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Nature's Biodegradable Marvel: Unveiling the Structure, Creation, and Future of Bacterial Polyhydroxyalkanoate Lipid Granules

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Abstract
Polyhydroxyalkanoates are biopolymers composed of hydroxyalkanoate monomers, serving as carbon and energy stores in bacteria. PHAs accumulate as granules under unbalanced growth conditions. Poly(3-hydroxybutyrate), a crystalline form of PHA, is versatile for bioplastics and blends. Categorized into short-chain-length and medium-chain-length types, PHAs offer diverse thermal and mechanical properties. Efficiently produced by bacteria, fungi, and microalgae like *Bacillus megaterium* and Pseudomonas species, PHAs are synthesized from acetyl-CoA or acyl-CoA, polymerized by PHA synthases. Regulation of PHA metabolism responds to environmental cues. PHAs replace synthetic plastics in packaging, agriculture, medicine, and more, promoting environmental sustainability and biotechnological innovation.

Introduction
Polyhydroxyalkanoates (PHAs) are biopolymers composed of various hydroxyalkanoate monomers, serving as energy and carbon storage materials in bacteria. PHAs were first observed as refractile bodies inside bacterial cells by Beijerinck in 1888. The composition of PHAs was later identified by Lemoigne in 1926. These polymers accumulate as granules (0.2–0.5 µm) within the cytoplasm of various bacterial cells and are produced by a range of prokaryotic microorganisms, typically under unbalanced growth conditions. The first PHA to be isolated and characterized was Polyhydroxybutyrate (PHB), specifically Poly(3-hydroxybutyrate) (P3(HB)). P3(HB) is highly crystalline due to its linear chain structure, which includes both amorphous and crystalline phases. It can exist as a virgin polymer or as part of copolymers and blends. Bacteria produce P3(HB) as a carbon reserve, and it is manufactured industrially through bacterial fermentation. PHA polymers are stored in bacterial cells as well-defined granules consisting of polyester, proteins, and lipids. The composition of these granules was first studied by Griebel in 1968, who found that PHB granules in *Bacillus megaterium* were composed of 97.7% polyester, 1.87% proteins, and 0.46% lipids or phospholipids.

Structure
Polyhydroxyalkanoates (PHAs) are biopolyesters made from around 150 known hydroxyalkanoic acids. These bacterial storage polyesters are categorized based on the carbon atoms in their monomeric units into short-chain-length (SCL) PHAs (3–5 carbon atoms) and medium-chain-length (MCL) PHAs (6–14 carbon atoms). PHAs are naturally hydrophobic and crystalline, composed exclusively of chirally pure (R)-configuration monomers. Despite variations in chain lengths (SCL, MCL, or long-chain-length PHAs), all PHAs share a

common general structure, with differences arising in the main chain of their monomer at position –(CH_2) n (where 'n' ranges from 1 to 4) and in the number of repetitions of this monomer to form a polyester chain, denoted as ‘X’. This monomer repetition can range from 100 to 30,000 times, reflecting the structural diversity and varying properties of these polyesters.

Properties of PHB granules

PHAs have diverse properties, with thermal and mechanical characteristics being crucial. Thermal characterization is essential for determining their applications in bioplastics, biomedical implants, and plastic replacements. The thermal properties are defined by the glass transition temperature (Tg) for the amorphous phase and the melting temperature (Tm) for the crystalline phase. Research indicates that as the side chain carbon number increases from 1 to 7, Tg decreases and Tm rises, with melting temperatures ranging from 45 to 69 °C.

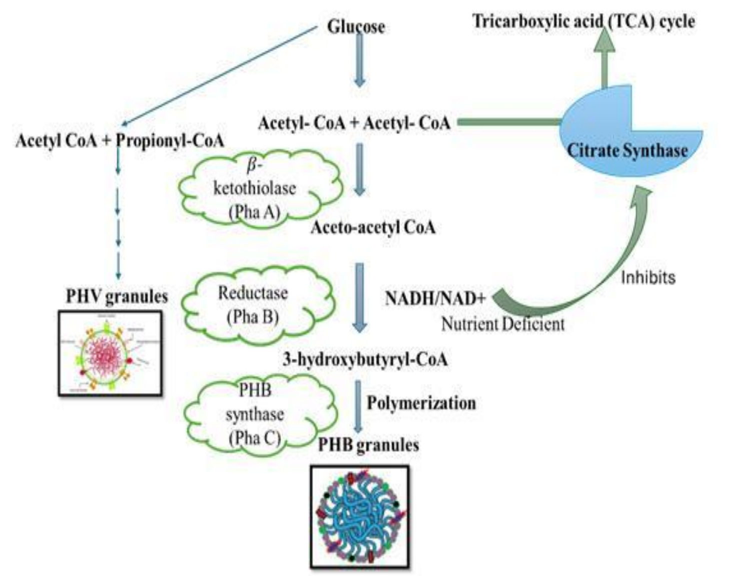
Microbes Best Suitable for PHA Production

