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Engineering *Ralstonia Eutropha* to Convert CO_2/Waste Stream into Useful Chemicals Using a Synthetic Biology Approach

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Abstract

CO_2 emissions in the atmosphere are increasing dramatically every year. Some organisms such as *Ralstonia eutropha*, have the ability to assimilate CO_2 and convert it into value-added chemicals. The use of synthetic biology approaches may contribute for the bio-based economy to synthesize products such as bio-plastics. *R. eutropha* can be used as a cell factory for chemical production due to its diverse biochemical pathways for growth and biosynthesis that can achieve industrial production scale, but synthetic biology approaches still have to be enhanced pursuing the creation of robust industrial strains capable of producing chemicals that can compete with petroleum-derived product prices.

Keywords Bio-plastics; Carbon dioxide; Polyhydroxybutyrate (PHB); *R. eutropha*; Synthetic biology.

1. Introduction

Ralstonia eutropha (also known as *Cupriavidus necator*) is a soil bacteria isolated about 50 years ago. This gram-negative bacterium is widely regarded as the knallgas bacterium for its hydrogen-oxidizing capability. *R. eutropha* is capable of growing under heterotrophic, autotrophic and mixotrophic conditions, which is of major interest to biological engineers. *R. eutropha* is capable of metabolising different carbon sources such as sugars, organic acids and aliphatic acids, and it has a CO_2-fixing ability under autotrophic conditions using H_2 or formate as an energy source [1]. One of the most attractive features of *R. eutropha* is its ability to accumulate intracellular poly[(*R*)-3-hydroxybutyrate] (PHB) polymers. PHB are biodegradable polymers that have thermoplastic and mechanical properties similar to petroleum-based plastics and can be accumulated up to 70% of total cell weight in *R. eutropha. R. eutropha* is of biotechnological interest due to its capability to synthesize other value-added chemicals such as propylene, acetone, cyanophycin, feluric acid, 2-methylcitric acid, diesel range methyl-ketones, isobutanol and other chemicals, which contribute to a bio-based economy. Some of these chemicals have been produced under chemolithoautotrophic conditions, using CO_2 and H_2 as the carbon source and electron donor, respectively [2].

2. MATERIALS AND METHODS

For the expression of recombinant strains of *R. eutropha*, broad-host-range (bhr) plasmids are the vectors of preference. pBBR1MCS1 (4.7kb) is a bhr cloning vector that confers plasmid mobility, as well as a pBBR1 origin of replication that is replicable in *R. eutropha* [3]. This medium copy number plasmid is ideal for constructing recombinant plasmids for *R. eutropha*, where strong promoters maximize protein expression. P_{BAD} promoter is one of the best promoters used for gene expression in *R. eutropha*, where L-arabinose acts as the inducer [4]. Therefore, the plasmid B2(CamR) has been constructed in our laboratory for gene expression in *R. eutropha*. B2(CamR) was constructed with the bhr cloning vector pBBR1MCS1 [5] and pBbA8k-RFP (3.2 kb) [6] backbones.

Isolation of plasmids, restriction enzyme digestions, polymerase chain reaction (PCR) (Eppendorf Mastercycler), agarose gel electrophoresis, DNA gel extraction, PCR purification, and DNA ligation were performed according to standard procedures and recommendations by the manufacturers. Transformation of *E. coli* DH5α was carried out using the $CaCl_2$ method and transformation of *R. eutropha* H16 was carried out by electroporation (Eppendorf Eporator), both transformations were carried out by standard procedures and recommendations by the manufacturers. See Table 1 for bacterial strains, plasmids and primers used.

Table 1. Bacterial strains, plasmids and primers used in this study. Restriction enzyme sequences are underlined.

