Plastic Produced by *Escherichia coli*

By SONG Jianlan (Staff Reporter)

Prof. ZHANG Xueli, principal investigator of TIB.

Human activity is now producing as much as 50 million tons of plastic waste a year. This has caused many environmental issues, as this durable material is extremely hard for nature to decompose and break down – in a landfill plastic bags can last up to 500 years. As a result large amounts of garbage can easily accumulate over time.

A solution to this problem is to substitute high-density polyethylene (HDPE) plastic packaging materials with biodegradable plastic materials. This will not only ease the pressure imposed on the natural environment, but also help relieve human beings from the dependence on fossil resources, as it takes no crude oil, gas or coal to produce such bio-plastic materials.

"Such novel materials are produced from renewable biomass resources, such as cassava and straw, through biological conversion, and can easily be decomposed by microbes in natural environments," introduces Xueli ZHANG, principal investigator at the Tianjian Institute of Industrial Biotechnology (TIB), CAS. His team is engaged in design, construction and optimization of industrial microbial strains, drawing on systems biology and synthetic biology. "Our major tasks are to construct efficient 'cell factories' for production of bulk chemicals, amino acids and plant-derived natural products," he introduces.

"To a large extent genetic engineering or modification remains an important tool for us; the difference is, it is taking a new life due to the developments in new biology," he explains when asked of the methods they employed to optimize the genome of the involved microbes.

ZHANG's institute, TIB has committed to the R&D and transfer of green biotechnologies for low-carbon development and industrial advancement. His team, one of the groups deployed in the R&D network of the CAS Key Lab for System Microbiology Engineering, has successfully tackled a series of technical barriers in synthesizing monomers of polybutylene succinate (PBS) and polylactic acid (PLA) both biodegradable plastics, through fermentation. Besides, his team has also successfully constructed optimal cell factories for synthesis of a series of medicine compounds including ginsenoside (effective constituent of an anti-cancer drug), lycopene, and beta-carotene.

Actually there exist in nature some microbes that can produce succinic acid, the monomer for PBS in the process of their fermentation based on sugar, including some species of *Actinobacillus*, *Anaerobiospirillum* and *Mannheimia*. Unfortunately, more or less they have some setbacks. For example none of these microbes is good in terms of conversion rate, a property critical in production costs: at the best only 0.8 g succinic acid can be converted from 1.0 g of sugar, with a significant percentage of carbon atoms lost to the synthesis of other organic acids. On the other hand, they require rich medium for fermentation. These setbacks could lead to increased costs and very complicated downstream extraction processing, largely lowering the value of such microbes in industrial application.

Images/photos by courtesy of Prof. ZHANG X.

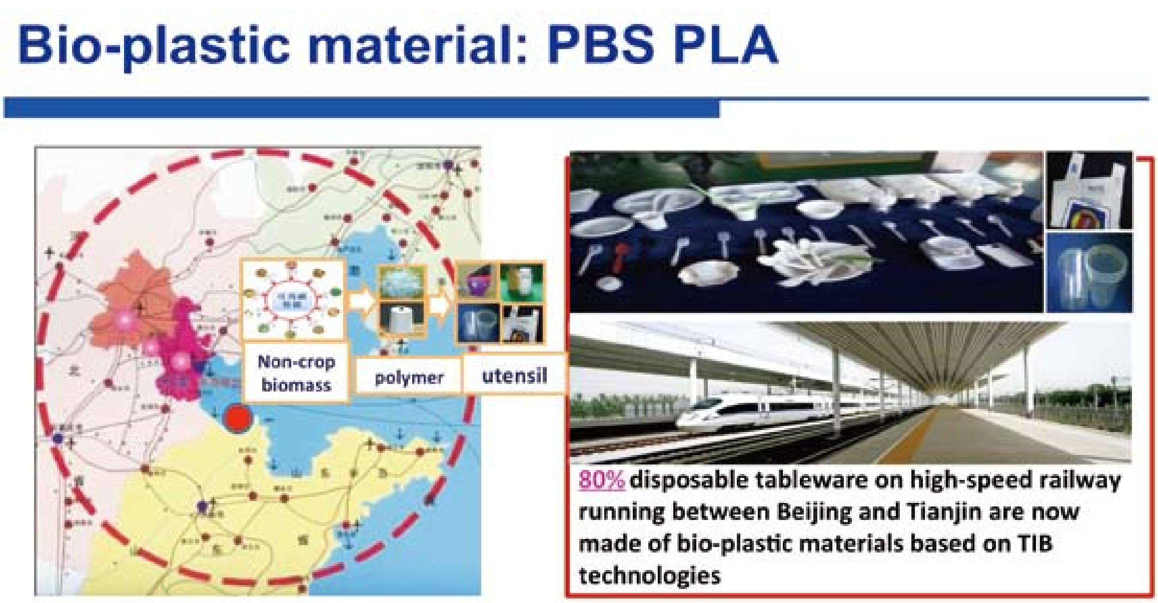
ZHANG at work.

"Therefore we forsook them for *Escherichia coli*," ZHANG says when explaining their strategy: "because their genetic background is very clear and hence easier for us to modify and optimize via techniques of systems microbiology, despite the fact that they only accumulate very little succinic acid as the result from their fermentation."

Based on the genome-scale metabolic network of *Escherichia coli*, ZHANG's team designed an optimal synthesis pathway of succinic acid, channeling all the carbon atoms of sugar to the synthesis of succinic acid. According to this pathway, the team knocked out the genes involved in competing synthesis pathways (namely those for products other than succinic acid), and activated phosphoenolpyruvate carboxykinase to increase the energy supply for the synthesis of succinic acid. Further, they enhanced the cellular growth and the succinic acid production through metabolic evolution technology. After 2,300 generations of evolution, a genetically modified strain of *E. coli* emerged as the first generation of cell factory for succinic acid production.

