PHA AS A BIOFILM COMPONENT IN ENDOPHYTIC BACTERIA IN HARSH ENVIRONMENTS

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The formation of microbial biofilms represents a ubiquitous adaptive strategy, allowing microorganisms to persist and thrive in diverse ecological niches, ranging from beneficial symbioses to pathogenic associations (Hobley et al., 2015). PHA serves as an energy store in biofilms. These biodegradable polymers, structurally akin to synthetic plastics, are produced by various microorganisms as intracellular inclusions under conditions of nutrient limitation with an excess carbon source (Kankonkar & Khandeparker, 2022). Their synthesis within bacterial cells, particularly those residing in biofilms, can therefore be critical for survival and proliferation in dynamic and often nutrient-depleted niches, such as those encountered by endophytic bacteria in harsh environments (Agarwal et al., 2024). The intricate interplay between PHA synthesis and biofilm formation in endophytic bacteria, particularly within the context of environmental extremophiles, remains an underexplored area of research. The aim of our research was to study the possibility to extract PHA from endophytic microbial communities associated from Antarctic vascular plants.

Material and methods. Two types of microbial communities (air-liquid-solid, ALS and liquid-solid, LS) were cultivated stationary in minimal salt medium (MSM) at 25ºC, 6 days. The ability of microbial communities to accumulate PHA was verified using Sudan Black staining with light microscopy and the fluorescent dye Nile Red with Confocal Laser Scanning Microscopy (Leica TCS SPE Confocal system with a coded DMi8 inverted microscope (Leica, Germany) and Leica Application Suite X (LAS X) Version 3.4.1 software). After harvesting the biofilms via centrifugation (160000 rpm, 20 min), the cells were washed with saline twice and dried out. After dried biomass was disintegrated using an ultrasound bath, PHA were extracted with chloroform for 24 h and precipitated with cold ethanol at 4°C overnight. The precipitated PHA was washed out with cold ethanol and dried at 40ºC.

Results. PHA accumulation was confirmed in both microbial communities using Sudan Black staining. However, Nile Red fluorescence only visualized lipid complexes within the ALS community type. Despite this histochemical difference, the standard extraction protocol yielded a consistent concentration of 4 mg of PHA per gram of ADM for each community. Critically, the biomass productivity of the ALS community was found to be fivefold greater than that observed in the LS community.

Conclusions. While both microbial communities accumulated PHA at the same concentration, the ALS community demonstrated fivefold greater biomass productivity, establishing it as the more efficient candidate for large-scale PHA production.