Strains, plasmids or primers	Important features or primer sequences	Ref.
Strains		
***E. coli* DH5α**	High efficiency transformation strain.	[7]
***R. eutropha* H16**	Wild type gentamycin resistance (Gen^r).	
Plasmids		
pBBR1MCS1	Source of chloramphenicol resistance (Cam^r), mob gen and pBBR1 origin of replication.	[5]
pBbA8k-RFP	Source of P_{BAD} promoter and RFP.	[6]
Primers (5' → 3')		
AvrII-Cam-pBBR1	GATC<u>CCTAGG</u>ATTGTTATCCGCTCACAATTCCACACAACATAC	
pBBR1MCS1-PstI	GATC<u>CTGCAG</u>AAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTG	

The 4.3 kb amplified fragment harbouring Cam^r, mob gene and pBBR1 oriV from pBBR1MCS1 backbone (where AvrII-Cam-pBBR1 and pBBR1MCS1-PstI were used as primers) was ligated with the 2.4 kb digested fragment from pBbA8k-RFP, resulting in the 6.7 kb plasmid B2(CamR). The Cam^r gene

amplified from plasmid pBBR1MCS1 was cloned into plasmid pBbA8k-RFP between AvrII and PstI sites (see Fig. 1).

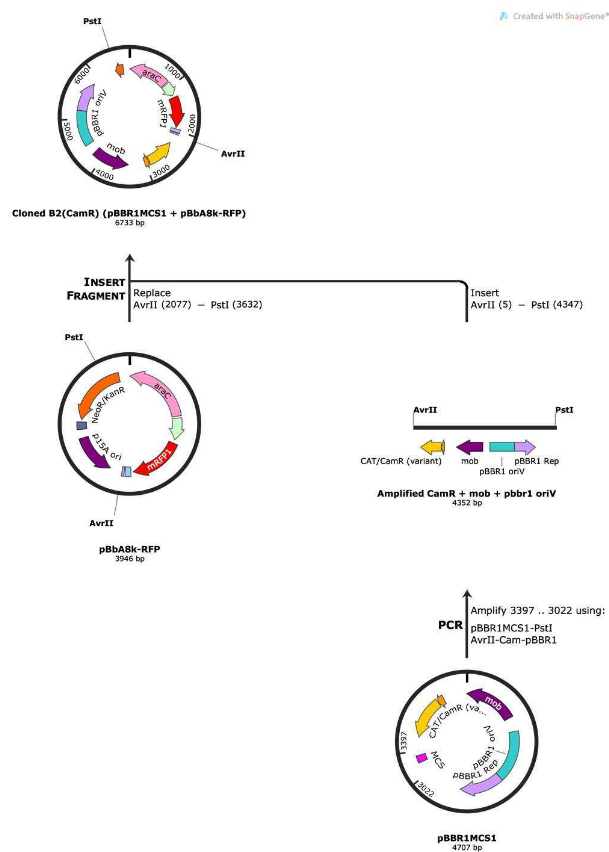

Figure 1. Construction of plasmid B2(CamR) for *R. eutropha.*

3. RESULTS

The constructed plasmid B2(CamR) (see Figure 2) conferring Cam^r has been tested and found to replicate in *R. eutropha* by electroporation, where many colonies were obtained after 48hrs at 30°C.

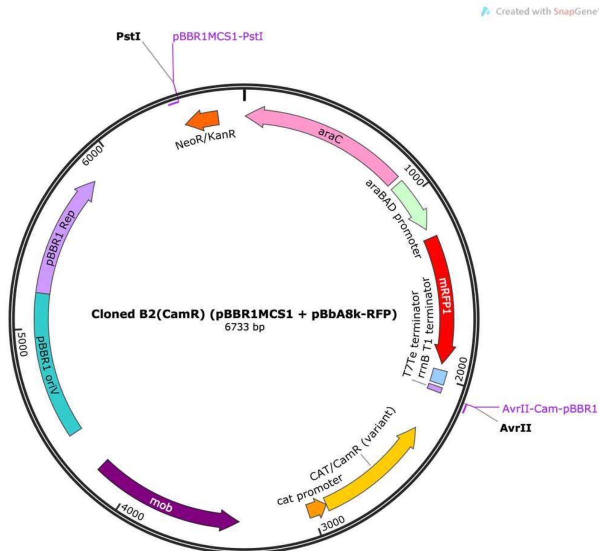

Figure 2. Plasmid B2(CamR).

4. CONCLUSIONS

The above results revealed that plasmid B2(CamR) can be transformed in *R. eutropha* by electroporation. This plasmid will be of great importance for future experiments in the laboratory to engineer *R. eutropha* for biomanufacturing purposes.

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