TAILOR-MADE BIODEGRADABLE POLYMERS MADE BY BACTERIA FROM BIOMASS

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FAPESP Process 2012/51533-6 | Term: Jul 2013 to Jun 2015 | FAPESP / BE-Basic

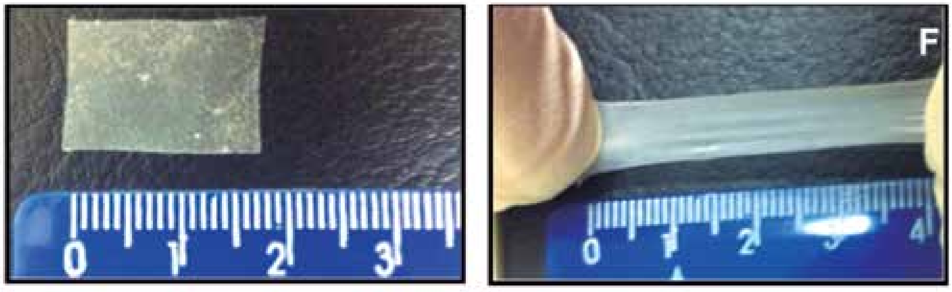

Figure 1. Elasticity of copolymers P(HB-co-20mol%HHx) films produced by recombinant B. sacchari (Mendonça, 2014

The project combined different strategies to produce the biopolymer P(HB-*co*-HHx) controlling monomer composition and contributing to reduce the cost of production. Recent papers and patents have shown that P(HB-*co*-HHx) is a promising biodegradable copolymer of the polyhydroxyalkanoate family (PHA), combining the short-chain (SC) monomer hydroxybutyrate (HB) and the medium-chain (MC) monomer hydroxyhexanoate (HHx), improving properties and applications, when compared with PHA with only SC or MC monomers. Since it can be processed into films, it is interesting to reduce its cost of production to make it a commodity. *Aeromonas spp* naturally produces a copolymer with 5-25 mol% HHx units, however only 10-20% of cell dry weight (CDW) is accumulated as PHA. On the other hand, *Burkholderia sacchari* accumulates up to 80% of CDW as PHB, but incorporates up to 2mol% HHx.

HB fractions are obtained feeding bacteria with a sugar or sugarcane bagasse derivatives, however HHx incorporation depends on feeding with more scarce and expensive medium-chain fatty acids (MCFA). Raw material can represent up to 40% of costs of PHA production thus, is of high interest to find sustainable alternatives with lower cost. It was found that MCFA could efficiently be produced with a mixed microbial fermentation (MMF) technology by anaerobic digestion, with the potential to transform acidified organic waste streams (acetic acid, ethanol etc.) into MCFA mixtures with a hexanoic acid yield of 0.85 mol C/mol C substrate in synthetic medium. MMF was adapted to and optimized for food residues and real feedstocks (e.g. sugarcane bagasse) to generate MCFA-containing streams appropriate to feed bacterial PHA production. This involved setting required effluent standards of MMF for the type and concentration of the fatty acids, or for other disturbing components (N or water content). Operation conditions and appropriate organic waste streams were selected, based on the requirements to feed PHA production. The global objective of the project was to start from a single bacterial species, introduce improvements to produce tailor-made copolymers, controlling monomer composition A relevant issue was to combine and to improve two biological processes (MMF and PHA production) to contribute to sustainably produce an environmentally friendly material starting from low-grade biomass.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

A *Burkholderia sacchari* recombinant was constructed with the ability to incorporate up to 22 mol% 3-hydroxyhexanoate (HHx) units in a copolymer containing also 3-hydroxybutyrate (HB), a considerable increase considering the value of 1.6 mol% HHx incorporated by the wild type strain. The composition of the copolymer P(HB-*co*-HHx) was modulated depending on the combination of the genes expressed and the glucose/hexanoic acid concentration provided. 3-hydroxyvalerate (HV) and 3-hydroxyheptanoate (HHp) monomers could be also incorporated under specific conditions.

Simultaneously to strain improvement, ChainCraft B.V. (Amsterdam, The Netherlands) applied mixed microbial fermentation technology (MMF) to convert residues and biomass into mixtures of medium-chain fatty acids (MCFA) appropriate to be supplied to the recombinants resulting polymers with variable MC monomer compositions, thus with variable properties needed to different uses. Regarding to metabolic fluxes analysis (MFA), based on data from bioreactor experiments with *B. sacchari*, harboring the phaPCJ operon from a locally isolated *Aeromonas sp*, a metabolic model was proposed representing the experimental data obtained. The model predicted that the strain presented a cyclic Entner-Doudoroff pathway for PHA synthesis in the intervals tested. Despite the considerable improvements obtained in converting hexanoic acid to HHx, the strain is operating at 50% of the maximum theoretical value, thus further improvements are currently under study, based on MFA. Analysis of the properties of the copolymers showed that glass transition temperature (*Tg*) and melting temperature (*Tm*) depend on HHx content. According to the properties exhibited by these copolymers, application on flexible porous films or controlled release systems for medicine and dentistry are suggested.

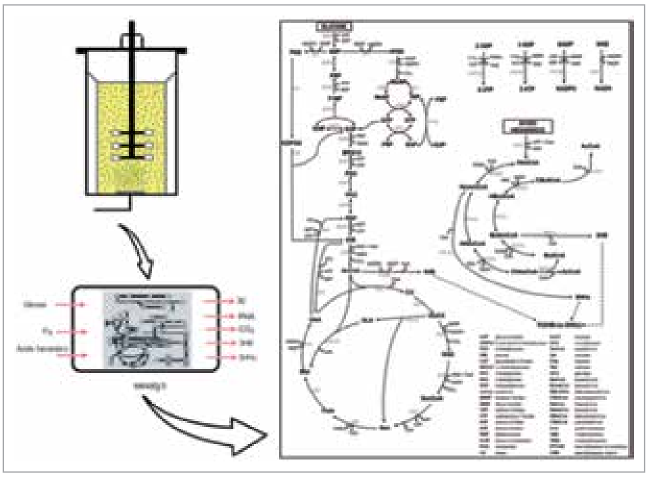

Figure 2. Metabolic Flux Analysis: metabolic model proposed to B. sacchari (Mendonça, 2014)

MAIN PUBLICATIONS

Alexandrino PMR, Mendonça TT, Bautista LPG, Cherix J, Lozano-Sakalauskas GC,Fujita A, Ramos Filho E, Long P, Padilla G, Taciro MK, Gomez JGC, Silva LF. 2015. Draft genome sequence of the polyhydroxyalkanoate-producing bacterium *Burkholderia sacchari* LMG 19450 isolated from Brazilian sugarcane plantation soil. *Genome Announcements*. **3**: e00313-15.

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