INDO SWISS JOINT RESEARCH PROGRAMME (ISJRP)

JOINT RESEARCH PROJECT

FINAL REPORT

Submitted by the Swiss Principal Investigator(s)

Part 1 - General Information

<table>
<thead>
<tr>
<th>Project Title:</th>
<th>Utilization of xylose for production of polyhydroxyalkanoates (PHAs)</th>
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<tbody>
<tr>
<td>Swiss Principal Investigator(s):</td>
<td>Dr. Qun Ren</td>
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<td>9014 St. Gallen</td>
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<td>Project Start:</td>
<td>July 1, 2009</td>
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<td>Project Duration:</td>
<td>37 months + 3 months extension</td>
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<td>Indian Principal Investigator(s):</td>
<td>Rakesh K Jain (deceased)</td>
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Part 2 - Scientific Information

A) SYNTHESIS OF THE PROJECT

Plastics have become an integral part of the contemporary life because of many desirable properties such as durability and resistance to degradation. However, an increase in public concern about the harmful effects of petrochemical-derived plastic materials to the environment has created much interest in the development of biodegradable plastic materials. These biodegradable plastic materials must retain the desired properties of the conventional synthetic plastics, and should be completely degraded without leaving any undesirable residues when discarded. Among the candidates of the biodegradable plastics, polyhydroxyalkanoates (PHAs) have been drawing much attention because of their material properties being similar to various synthetic thermoplastics and elastomers currently in use and their complete biodegradability to water and carbon dioxide (or methane under anaerobic conditions) upon disposal by microorganisms in various environments such as soil, sea, lake water and sewages. PHAs are produced by a number of bacteria under nutrient depletion conditions as intracellular storage materials of carbon and energy. Composition of the carbon substrate used for fermentation and deployment of appropriate bacterial strain control the copolymer production where substrate cost represents nearly 40% of the total cost. The price for PHAs is still too high to be used as
bulk materials as compared to that of the chemically synthesized ones. Therefore, the isolation and development of strains that overproduce PHAs from a cheap carbon source is desired. Cheaper raw materials, such as whey, wastewater from olive mills, molasses, corn steep liquor, starchy wastewater, palm oil mill effluent, have been used as nutrient supplements for bacterial PHA production. However, despite the fact that these are abundant and relatively inexpensive commodities, development of a truly cost effective PHA fermentation requires an even less expensive feedstock. Therefore, the aim of the present work is to produce PHAs using xylose-degrading microorganisms or heterologous host(s) capable of utilizing xylose, a major waste product of the paper industry.

B) RESULTS

Part One

Mcl-PHA production in engineered P. putida KT2440 strain by sequential feeding of xylose and octanoic acid (http://www.biomedcentral.com/content/pdf/1472-6750-12-53.pdf)

Background

Pseudomonas putida KT2440 is able to synthesize large amounts of medium-chain-length polyhydroxyalkanoates (mcl-PHAs). To reduce the substrate cost, which represents nearly 50% of the total PHA production cost, xylose, a hemicellulose derivate, was tested as the growth carbon source in an engineered P. putida KT2440 strain.

Results

The genes encoding xylose isomerase (XylA) and xylulokinase (XylB) from Escherichia coli W3110 were introduced into P. putida KT2440. The recombinant KT2440 exhibited a XylA activity of 1.47 U and a XylB activity of 0.97 U when grown on a defined medium supplemented with xylose. The cells reached a maximum specific growth rate of 0.24 h^{-1} and a final cell dry weight (CDW) of 2.5 g L^{-1} with a maximal yield of 0.5 g CDW g^{-1} xylose. Since no mcl-PHA was accumulated from xylose, mcl-PHA production can be controlled by the addition of fatty acids leading to tailor-made PHA compositions. Sequential feeding strategy was applied using xylose as the growth substrate and octanoic acid as the precursor for mcl-PHA production. In this way, up to 20% w w^{-1} of mcl-PHA was obtained. A yield of 0.37 g mcl-PHA per g octanoic acid was achieved under employed conditions.

Conclusions

Sequential feeding of relatively cheap carbohydrates and expensive fatty acids is a practical way to achieve more cost-effective mcl-PHA production. This study is the first reported attempt to produce
mcl-PHA by using xylose as the growth substrate. Further process optimizations to achieve higher cell density and higher productivity of mcl-PHA should be investigated. These scientific exercises will undoubtedly contribute to the economic feasibility of mcl-PHA production from renewable feedstock.

