Strains and Genes that Facilitate Genetic Manipulation of Hyperthermophiles

TECHNOLOGY CASE
1576

INVENTORS

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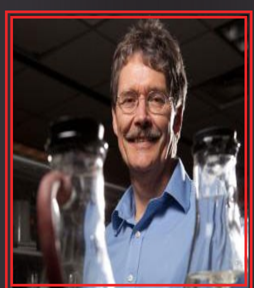

PATENT INFORMATION

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Introduction

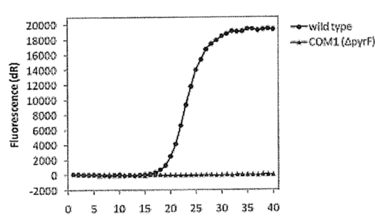
It would be difficult to overestimate the contribution of genetic manipulation to the study of any biological system, and it is an essential tool for the metabolic engineering of biosynthetic and substrate utilization pathways. This is particularly true for the archaea since, in spite of their environmental and industrial importance, coupled with their unique molecular features, much remains to be learned about their biology. The marine hyperthermophilic anaerobe *Pyrococcus furiosus* is of special interest not only for its ability to grow optimally at 100° C and the implications of this trait for its biology but also for industrial applications of its enzymes, as well as its capacity to produce hydrogen efficiently in a highly efficient manner. The development of genetic systems in the archaea, in general, presents many unique challenges given the extreme growth requirements of many of these organisms. To date, genetic systems of various levels of sophistication have been developed for representatives of all major groups of archaea, including halophiles, methanogens, thermoacidophiles, and hyperthermophiles. One of the most significant barriers to genetic manipulation of archaea, in general, and hyperthermophiles, in particular, is the lack of selectable markers. Antibiotic selection strategies used in mesophilic bacteria are typically ineffective because the molecular machineries of archaea are not affected by the antibiotic.

Summary

The UGA teams led by Drs. Janet Westpheling and Michael W.W. Adams developed methods for transforming *P. furiosus* with any given polynucleotide. The number of transformants may be at least 10^3/ µg(DNA) and such number can be even higher than 10^5/ µg(DNA). The polynucleotide can be circular or linear. Using this method, several modifications of *P. furiosus* have been developed. Plasmids of the invention are stable and remain unchanged for more than 100 generations of the recipient organism. Highly competent strains of *P. furiosus* have been developed and those have been transformed at frequencies much higher than wild type (e.g., DSM3638). Isolated P. furiosus of the invention are suitable to undergo diverse, industrially useful modifications, for the controlled production of enzymes, production of chemicals (including H_2).

Advantages and Some Potential Applications

- Facile and reproducible leading to a very high titer of transformants
- Applicable to other hyperthermophiles and, *potentially*, to other extremophiles
- Provides strains (e.g. COM1) that are suitable to undergo subsequent modifications of interest to the end user



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Innovative Solutions to Global Needs

Overcoming DNA Restriction Barriers to DNA Transformation of the *Caldicellulosiruptor* Species

TECHNOLOGY CASE
1666

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USPTO Link

Introduction

Sugars contained within naturally occurring biomass can be transformed into ethanol, other fuels and commodity biochemicals. A specific type of biomass, lignocellulose (the main component of agricultural residues, such as corn stover and plant bagasses, and of wood residues, such as sawmill and paper mill discards) has great potential as a biofuel source, since it contains several high volume sugar components, including cellulose. However, the carbohydrate polymers in lignocellulose are tightly bound to the lignin fraction and their direct conversion into biofuels is hindered by this feature ("recalcitrance"). Historically, the cost of biomass-based fuels has not been competitive relative to oil or other energy resources because the biochemical conversion step, where plant sugar is converted to fuel, is made difficult by recalcitrance and has been a primary bottleneck. A key challenge in converting lignocellulosic biomass into fuels is that plants have built up a natural protection to fermentation microbes and enzymes, due to the afore-mentioned factors.

