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Production of 4-methyl-1-pentanol biofuel from monomers of poly(3-hydroxy-4-methylvalerate)

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Sustainable alternatives for petroleum-based products such as plastics and gasoline must be developed promptly. *Herbaspirillum seropedicae* is an endophyte that naturally produces polyhydroxyalkanoates (PHAs), like many bacteria. PHAs are widely considered to be bioplastics, and poly-3-hydroxybutyrate, PHB, which is most commonly produced by many bacteria is stiff, brittle and has few applications. Co-polymers like poly-3-hydroxybutyrate-co-3-hydoxyvalerate, P(3HB-co-3HV), on the other hand, are more flexible, durable and more like polypropylene. What is not appreciated or reported in the literature is that monomers of other PHAs, e.g., poly(3-hydroxy-4-methylvalerate), PH(4me)V,can be chemically or biochemically reduced to branched-chain higher alcohols which are next generation biofuels, for PH(4me)V conversion is to 4-methyl-1-pentanol. Development of biofuels from PHAs with higher energy density and lower vapor pressure than ethanol, e.g., 4-methyl-1-pentanol, significantly expands use of PHAs as alternatives for petroleum based products and well beyond use as bioplastics.

Our previous studies showed that *H. seropedicae* accumulates PHB when grown on glucose in which two acetyl-CoAs are condensed to form the 3HB monomer substrate. We also showed that the co-polymer, P(3HB-co-3HV), was produced when this bacterium was grown on glucose and nonanoic acid as co-substrate. Beta-oxidation of nonanoic acid results in formation of propionyl-CoA which condenses with acetyl-CoA to form the 3HV monomer substrate. We also found that a PrPC mutant of this bacterium, in which propionate is not effectively catabolized, produced significantly higher amounts of P(3HB-co-3HV). For production of a PHA with a six carbon monomer, we propose to overexpress an ilvIHCD operon in *H. seropedicae* such that a high level of 2-ketoisovalerate, valine metabolite, is produced. Condensation of the CoA derivative of 2-ketoisovalerate (i.e.,isobutyryl-CoA) with acetyl-CoA forms PHA monomer substrate, 3-hydroxy-4-methylvaleryl-CoA. The PHA produced is PH(4me)V, a naturally occurring PHA that should accumulate in *H. seropedicae*. Monomers of this PHA will then be isolated and reduced to the corresponding aldehyde and alcohol, i.e., PHA monomer 3-hydroxy-4-methyl pentanoic acid (3-hydrox-4-methyl valeric acid) to 4-methyl pentanoic acid then to 4-methyl pentanal and then to 4-methyl pentanol. Removal of the 3-hydroxy group will make use of part of a glutamate fermentation pathway used by some Clostridia that involves conversion of 3-hydroxybutyryl-CoA to butyryl-CoA (Dawes & Sutherland, 1992). Also, extracts of Clostridium butyricum can be used to reduce a fatty acyl-CoA to the corresponding aldehyde and alcohol (Day et al., 1970). A large number of PHA depolymerases have also been described, e.g., in various Clostridia.

The 4-methyl pentanol produced will then be tested as fuel like ethanol. Our proposed study will focus on PH(4me)V production in the lab using standard methods for PHA production, isolation and characterization. Recombinant *H. seropedicae* overexpressing an ilvIHCD operon from another Herbaspirillum sp., an Oxalobacteraceae or Burkholderiales will be tested for PH(4me)V production.

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