Strains and Genes that Facilitate Genetic Manipulation of Hyperthermophiles

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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 $10^3/\mu g(DNA)$ and such number can be even higher than $10^7/\mu g(DNA)$. 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
Overcoming DNA Restriction Barriers to DNA Transformation of the *Caldicellulosiruptor* Species

**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. cerevisiae* 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
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.
Advantages and Some Potential Applications

- The *Caldicellulosiruptor* 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.