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ROLE OF yqiG PSEUDOGENE IN Escherichia coli TOWARDS BIOHYDROGEN PRODUCTION

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ROLE OF *yqiG* PSEUDOGENE IN *Escherichia coli* TOWARDS BIOHYDROGEN PRODUCTION

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Biohydrogen gas has a great potential as alternative energy and it is considered as environmental friendly energy source. Extensive studies have been carried out on the production of biohydrogen from *Escherichia coli (E. coli). E. coli* was used due to its accessibility of full genome and well characterized proteins. Interestingly, there are some of pseudogenes present in *E. coli* genome. The pseudogenes were considered as non-functional genes as result of genetic variability. In this study, we found that *yqiG* is one of the pseudogenes in *E. coli* that is essential for biohydrogen production. The deletion of *yqiG* has reduced the biohydrogen productivity in comparison to the *E. coli* wild type as control. The objective of this study is to investigate the role of *yqiG* pseudogene of *E. coli* during hydrogen production. The *yqiG* single gene deletion strain and ASKA strains were used throughout the study. The *yqiG* mutant strain has significantly interrupted biohydrogen productivity and suppressed glucose consumption up to 50% compared to wild type, respectively. In addition, overexpressed *yqiG* protein in the wild type via pCA24N-YqiG had increased the hydrogen productivity to 56 µmol H_2/mg protein from glucose compared to control which only 38 µmol H_2/mg protein. Similar pattern was observed from formate fermentation whereas wild type producing *yqiG* protein dominated the biohydrogen productivity about 141.8 µmol H_2/mg protein. Higher expression of formate hydrogen lyase components (*fdhF*, *hycE* and *fhlA)* were observed in mutant *yqiG* strain. In contrast, the *yqiG* mutation causes down regulation of *pflB* and *pykF* of glycolysis as well as formic acid accumulation in the fermentation broth compared to the wild type strain. The *pflB* and *pykF* encode pyruvate kinase and pyruvate formate lyase, respectively. We conclude that the *yqiG* protein is important in the conversion of phosphoenolpyruvate to pyruvate and utilization of pyruvate to formate for hydrogen production. Thus, *yqiG* pseudogene makes an important contribution during hydrogen metabolism in *E.coli*.

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PERANAN PSEUDOGENE *yqiG* DALAM *Escherichia coli*
KE ARAH PENGELUARAN BIOHIDROGEN

Oleh

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Gas biohidrogen berpotensi besar sebagai tenaga alternatif dan ia dianggap sebagai sumber tenaga mesra alam. Kajian yang menyeluruh sudah dijalankan terhadap pengeluaran biohidrogen daripada *Escherichia coli* (*E.coli*). *E. coli* diguna disebabkan oleh kebolehcapaian terhadap genomnya yang menyeluruh dan protein-proteinnya yang sudah dicirikan. Menariknya, terdapat beberapa pseudogene wujud di dalam genom *E. coli*. Pseudogene dianggap sebagai gen yang tidak berfungsi akibat daripada kebolehubahan genetik. Di dalam kajian ini, kami mendapati bahawa *yqiG* merupakan salah satu pseudogene dalam *E. coli* yang mana penting untuk pengeluaran biohidrogen. Penghapusan *yqiG* telah mengurangkan produktiviti biohidrogen dalam perbandingan kepada *E. coli* jenis liar sebagai kawalan. Objektif kajian ini adalah untuk menyiasat peranan pseudogene *yqiG* daripada *E.coli* semasa penghasilan biohidrogen. Strain tanpa satu gen *yqiG* dan strain ASKA telah digunakan sepanjang kajian ini dijalankan. Strain mutan *yqiG* dengan ketara menganggu produktiviti biohidrogen dan mengurangkan penggunaan glukosa sehingga 50% berbanding strain jenis liar, masing-masing. Di samping itu, ekspresi berlebihan YqiG dalam jenis liar melalui pCA24N-YqiG telah meningkatkan produktiviti hidrogen kepada 56 μmol H_2/mg protein daripada glukosa berbanding strain kawalan, 38 μmol H_2/mg protein. Bentuk yang sama diperhatikan daripada penapaian yang mana strain jenis liar yang menghasilkan protein *yqiG* mendominasi produktiviti biohidrogen sebanyak 141.8 μmol H_2/mg protein. Keputusan yang diperolehi adalah selaras dengan keputusan analisis transkripsi dengan ekspresi yang lebih tinggi kepada komponen format hidrogen lyase (*fdhF*, *hycE* dan *fhlA*). Sebaliknya, mutasi *yqiG* menyebabkan regulasi negatif kepada glycolysis *pflB* dan *pykF* serta pengumpulan asid formik dalam medium penapaian berbanding strain jenis liar. *pflB* dan *pykF* mengekod kepada pyruvate kinase dan pyruvate format lyase masing-masing. Kami menyimpulkan bahawa protein *yqiG* ini adalah penting dalam penukaran phosphoenolpyruvate ke pyruvate dan penggunaan pyruvate ke formate untuk pengeluaran biohidrogen. Oleh itu, pseudogen *yqiG* menyumbang kepada metabolisme hidrogen dalam *E.coli.*

