Non-natural biomedical polymers produced from microorganisms

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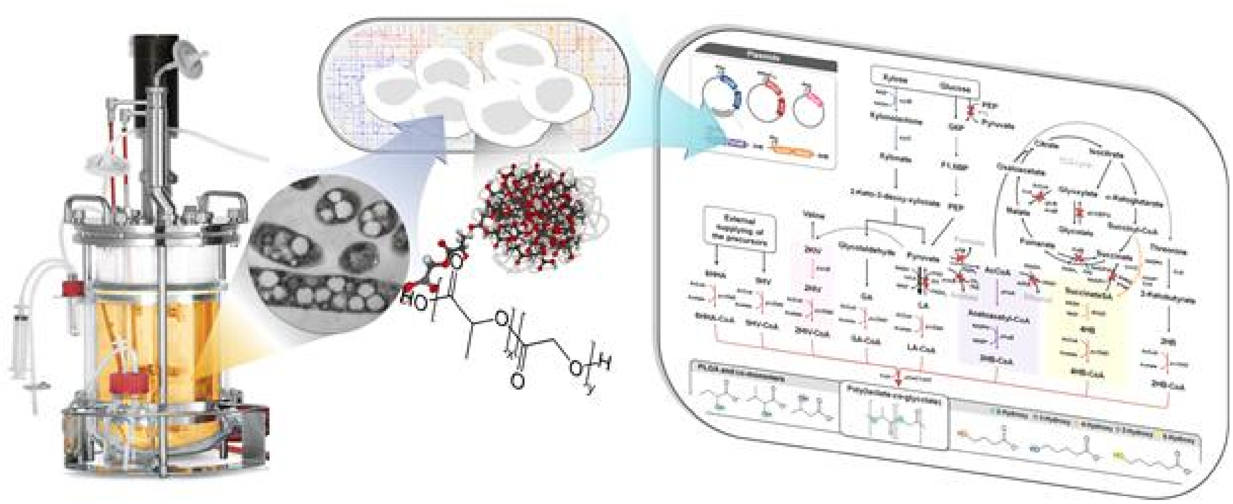

The schematic diagram shows the overall conceptualization of how metabolically engineered E. coli produced a variety of PLGAs with different monomer compositions, proposing the chemosynthetic process of non-natural polymers from biomass. The non-natural polymer PLGA and its other copolymers, which are produced by engineered bacteria developed by taking a systems metabolic engineering approach, accumulate in granule forms within a cell. Credit: KAIST

Renewable non-food biomass could potentially replace petrochemical raw materials to produce energy sources, useful chemicals, or a vast array of petroleum-based end products such as plastics, lubricants, paints, fertilizers, and vitamin capsules. In recent years, biorefineries which transform non-edible biomass into fuel, heat, power, chemicals, and materials have received a great deal of attention as a sustainable alternative to decreasing the reliance on fossil fuels.

A Korean research team headed by Distinguished Professor Sang Yup Lee of the Chemical and Biomolecular Engineering Department at the Korea Advanced Institute of Science and Technology (KAIST) established a biorefinery system to create non-natural polymers from natural sources, allowing various plastics to be made in an environmentally-friendly and sustainable manner. The research results were published online in *Nature Biotechnology* on March 7, 2016. The print version will be issued in April 2016.

The research team adopted a systems metabolic engineering approach to develop a microorganism that can produce diverse non-natural, biomedically important polymers and succeeded in synthesizing poly(lactate-co-glycolate) (PLGA), a copolymer of two different polymer monomers, lactic and glycolic acid. PLGA is biodegradable, biocompatible, and non-toxic, and has been widely used in biomedical and therapeutic applications such as surgical sutures, prosthetic devices, drug delivery, and tissue engineering.

Inspired by the biosynthesis process for polyhydroxyalkanoates (PHAs), biologically-derived polyesters produced in nature by the bacterial fermentation of sugar or lipids, the research team designed a metabolic pathway for the biosynthesis of PLGA through microbial fermentation directly from carbohydrates in *Escherichia coli* (*E. coli*) strains.

The team had previously reported a recombinant *E. coli* producing PLGA by using the glyoxylate shunt pathway for the generation of glycolate from glucose, which was disclosed in their patents KR10-1575585-0000 (filing date of March 11, 2011), US08883463 and JP5820363. However, they discovered that the polymer content and glycolate fraction of PLGA could not be significantly enhanced via further engineering techniques. Thus, in this research, the team introduced a heterologous pathway to produce glycolate from xylose and succeeded in developing the recombinant *E. coli* producing PLGA and various novel copolymers much more efficiently.

In order to produce PLGA by microbial fermentation directly from carbohydrates, the team incorporated external and engineered enzymes as catalysts to co-polymerize PLGA while establishing a few additional metabolic pathways for the biosynthesis to produce a range of different non-natural polymers, some for the first time. This bio-based synthetic process for PLGA and other polymers could substitute for the existing complicated chemical production that involves the preparation and purification of precursors, chemical polymerization processes, and the elimination of metal catalysts.

Professor Lee and his team performed in silico genome-scale metabolic simulations of the *E. coli* cell to predict and analyze changes in the metabolic fluxes of cells which are caused by the introduction of external metabolic pathways. Based on these results, genes are manipulated to optimize metabolic fluxes by eliminating the genes responsible for byproducts formation and enhancing the expression levels of certain genes, thereby achieving the effective production of target polymers as well as stimulating cell growth.

The team utilized the structural basis of broad substrate specificity of the key synthesizing enzyme, PHA synthase, to incorporate various co-monomers with main and side chains of different lengths. These monomers were produced inside the cell by metabolic engineering, and then copolymerized to improve the material properties of PLGA. As a result, a variety of PLGA copolymers with different monomer compositions such as the US Food and Drug Administration (FDA)-approved monomers, 3-hydroxyburate, 4-hydroxyburate, and

6-hydroxyhexanoate, were produced. Newly applied bioplastics such as 5-hydroxyvalerate and 2-hydroxyisovalerate were also made.

The team employed a systems metabolic engineering application which, according to the researchers, is the first successful example of biological production of PGLA and several novel copolymers from renewable biomass by one-step direct fermentation of metabolically engineered E.coli.

Professor Lee said,

"We presented important findings that non-natural polymers, such as PLGA which is commonly used for drug delivery or biomedical devices, were produced by a metabolically engineered gut bacterium. Our research is meaningful in that it proposes a platform strategy in metabolic engineering, which can be further utilized in the development of numerous non-natural, useful polymers."

Director Ilsub Baek at the Platform Technology Division of the Ministry of Science, ICT and Future Planning of Korea, who oversees the Technology Development Program to Solve Climate Change, said,

"Professor Lee has led one of our research projects, the Systems Metabolic Engineering for Biorefineries, which began as part of the Ministry's Technology Development Program to Solve Climate Change. He and his team have been continuously achieving promising results and attracting greater interest from the global scientific community. As climate change technology becomes more important, this research on the biological production of non-natural, high value polymers has a great impact on science and industry."

More information: One-step fermentative production of poly(lactate-co-glycolate) from carbohydrates in Escherichia coli, *Nature*

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