**Investigating the novel ISS *Methylobacterium species* for PHA biosynthesis**

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Polyhydroxyalkanoates (PHAs) are gaining attention in the family of polyesters polymers due to their biodegradability and biocompatibility. The polymer molecular structure is generally directed by the biosynthetic pathways of microbes employed for PHA synthesis, influenced by the bacterial species, substrate utilized, and the culturing conditions provided. There are more than 100 PHA polymer monomeric units, and the composition of these sub-units in polymer structure determines their physical and thermo-mechanical features. This variability has produced PHAs with drastically different polymer properties. In this context, it is interesting to explore PHA producers with properties having desirable functionality. In this study, novel microbial species, namely *Mehylobacterium ajmalii* (JC-0089 and JC-0023) and *Methylorubrum rhodesium* (JC-1176) isolated from the International Space Station were investigated for the potential of PHA synthesis. Methane gas, Luria broth, and soyabean oil are the chosen substrates for this study. The research focuses on comprehensive *in-silico* analysis of the whole genome sequences and metabolic pathways entailed in the substrate to PHA production. This analysis showed a lack of methane monooxygenase, due to which methanol was used instead of methane gas as a C1 carbon source. In addition, nitrogen-limited growth conditions with 7:3 carbon source to minimal media ratio were provided for PHA accumulation, followed by molecular gene targeting, positive for PHA synthase gene (*phaC*). Further, the optimized solvent extraction method accomplishes the PHA extraction of 59.71% in JC-1176, 44.9% in JC-0089 and 48.7% in JC-0023 per gram of cell biomass. FTIR peak at 1723 confirmed the extracted product as PHA. Using biophysical techniques like XRD, NMR, AFM, DSC, TGA, etc., can report the physical and thermal properties of the extracted PHA polymers. The findings further elucidate the relationship between carbon substrate and the biochemical pathways resulting in polymer characteristics.