Lipase-catalyzed production of biodiesel

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Biodiesel refers to fatty acid methyl esters (FAME) synthesized from natural triglycerides (TG) through trans-esterification reaction with alcohols. It has attracted a great attention during the past decade as a renewable and biodegradable fuel. As a supplementary fuel for diesel engine, it greatly reduces the amount of SO_2, halogens, soot and CO in the exhaust gas, which are major contributors to environmental pollution. Chemical process using alkali-catalyst has been the major and commercialized process for the transesterification of TG to biodiesel. But it has several drawbacks, such as, difficulties in the recovery of pure products (FAME and glycerol), the need for removal of catalyst, and the energy-intensive nature of the processes. Conversion of TG to methyl- or other short-chain alcohol esters can be also catalyzed by lipase in a single transesterification reaction. Lipase-catalyzed production of biodiesel is a low energy-demanding process and simplifies the process of product recovery. At present, the main hurdle of commercialization of this system is the cost of lipase production.

To develop a cost-effective process for lipase-catalyzed biodiesel production in this study, we have improved the biocatalyst. Several recombinant lipases of microbial source were evaluated for their efficacy of bioconversion of soy oil to biodiesel. GC and TLC analyses of biodiesel, produced by each lipase, revealed that lipase B of *Candida antarctica* (CalB) showed higher methanolysis activity and produced considerably lower amount of fatty acids even under 2% of water content than other lipases. Directed evolution has been performed for the improvement of catalytic activity and methanol resistance of CalB. A mutant lipase, CalB14 acquired from seven thousand tested mutants showed 11 times greater activity and higher resistance to methanol compared to the wild type. CalB14 could be efficiently produced up to about 0.8 g per liter by recombinant *Saccharomyces cerevisiae*. Concentrated fermentation broth containing recombinant CalB14 could be directly used for biodiesel production with higher conversion rate than wild type. For the repeated use of CalB14 as a biocatalyst for biodiesel production, two different strategies of enzyme immobilization using yeast cell surface display system and adsorption to hydrophobic resin have been investigated. Biodiesel production with such immobilized lipase will be discussed.