Sequestration of Carbon Dioxide with Hydrogen to Useful Products

TECHNOLOGY CASE
1563

INVENTORS

Michael W. W. Adams,

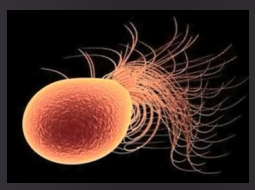
R.M. Kelly.

PATENT INFORMATION

US Publ. 20150211030

CONTACT INFO

Gennaro Gama, Ph.D.
Ph: 706-583-8088
Fx: 706-542-3837
GJG@uga.edu
Homepage:
http://research.uga.edu/gateway/



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Introduction

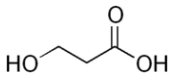
Carbon dioxide is chemically stable and unreactive, and must be reduced to enable its incorporation into biological molecules. Autotrophic microorganisms are able to utilize carbon dioxide as their sole carbon source and a variety of pathways are known to activate and incorporate it into biomolecules essential for growth and replication. Recently, carbon dioxide fixation pathways have received interest for biotechnological applications, since this could provide biological routes for *de novo* generation of fuels and small (C_1 to C_6) organic molecules. There are currently at least six natural pathways for the incorporation of inorganic carbon dioxide into cellular carbon . The most recently discovered of these are found exclusively in extremely thermophilic archaea: the 3-hydroxypropionate/4-hydroxybutyrate (3HP/4HB) carbon fixation cycle and the dicarboxylate/4-hydroxybutyrate (DC/4HB) cycle.

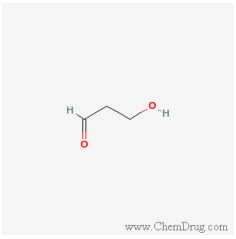
Summary

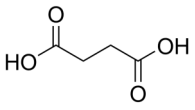
As a product of a collaboration between UGA and NCSU researchers, certain enzymes from an extremophile and associated with the (3HP/4HB) cycle have been identified and characterized biochemically in their native or recombinant form. Organisms were specially engineered to benefit from this cycle, and these organisms were capable of producing 3-HPA in good yield, from H_2 and CO_2. Conversion of CO_2 and H_2 into 3-HPA can be accomplished in cell-free extracts as well. Levels of production of 3HPA were determined to be substrate-dependent and ranged from 15% up to 100% of theoretical yield. Yields ranged from 70 nmol/mL to 155 nmol/mL.

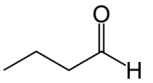
Advantages and Some Potential Applications

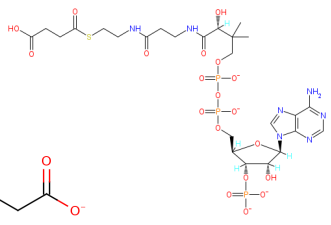
- Facile and reproducible leading to a very high titer of transformants
- Based on a well-established microorganism
- Strains can be engineered to be capable to produce a multitude of commodity chemicals in very good titer (e.g., 3HP and other C_3 chemicals, C_4 chemicals derived from hydroxybutyrate)
- Eliminates the need for culturing extremophiles at very high temperatures

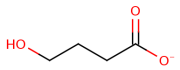












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Innovative Solutions to Global Needs

Strains and Genes that Facilitate Genetic Manipulation of Hyperthermophiles

TECHNOLOGY CASE
1576

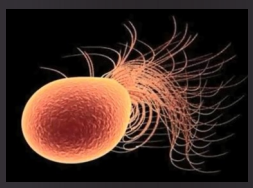
INVENTORS

Michael W. W. Adams,
J.A. Farkas, G. Lipscomb,
and Janet Westpheling

PATENT INFORMATION

US Pat. 8927254

CONTACT INFO
Gennaro Gama, Ph.D.
Ph: 706-583-8088
Fx: 706-542-3837
GJG@uga.edu
Homepage:
http://research.uga.edu/gateway/



