofilms formed using PRBs and perchlorate-tolerant fungi for efficient perchlorate reduction under extreme environmental conditions, offering promising candidates for bioremediation applications on Mars and perchlorate contaminated sites on Earth. Future work will focus on refining the quantification of biofilm biomass and efficiency of Perchlorate reduction in contaminated soils and mass culturing of selected biofilms.

Keywords: Perchlorate-reducing bacteria, Biofilm formation, Martian soil, Serpentine soil, Extracellular polymeric substances

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