Various microbes, including bacteria, fungi, and microalgae, are capable of biosynthesizing polyesters like PHAs. Since their discovery in the 20th century, hundreds of bacterial species and strains have been identified as PHA producers. *Bacillus megaterium*, Pseudomonas species (aeruginosa, putida, fluorescens, oleovorans), *Ralstonia eutropha*, and *Cupriavidus necator* are extensively studied for efficient PHA production. Microalgae such as *Nostoc muscorum*, *Chlorella minutissima*, and *Botryococcus braunii* are also investigated for their PHA production capabilities. Fungal sources like *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, and *Yarrowia lipolytica* have shown promise in PHA production. Besides PHB producers like *Cupriavidus necator* and *Bacillus spp*., certain actinomycetes, particularly Streptomyces, have been studied for their ability to accumulate PHB granules.

PHB Synthesis

Polyhydroxybutyrate (PHB) is produced by microorganisms under nutrient stress, with excess carbon but limited other nutrients, in both gram-positive and gram-negative bacteria. Key enzymes in PHB biosynthesis are PhaA, PhaB, PhaC, and PhaE, with PhaP aiding in PHA accumulation and morphology. Sugars and fatty acids are converted into PHAs via three pathways involving acetyl-CoA or acyl-CoA, ending in polymerization by PHA synthases. PhaC, discovered nearly thirty years ago, was the first identified PHA synthase. Class I synthases, found in *Ralstonia eutropha*, produce short-chain-length PHAs, while Class II synthases, found in *Pseudomonas putida*, produce medium-chain-length PHAs.

Figure: Metabolic biosynthetic pathways and regulatory circuits for PHA synthesis in *Cupriavidus necator* (earlier called as *Ralstonia eutropha*). PHA synthesis is influenced by the NADH/NAD+ ratio. During cell growth, high CoA levels result from the rapid acetyl-CoA flux into the TCA cycle. When nutrients like nitrogen are depleted, the NADH/NAD+ ratio rises, inhibiting TCA cycle enzymes. This reduces acetyl-CoA flux, lowers CoA levels, and lifts the inhibition of β-ketothiolase. →



Mechanisms Regulating PHA Metabolism

Bacteria regulate PHA metabolism in response to environmental and nutritional changes through various mechanisms: Enzymatic activity control, Global regulators in PHA metabolism control, Role of alternative sigma factors, Stringent response control, Regulation via quorum sensing, Control by PhoB under phosphate limitation, Oxygen-Responsive Regulators, Regulation by the Carbon Catabolite Regulator (Crc), The NtrB-NtrC Two-Component System, Regulatory Small RNAs, The GacS-GacA Two-Component System.

Application of PHB

Polyhydroxyalkanoates (PHAs) are polyesters valued for their polymeric properties, making them a sustainable and eco-friendly alternative to synthetic polymers and plastics. PHA bioplastics share similar characteristics with petrochemical-based plastics, positioning them as a viable source for sustainable bioplastic production. These microbial polyhydroxyalkanoates are non-toxic and environmentally friendly, ensuring their use as biomaterials does not harm the environment. PHAs can be used in a variety of applications, including packaging materials, agricultural mulching films, 3D printing materials, textiles, medical implants, drug delivery carriers, animal nutritional supplements, pharmaceuticals, fine chemicals, and innovative biofuels. By replacing synthetic plastics with PHA bioplastics, we can promote ecosystem conservation. PHAs thus offer a wide range of applications as eco-friendly biomaterials.

Conclusion

Polyhydroxyalkanoates (PHAs) represent a breakthrough in sustainable materials, offering a biodegradable and eco-friendly alternative to traditional plastics. With applications from packaging to medical implants, PHAs have the potential to transform multiple industries. The intricate metabolic pathways and regulatory mechanisms involved in PHA synthesis highlight bacterial adaptability in producing these valuable biopolymers. Continued research and technological advancements are expected to enhance PHA production efficiency, promoting environmental conservation and sustainable product development. Embracing PHAs reduces dependence on synthetic plastics, paving the way for a greener future.

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