USPTO Links

Introduction

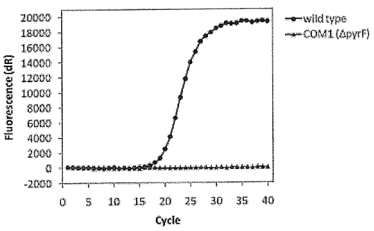
It would be difficult to overestimate the contribution of genetic manipulation to the study of any biological system, and it is an essential tool for the metabolic engineering of biosynthetic and substrate utilization pathways. This is particularly true for the archaea since, in spite of their environmental 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

Methods for Expressing Polypeptides in Hyperthermophiles

TECHNOLOGY CASE
1889

INVENTORS

Michael W. W. Adams, R.M. Kelly, M. Basen, G. Lipscomb, and A. Menon

PATENT INFORMATION

US Publ. 20140248687

CONTACT INFO

Gennaro Gama, Ph.D.
Ph: 706-583-8088
Fx: 706-542-3837
GJG@uga.edu
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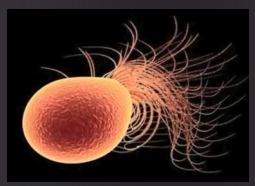

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Introduction

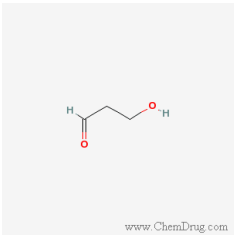
Since the discovery of hyperthermophiles in the 1980s , hyperthermophiles have attracted a great deal of attention due to their ability to grow optimally at temperatures above 80°C. Virtually all are classified within the archaeal domain rather than the bacterial domain. In addition to their evolutionary implications, hyperthermostable enzymes are of high biotechnological interest, since many industrial processes are facilitated by elevated temperatures and organisms that grow under such conditions can be used without risk of contamination. Although the ability to metabolically engineer microorganisms is a prerequisite for their utility as whole-cell biocatalysts, the genetic manipulation of hyperthermophiles is a very recent development. Targeted modifications of the chromosome have been reported for some microorganisms growing optimally near 80°C or so, which include *S. acidocaldarius* [T_{opt} = 80°C] and *S. solfataricus* [T_{opt} = 75°C].

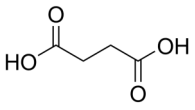
Summary

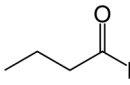
Insprired by their previous work (US Publ. 20120135411) on the extremophile *P furiosus* [T_{opt}=100°C], he group led by Prof. Michael Adams (Biochemistry and Molecular Biology) developed general methods for the engineering of archea. The method includes culturing a GM archaeon, wherein the archeon includes a heterologous polynucleotide that has a promoter operably linked to a coding region. Culturing can be carried out at temperatures *at least* 20°C lower than T_{opt} for any given archeon. The method also allows for increased activity of the encoded polypeptide at temperatures below T_{opt}. The method is applicable to a myriad extremophiles such are *Thermococci, Sulfolobi, Pyrococci, Anarocelli, Caldicellulosiruptor* and others. The coding regions encoding activity of—*for instance*— acetyl/propionyl-CoA carboxylase, malonyl/succinyl-CoA reductase, NADPH-dependent hydrogenase, or 4-hydroxybutyrate-CoA snthetase activity have been used in this work and corresponding engineered organisms were successfully cultured at relatively low temperatures [< T_{opt}]. Several of the engineered organisms were tested for their ability to produce certain commodity chemicals such as 3-HP, and succinic semialdehyde, for example.

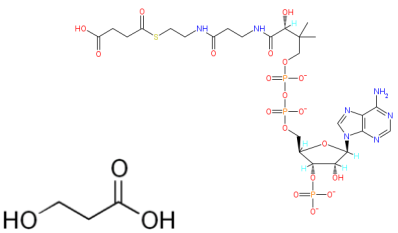
Advantages and Some Potential Applications

- Facile and reproducible leading to a very high titer of transformants
- Applicable to a myriad of extremophiles
- Strains can be engineered to be capable to produce a multitude of commodity chemicals in very good titer (e.g., 3HP and other C_3 chemicals, C_4 chemicals derived from succinate)









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