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CHARACTERIZATION OF EXTRACELLULAR POLYMERIC SUBSTANCES OF CYANOBACTERIAL, FUNGAL AND BACTERIAL BIOFILM COMPLEXES BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY AND FOURIER TRANSFORM INFRARED SPECTROSCOPY

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Biofilms are complex communities of multiple microbial species that are attached to surfaces or interfaces in nature. They secrete self-produced extracellular polymeric substances (EPS) for their structure and protection. Such biofilms can also be developed *in vitro* using beneficial microbes for biofertilizers, pharmaceuticals, fuels and electricity. Resident microbes in biofilms establish EPS biochemical-based complex network interactions that govern their functioning. The EPS is considered the "dark matter of biofilm" due to its complexity. Identifying the EPS biochemicals and understanding their contribution to the network interactions is important to achieve improved qualitative productivity and innovation in this field of research. Therefore, a study was designed to analyze biochemical parameters of fungal-bacterial biofilms (FBBs), fungal-cyanobacterial biofilms (FCBs), cyanobacterial-bacterial biofilms (CBBs), and fungal-cyanobacterial-bacterial biofilms (FCBBs). Microbes used were *Aspergillus niger*, *Nostoc* sp., and gram (-) *Stenotrophomonas maltophilia* and gram (+) *Bacillus subtilis*, as fungal (F), cyanobacterial (C) and bacterial (B) counterparts, respectively. ATR-FTIR and LCMS were used to characterize the EPS produced by the different biofilms. Results revealed that FCBBs were at the forefront of producing lipids, proteins, and polysaccharides. In FCBBs, gram (-) *S. maltophilia* was more productive than the gram (+) *B. subtilis*, and the EPS production was affected by the F:C:B ratio of the inoculum. In addition, ecologically and industrially important three biochemicals, i.e. Rescinnamine, Colchicine, and Syrosingopine, were found in biofilm-EPS. In conclusion, the productivity of biofilms can be improved by manipulating microbial composition. Further research is needed to develop more productive (75 – 95%) biofilms for various biotechnological applications. Also, LC-MS and ATR-FTIR spectroscopy can be recommended as effective tools in comparative studies in evaluating structural and functional properties of fungal, bacterial and cyanobacterial biofilm complexes.

Keywords: Biofilms, EPS biochemicals, Network interactions