Summary

The group led by Dr. Janet Westpheling at UGA's Dept. of Genetics developed a method to more efficiently decompose recalcitrant biomass, which could potentially lower the cost of producing biofuel. The method centers around the bacterium *Caldicellulosiruptor bescii ("Caldi")*, which has unique characteristics that make it more conducive for processing lignocellulose. Genetic modifications of Caldi improve the efficiency of converting biomass into, for instance, fuels.

To overcome recalcitrance issues, the inventors have developed methods for modifying Caldi. These researchers are the first to report success of redesigning the *Caldicellulosiruptor* genus into a more effective biomass catalyst. The researchers have utilized this bacterium in the same manner that organisms such as *E. coli* and *S. cerevisia* are traditionally used in biofermentation. In this invention, the use of *Caldi* obviates the need for chemical pre-treatment of biomass. The engineered Caldi genome also includes sequences for cellulases, glycoside, hydrolase and transporters that are important in, and accelerate, biomass decomposition. The researchers have achieved the ability to conduct, in a simple manner, multiple and simultaneous modifications of Caldi. The ability to genetically manipulate "Caldi" species allows the functional identification of genes involved in biomass deconstruction to occur, and also allows the metabolic engineering of these strains for the production of biofuels and multiple other commodity biochemicals, such as alcohols, furans, lactones and long chain acids and keto-acids.

The researchers have also created specific techniques for selecting deletions with the ability to remove the selectable marker, thus allowing the construction of multiple mutations in the same strain. The same methods will also allow multiple gene knockouts and other modifications, or the technique could be used for replacing promoters and regulatory regions. These general procedures can potentially be utilized across other extremophile organisms

Advantages and Some Potential Applications

- The bacterium *Caldi cellulosiruptor* has special decomposition properties that enable it to be more effective in converting lignocellulosic biomass into fuel
- Provides the basis for engineering a versatile organism for the production of multiple chemicals of industrial interest
- The proprietary modification technique can be applied to other members of the genus, as well as other extremophile organisms

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Metabolic Engineering of *Caldicellulosiruptor bescii* for the Production of Biofuels and Bioproducts

TECHNOLOGY CASE
1936

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Introduction

Biomass is a renewable resource that can be processed into myriad of chemicals and has shown promise to replace petroleum-based fuels and other materials. Sugars contained within naturally occurring biomass can be transformed into ethanol and other products. However, the cost of biomass-based fuels historically has not been competitive relative to oil or other energy resources. The biochemical conversion step, where plant sugar is converted to fuel, has been very expensive. The presence of lignin, lignocellulose and other compounds hinder direct processing of biomass by bacteria, yeasts and enzymes. This hindrance, also known as “biomass recalcitrance”, often requires expensive pretreatment of biomass prior to its transformation into useful products such as biofuels.

Caldicellulosiruptor bescii, a thermophilic bacterium, has a high affinity for decomposing lignocellulosic biomass, which includes agricultural residues such as rice straw, switchgrass, as well as hard- and softwoods. Lignocellulosic biomass consists of cellulose, hemicellulose, pectin and lignin. *Caldicellulosiruptor* are among the most thermophilic and cellulolytic bacteria, which enables them to degrade untreated biomass. This characteristic makes this organism especially appealing to biomass processing. However, its inability to produce compounds of economic interest require that the organism be modified in order to enable its use in industrial processes for the production of biofuels and other commodity chemicals.

Summary

The group led by Prof. Janet Westpheling at UGA’s Dept. of Genetics invented a method, involving the use of *C. bescii*, to more efficiently decompose biomass. The *Caldicellulosiruptor* bacterium has unique properties for processing a type of lignocellulosic biomass. By genetically manipulating *C. bescii* through the introduction of key genes (thus altering the organism’s metabolism) that allow the production of desired chemicals from biomass, the researchers have shown that ethanol can be more easily created.

