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Production of high carbon atom biofuels from lignocellulosic biomass using fusion technology to create new chimera Clostridia species

Omar Bagasra, Zhabiz Golkar, Angelina Hargrove and Janelle Robinson
Claflin University, USA

Ethanol, the most widely used renewable liquid transportation fuel, has only 70% of the energy content of gasoline and its hygroscopicity makes it incompatible with existing fuel storage and distribution infrastructure. Advanced biofuels with high-energy content and physiochemical properties similar to petroleum-based fuels may be better alternatives, as they would allow use of existing combustion engine designs, distribution systems and storage infrastructure. Recently there has been an increased interest to convert sugars from lignocellulosic biomass into butanol. Due to its physical properties, the four-carbon butanol is a better replacement for gasoline than ethanol. Many different Clostridia have been utilized in butanol fermentation, although these gram-positive anaerobes coproduce butanol with a few byproducts, such as butyric acid, acetone, ethanol, therefore lowering its yield. From a biotechnology perspective, the lack of efficient genetic tools to manipulate Clostridia hinders metabolic engineering efforts for the optimization of butanol synthesis and the reduction of by-product formation. In addition, alcohols are toxic to microbes at higher concentrations. Because of these two major hurdles, we created new species of thermophilic Clostridia by a novel bacterial fusion technology. Therefore, instead of transfer of enzyme coding genes in cassettes by recombinant technology, we created entirely new microorganisms by fusing two species of Clostridia. This new Clostridia can carry out fermentation in a single vessel at relatively thermophilic temperatures (i.e. 45°C to 65°C). Use of this microorganism eliminates the need for multistep, multi-vessel, low temperature reaction system and brings about a single vessel system for the direct conversion of lignocellulosic biomass to butanol and other economical important chemicals. Because of its thermophilic quality, the alcohols are evaporated under vacuum at 65°C, eliminating the toxic effects of the alcohols. Since, new Clostridia microorganisms are not naturally occurring in wild by total genomic fusion process, we have tentatively named these strains Clostridium thermobutanolicum (which will be subject to verification by Nomenclature Committee of the International Society for Microbiology in the future).

ifarauc@yahoo.com