**Part two**

**Biosynthesis of poly(4-hydroxybutyrate) (P4HB) from xylose**

**Background**

One of the most promising PHAs for medical applications is the poly(4-hydroxybutyrate) (P4HB) which is the first and only PHA-based product approved by the FDA in 2007 for clinical application (TephaFLEX®) as absorbable suture. P4HB is a strong, flexible and absorbable material, which leads to wide variety of medical applications like tissue engineering and drug delivery. In addition, P4HB is biocompatible and extremely well tolerated in vivo given that the hydrolysis of P4HB yields 4HB which is already present in the human body [1]. For production of P4HB, Hein and coworkers reported that the introduction plasmid pKSSE5.3 carrying PHA synthase gene (phaC) from *Rastonia eutropha* and a 4-hydroxybutyric acid-coenzyme A transferase gene (orfZ) from *Clostridium kluyveri* enabled *E. coli* strains to produce P4HB when 4HB was supplied in the culture medium [2] (Fig. 1). The production of P4HB in recombinant *E. coli* has also been investigated by using glucose as the growth substrate and it was found that P4HB production is related to cell growth [2, 3]. In this study, we aim to use xylose instead of glucose because xylose as the carbon source has potential to reduce the total cost of P4HB production.
Summary of Results

In this study, we investigated different *E. coli* hosts for P4HB production. It was found that the best host was *E. coli* JM109. Different growth conditions were also studied such as temperature and the cell physiological stages for P4HB synthesis. Unlike what was previously reported, the P4HB synthesis was found to be un-related to cell growth, namely P4HB synthesis mainly takes place after the end of the exponential growth phase. Under the tested conditions, P4HB content in the range of 58 to 70% (ww⁻¹) and P4HB concentrations in the range of 2.76 to 4.33 g L⁻¹ could be obtained with a conversion yield $Y_{P4HB/Na4HB}$ of 92 %. These results were achieved here by one single batch culture, which was only possible previously through fed-batch high cell density cultures.
Part three

P4HB production by high cell density culture

Background

Much effort has been devoted to lowering the production cost of PHAs by developing better bacterial strains and more efficient fermentation and recovery processes. To date, only P3HB and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) are commercially available. Despite of the advantages of P4HB for medical applications, there is only one report available describing its production process[3]. In this report the P4HB concentration and P4HB content of 4.4 g L\(^{-1}\) and 36% were obtained, respectively, through fed-batch cultivation. During the current project we could achieve similar yields by a single batch culture (see Part two). We decided to investigate whether the yields achieved in our batch culture could be further improved by fed-batch culture. Thus, different feeding modes in fed-batch cultures were studied: pulse-feeding, linear-feeding and exponential-feeding. Different feeding rates were also tested. This study aims to develop a laboratory scale method of producing P4HB.

Summary of Results

In conclusion, P4HB synthesis from glycerol in recombinant E. coli could be stimulated by simply adding acetic acid at the beginning of the cultivation. The pulse feeding mode was the best for obtaining high P4HB concentrations compared to exponential or linear feeding modes. 15 g L\(^{-1}\) P4HB could be obtained in 64 h, which is more than 3 fold higher than what has been reported previously[3]. Our results demonstrate that recombinant E. coli is a good candidate for production of P4HB.

References

C) LIST OF PUBLICATIONS

Please list ALL publications produced under this ISJRiP project.

Publications in reviewed journals


Sylvaine Le Meur, Manfred Zinn, Thomas Egli, Linda Thöny-Meyer and Qun Ren. High level production of poly(4-hydroxybutyrate) (P4HB) by high cell density cultivations of recombinant *Escherichia coli*. To be submitted to Biotechnology and Bioengineering (impact factor 3.95).

Award

SGVC Science Award Winner 2012. Sylvaine Le Meur, Manfred Zinn, Thomas Egli, Qun Ren. Utilization of low-cost agricultural feedstock as carbon source for production of mcl-PHAs by engineered strains. (SGVC: Swiss Process and Chemical Engineers)

Invited oral presentations

Sylvaine Le Meur, Manfred Zinn, Qun Ren. Utilization of low-cost agricultural feedstock to produce mcl-PHAs by engineered strains. 5th Summer School in Advanced Biotechnology, Palermo, Italy. August 31- September 4, 2010.


Sylvaine Le Meur, Manfred Zinn, Thomas Egli, Qun Ren. Utilization of low-cost agricultural feedstock to produce mcl-PHAs by engineered strains. 1 DAY SYMPOSIUM Biocatalysis, Dübendorf, Switzerland. January 18, 2012.

**Poster presentations**

**Sylvaine Le Meur, Manfred Zinn, Qun Ren.** Utilization of low-cost agricultural feedstock to produce mcl-PHAs by engineered strain. International Symposium on Biopolymers ISBP 2010, Stuttgart, Germany. October 3-7, 2010.

**Sylvaine Le Meur, Manfred Zinn, Qun Ren.** Production of polyhydroxyalkanoates (PHAs) by engineered strains using low cost agricultural feedstock. The 4th Congress of European Microbiologists, Geneva, Switzerland. June 26-30, 2011.