Specifically, the researchers have developed methods for genetic manipulation of members of this *Caldicellulosiruptor* genus, enabling them to produce ethanol and hydrogen from biomass. For example, genes from *C. thermocellum*, which is the best-known thermophilic ethanol producer, were cloned and introduced into *C. bescii*. Other genes, from *Clostridium thermocellum* can similarly be introduced allowing the bacterium to convert acetate to acetaldehyde. Furthermore, the same approach can be used to produce hydrogen. Hence, the ability to engineer *C. bescii* offers the possibility of direct conversion of biomass to biofuels and bioproducts commodity biochemicals, such as alcohols,

Advantages and Some Potential Applications

- Modified *Caldicellulosiruptor bescii* bacterium has special decomposition properties that enable the more efficient conversion of lignocellulosic biomass into fuel.
- This novel invention of introducing key genetic traits into *C. bescii* will significantly reduce the high cost of biomass conversion by eliminating expensive enzymes, yeasts, and bacteria.
- Increased biomass-to-biofuel efficiency using this modified *C. bescii* will have a major impact on the energy industry by decreasing production cost, thus making it more economical to produce biomass-based ethanol versus petroleum-based fuels.

TECHNOLOGY CASE
2007

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Construction of a Stable Replicating Shuttle Vector for *Caldicellulosiruptor* species: Extending Genetic Methodologies to other Members of This Genus of This Genus

USPTO Link

Introduction

Biomass is a renewable resource that has shown promise to replace petroleum-based fuels while reducing greenhouse gas emissions. A key challenge towards achieving an economically viable biomass solution is that plants have built up a natural protection (or recalcitrance) against being converted to fuel. Therefore, more effort using special enzymes and microbes is needed to convert biomass into ethanol. In order to become more widely adopted, the high cost for biomass processing needs to be reduced.

A special type of bacterium, *Caldicellulosiruptor bescii*, has a high affinity for decomposing lignocellulosic biomass that includes agricultural residues such as rice straw, switchgrass, as well as hard- and softwoods. However, the products of *C. bescii's* degradation of biomass are not effective as fuels. That is, these naturally occurring processes do not produce compounds of great economic interest. However, given its high affinity for decomposition of, and its ability to grow on, untreated biomass, *C. bescii* makes an attractive candidate for genetic modifications that could better enable its use in the production of biofuels and other commodity chemicals.

The group led by Prof. Janet Westpheling at UGA's Dept. of Genetics developed a genetic tool that allows for transformations of *Caldi*, making it a more viable organism for the degradation of recalcitrant biomass and production of biofuels and commodity chemicals. The researchers have leveraged the special properties of thermophiles, organisms that grow at relatively high temperatures, which lead to better biomass conversion. The *Caldicellulosiruptor* genus is the most thermophilic cellulolytic microbes known, and therefore this genus was targeted towards improving its efficiency for biomass conversion.

To improve the effectiveness of the *Caldicellulosiruptor* species, those researchers constructed a replicating shuttle vector that enables advanced characterization and manipulation of genes and metabolic pathways to enhance biomass conversion. This is the first vector of its kind for members of this genus. Their technique also utilizes a native plasmid in this genus to generate a replicating vector.

Summary

The group led by Prof. Janet Westpheling at UGA's Dept. of Genetics developed a replicating shuttle vector based on a small plasmid for Caldicellulosiruptor bescii. The entire plasmid was cloned into an E. coli cloning vector. The shuttle vector enables modification of several members the genus (including C. hydrothermalis) providing them with features that are desirable to improve biomass-to-biofuel conversion. There was no evidence of DNA rearrangement during transformation and replication in C.

Advantages and Some Potential Applications

- The C*aldicellulosiruptor* genus comprises the most thermophilic cellulolytic microbes and therefore is well suited for biomass conversion
- Researchers have developed the first replicating shuttle vector for *Caldicellulosiruptor* species which is applicable to other members of the genus
- Applicable to other members of the genus, and other closely-related thermophiles, allowing for expansion of practical use of, and research on, these organisms.

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