From oil to bioplastics, a dream come true?

María A. Prieto*

Department of Molecular Microbiology, Biological Research Center, CSIC, Madrid, Spain

*Mailing address: Department of Molecular Microbiology, Biological Research Center, CSIC, C/Ramiro de Maeztu, 9, Madrid 28040, Spain. Tel.:34-918373112; Fax: 34-915360432; email: auxi@cib.csic.es

Human overpopulation combined with the current lifestyle urges the rational, efficient and sustainable use of natural resources to produce environmentally friendly plastic materials such as polyhydroxyalkanoic acids (PHA), whose production/degradation cycle reduces undesirable wastes and emissions. In this issue Sabirova et al. (15) presents results of paramount importance: a PHA hyper-producer mutant of the oil-degrading marine bacterium *Alcanivorax borkumensis* SK2 that deposits the PHA in the extracellular environment by a still unknown mechanism (Figure 1). This phenotype is only observed when grown on aliphatic hydrocarbons, one of the main components of petroleum.

Occurrence of PHA in bacteria has been known since 1926 when Lemoigne observed that *Bacillus megaterium* produced an intracellular polymer of hydroxybutyrate monomers, later called polyhydroxybutyrate (PHB), which is the most widely produced PHA by bacteria (6). Six decades later, other related biopolymers with longer side chains (medium chain length PHA or mcl-PHAs) were also described. Their production was first reported in *Pseudomonas putida* GPo1 (formerly known as *Pseudomonas oleovorans* GPo1) growing on alkanes (e. g., *n*-octane) (3). Since then, PHA has been
detected in some Archaea and a wide range of Gram-positive and Gram-negative bacteria in aerobic and anaerobic environments. Although there are many microorganisms able to produce PHA, mcl-PHA production had been repetitively restricted to *Pseudomonas* strains (3, 4, 7, 8, 19). As the new information provided by the bacterial sequenced genomes has started to indicate, the first conclusion that can be drawn from the contribution of Sabirova and coworkers is that the genus *Pseudomonas* has justifiably lost its exclusiveness in this phenotype. This conclusion is also extended to the metabolic pathways acquired through evolution by the paradigmatic strain *P. putida* GPo1 to synthesize mcl-PHA, supported by the observation that, *A. borkumensis* produces mcl-PHA from alkanes by a peripheral alkane oxidation pathway that links alkane catabolism to β-oxidation central pathway (17).

The strategies designed to produce mcl-PHAs in recombinant *Escherichia coli* cells expressing a heterologous mcl-PHA synthase use specific mutants deficient in certain steps of the β-oxidation pathway, to slow down or interrupt the ongoing cycle, that results in an accumulation of hydroxyacyl-CoA substrates for the mcl-PHA synthase (10, 11, 14). Likewise, mcl-PHA production was greatly increased in *P. putida* U engineered strains (*fadA-fadB* mutants on β-oxidation cycle), eliciting a strong intracellular accumulation of biopolymesters (9) (Figure 1). Another novelty of what Sabirova et al. discovered in their study is a new metabolic strategy to generate PHA hyper-producer strains by inactivating a specific thioesterase that channels the hydroxyacyl-CoA intermediates towards mcl-PHA synthesis, demonstrating once more, that metabolic engineering succeeds in improving microorganisms for biotechnological purposes.

Remarkably exciting was the fact that mcl-PHA is deposited extracellularly (Figure 1), since this is the first report describing this phenomenon. Other biopolymers can be
accumulated intracellularly or extracellularly in bacteria, but PHA was so far the paradigmatic example of a biopolymer that was only accumulated in the cytoplasm (3, 8). Depending on the organism, PHA production can reach levels as high as 90% of the cell dry weight (9). The cytoplasm space limits the amount of polymer that can be produced by a microbial cell, and the yield per volume is limited by the number of cells and the biopolymer fraction in the biomass. This increases the complexity of the production and downstream processes to obtain purified PHA, implying cell breakage procedures as well as separation processes of PHA from crude extracts (19). Some biotechnological relevant biopolymers, such as poly(γ-D-glutamate) (1), alginates (12) and hyaluronic acid (18), are examples of extracellular biopolymers (16). For these polymers, the cell volume is not a bottleneck, being bioreactor volume, water solubility and biopolymer viscosity the factors that hamper the yield. Taking into account that mcl-PHAs are water insoluble biopolymesters, their large scale production by recombinant strains such as A. borkumensis C9 will, undoubtedly, imply the development, implementation and optimization of new fermentation strategies considering novel systems for product recovering. Therefore the work by Sabirova et al (15) does not only show a new biological phenomenon, but more importantly it opens new avenues for research.

