DEVELOPMENT OF A MICROBIAL-CELL-FACTORY CONVERTING PLASTIC WASTE TO BIODEGRADABLE POLYMERS

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ABSTRACT

As plastic waste accumulation continues to surge uncontrollably, a planetary crisis is unfolding beneath our feet. Heightened concerns about the environmental impact of plastics underscore the need for urgent eco-friendly strategies, overcoming the conventional management practices. Despite plastics' durability and resistance to degradation [1,2], recent emphasis has shifted towards enzymatic breakdown, the ability of enzymes to target and shatter polymer bonds, leading to breakdown into oligo- and monomers [2–4]. The combination of chemical and biological valorization of these molecules generated through plastic degradation offers the potential for diverse product production, including lubricants, fuels, and innovative polymers [5]. Recent attention has increasingly focused on the biological valorization of the oligo- and monomers, serving as a carbon source for microorganisms. Engineered microorganisms, expressing plastic-degrading enzymes, have demonstrated the ability to metabolize plastic monomers like ethylene glycol (EG), terephthalic acid (TPA), or 6-hydroxyhexanoic acid, deriving from the degradation of polyethylene terephthalate (PET) and polycaprolactone (PCL), respectively. The valorization process holds the potential to yield value-added products such as polymers or chemicals [6–8]. In the present study, we focus on the creation of a consolidated bioprocess through the development of a microbial-cell-factory with plastic waste as feedstock. Bacterial strains of *Bacillus* genus, *B. subtilis* BPM12 and *B. subtilis* RIK, were utilized as platform. Strains were evolved for monomer (TPA and 6-hydroxyhexanoic acid) metabolism, through adaptive laboratory evolution (ALE) technique and transformed with recombinant plasmid, carrying expression gene of a recently discovered thermophilic polyesterase, *Dm*PETase [9] and phaCAB operon, for polyhydroxyalkanoates (PHAs) production. The engineered strains were tested for recombinant expression in Terrific Broth (TB) medium. Following up, the ability of recombinant bacteria to break down virgin polyesters (PCL and PET) as well as a mixture of different polyesters considered as post-consumer plastic waste, was monitored, through High-Performance Liquid Chromatography and mass loss, in liquid cultures of mineral salts medium (MSM), with polymers as the only carbon source. Eventually, the evaluation of PHAs synthesis was conducted utilizing Nile red staining, under fluorescent microscope or by Gas Chromatography-Mass Spectrometry (GS-MS) analysis.

KEYWORDS: plastic pollution, plastic degradation, microbial-cell-factory, genetic engineering, *Bacillus*

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