To further optimize this cell factory, the team investigated the genetic mechanism underlying its enhanced productivity of succinic acid, and identified the involved mutations. Through targeted manipulation of involved genes, they obtained the second generation of cell factory for succinic acid production.

An attractive part of this strain is, it feeds on sugar and simple mineral salts medium and yields the target product at a very high efficiency. The complete package of technologies has been transferred to a biotech company in Shandong Province of China. Capable of yielding 100 g of succinic acid in a litter of culture, it demonstrates a conversion yield of 1 g/g (or 1.53 mol/mol) in a pilot scale



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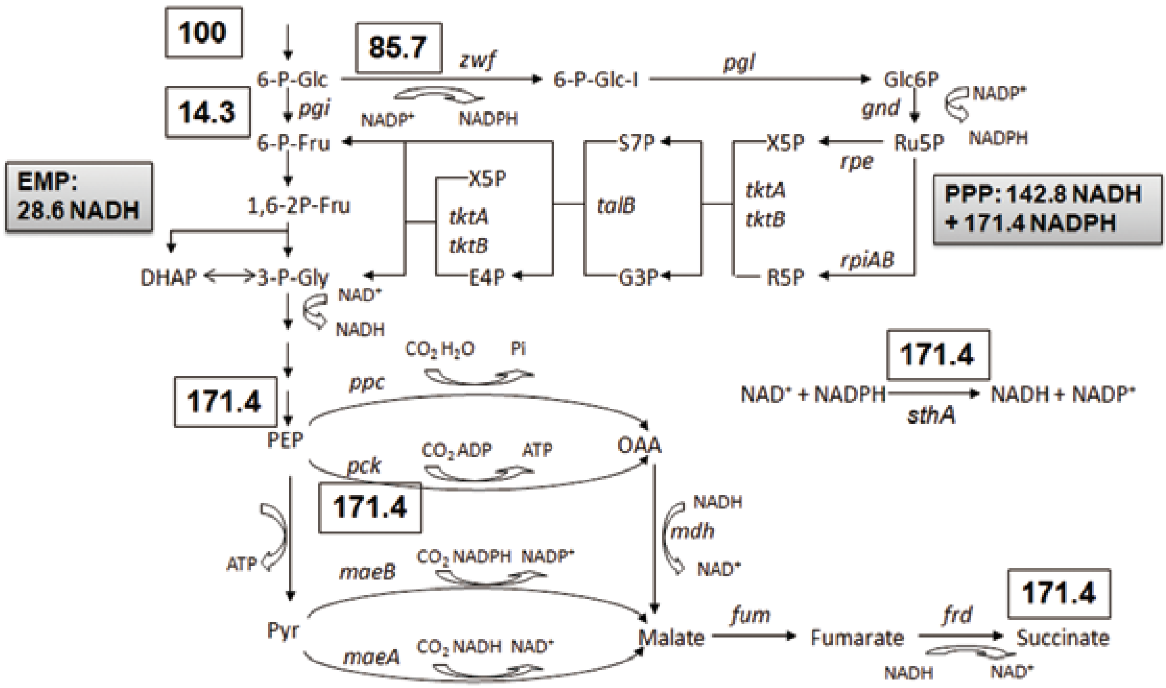
Bio-plastic materials produced from non-grain biomass based on TIB technologies.



EMP:	14.3 Glucose = 14.3 PEP + 28.6 NADH
PPP:	85.7 Glucose = 142.8 PEP + 142.8 NADH + 171.4 NADPH + 85.7 CO_2
EMP+PPP:	100 Glucose = 171.4 PEP + 171.4 NADH + 171.4 NADPH + 85.7 CO_2
SthA:	171.4 NADPH + 171.4 NAD^+ = 171.4 NADH + 171.4 $NADP^+$
PEP-Suc:	171.4 PEP + 171.4 CO_2 + 342.8 NADH = 171.4 Succinate + 171.4 ATP
Total:	**100 Glucose + 85.7 CO_2 = 171.4 Succinate + 171.4 ATP**

Shown is an optimized synthesis pathway for succinic acid, the monomer for a biodegradable plastic PBS, in *Escherichia coli* designed by ZHANG's group at TIB. Based on this they successfully developed a strain highly efficient at production of this PBS monomer.

test conducted in a fermentation tank of 10 m^3. As a result, the comprehensive costs for succinic acid production were reduced to 12,000 yuan/ton, a decrease of 20% compared with petrochemical method. It is anticipated that an industrial production line with an annual capacity of 50,000 tons will be completed by the end of 2015, representing the biggest of such kind in the world.

Employing similar methods, ZHANG's team successfully removed the technical barriers in fermentation-based production of D-lactic acid, the monomer for PLA, and constructed an optimal cell factory based on *E. coli*. Now this technology has been transferred to another company in Shandong and proven successful in a pilot test, yielding 130 g of D-lactic acid per liter of culture, and demonstrating a conversion yield of 0.95 g/g. It is anticipated that an industrial production line capable of producing 3,000 tons of D-lactic acid a year will be built within 2015.

An encouraging sign is, biodegradable plastic materials based on technologies developed by ZHANG's group is now being used on trains running on the high-speed railway between Beijing and Tianjin: about 80% of disposable tableware, cups and plastic bags on these trains are made of PBS/PLA developed via their technologies.

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