PHA biodegradation is performed in bacteria by at least two different pathways. One involves an intracellular degradation process as a self-service system to re-use storage carbon sources. The other is carried out extracellularly, where exogenous PHA is utilized as a carbon/energy source (5). Previously, it was assumed that the only source of extracellular PHA was the compound released after lysis of PHA producers. The PHA spread into the environment can be further hydrolyzed by secreted depolymerases into water soluble oligomers and monomers useful as carbon source for the microbial
community (5). The mechanism used by the A. borkumensis mutant to secrete PHA is unknown at present. The electron microscopy results presented seems to rule out cell wall lysis as the mechanism and it may well happen that we are confronting a new secretion mechanism for PHA not previously envisioned. Molecular insights will also provide new clues in the future to understand the machinery involved in the still not well established mechanism of PHA granule formation. In this sense, the latest findings in paradigmatic PHA-producers like Pseudomonas aeruginosa support a budding model for granule formation which involves four steps: i) the attachment of the PHA synthase to the inner surface of cytoplasmic membrane, ii) formation of oligomers that remain bound to the polymerase and therefore, associated to the inner surface of cytoplasmic membrane, iii) polymer elongation in the hydrophobic environment found between the phospholipid monolayers of cytoplasmic membrane, iv) budding granule formation probably directed by structural granule associated proteins (phasins) (13). Taking into account this proposal, it is not obvious how this budding mechanism can explain the PHA secretion observed in A. borkumensis C9 and therefore, the mechanism for granule formation might require reconsideration.

Finally, it is now assumed that the conversion of raw material in frequently used products, such as plastics, is a major and critical goal in the development of sustainable processes. In the same way, better bioremediation strategies are required for petroleum removal in oil polluted environments due, for instance, to crude oil spills or to uncontrolled release of industrial wastes from oil refineries. Interestingly, A. borkumensis is highly specialized in the assimilation of aliphatic hydrocarbons, and it makes up the main fraction of the biomass in oil-polluted marine environments. Moreover, the availability of its complete sequenced genome makes this strain a hydrocarbonoclastic microorganism of reference for exploration of new bioremediation
strategies as a practical oil-removal technology (2). The contribution of Sabirova et al., allows us to envision this strain as a superbug able not only to devour hydrocarbons from oil-contaminated environments, but also to efficiently biotransform them into extracellular PHA for biotechnological uses. From the viewpoint of bioremediation, hydrocarbons from marine oil spill accidents might be at the same time removed and converted into a widespread utilizable carbon source for the microbial world. Could this all just be a dream?
REFERENCES


Figure 1. Schematic overview of some characteristics of *Alcanivorax borkumensis* and *Pseudomonas putida* mcl-PHA hyper-producer strains. Several *Pseudomonas* strains are able to transform aliphatic hydrocarbons, one of the main components of petroleum, in mcl-PHA. Nevertheless, most of them can exclusively use fatty acids and carbohydrates as precursors (renewable resources). *A. borkumensis* SK2 is highly specialized in the assimilation of aliphatic hydrocarbons in oil-contaminated sea water but it can poorly transform such precursors in mcl-PHA. Metabolic engineered strains are in both examples based on the accumulation of hydroxyacyl-CoA substrates for the mcl-PHA synthase. Whereas *P. putida* strain over-accumulated the biopolymesters intracellularly, overproduction in *A. borkumensis* results in deposits of the mcl-PHA in the extracellular environment by a still unknown mechanism (indicated as a question mark). Sabirova and coworkers have isolated an *A. borkumensis* mutant strain specialized in the biotransformation of aliphatic hydrocarbons from oil into extracellular bioplastic.