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

APS	Ammonium Persulfate
Bp	Base pair
CBB	Coomassie Brilliant Blue
cDNA	Complementary DNA
CM	Chloramphenicol
CU	Chaperone Usher
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
FDH_N	Formate dehydrogenase-N
FDH_O	Formate dehydrogenase-O
FHL	Formate Hydrogen lyase System
FUP	Fimbrial Usher Protein
GC	Gas Chromatography
H_2	Hydrogen
HPLC	High Performance Liquid Chromatography
HCl	Hydrochloric acid
Hyd	Hydrogenase
IS	Insertion Sequence
IPTG	Isopropyl β-D-1-Thiogalactopyranoside
Km^R	Kanamycin Resistance Gene
LB	Luria Bertani
mRNA	Messenger RNA
MFC	Microbial Fuse Cell
$MgSO_4$	Magnesium Sulphate
NaoCl	Sodium hypochlorite
NaCl	Sodium Chloride
Ni-NTA Agarose	Nickel-charged Affinity resin
OMP	Outer Membrane Protein
psig	Pounds per Square Inch gauge
PCR	Polymerase Chain Reaction
PMSF	Phenylmethylsulfonyl Fluoride
PEP	Phosphoenolpyruvate
PTS	Phosphotransferase system
qPCR	Quantitative Polymerase Chain Reaction
RNA	Ribonucleic Acid
rpm	Rotation per minute
SDS-PAGE	Sodium dodecyl sulfate Polyacrylamide gel Electrophoresis
TAE	Tris-acetate
TEMED	Tetramethylethylenediamine

CHAPTER 1

INTRODUCTION

1.1 Research overview

Biohydrogen is an attractive energy source due its combustion only produces water vapour as by-products. It is known as environmental friendly fuel since it can be produced from renewable sources such as biomass and organic materials. Compared to other energy fuel such as fossil fuel and methane gas, biohydrogen contain higher energy content (Mohd Yasin *et al.,* 2011). Hydrogen energy research became worldwide phenomena for clean energy production (Patterson *et al.,* 2013; Pawar and van Niel, 2013). Photosynthetic hydrogen production is one of the biological methods for biohydrogen production. It will convert solar energy into hydrogen in the presence of sunlight by photosynthetic bacteria. However, due to low efficiency of light consumption and reactor design difficulties make it not suitable for large scale application (Das and Veziroglu, 2008; Hawkes *et al.,* 2007). Fermentative hydrogen production is the best option of sustainable technology whereas it can consume organic feedstocks, waste biomass or lignocellulosic materials. The fermentation process can be carried out at ambient temperature and lower atmospheric pressure (Hallenbeck and Ghosh, 2009; Hallenbeck, 2014). A lot of studies were reported on biohydrogen production from *Escherichia coli (E. coli).* The modification of *E. coli* through metabolic engineering approaches have delivered a great impact on the enhancement of biohydrogen production (Maeda *et al.,* 2007a; Seol *et al.,* 2014; Tran *et al.,* 2014). Metabolic engineering has increased the biohydrogen production by eliminating some of pathways that are not contributed to hydrogen production (Ghosh *et al.,* 2013).

In recent research, pseudogenes in *E. coli* have been found to influence hydrogen production (Mohd Yusoff *et al.,* 2013). Pseudogenes are usually considered as non-functional. They are derived from an active gene which has evolutionarily lost its function as a result of a defect in transcription and translation. Hence, pseudogenes are considered junk genes due to the presence of inappropriate stop codons, repetitive element frame shifts and lack of transcription elements (Balakirev and Ayala, 2003; Harrison *et al.,* 2001).

There are more than 4500 genes in the *E. coli* genome and 178 of the genes are known as pseudogenes (Zhou and Rudd, 2013). According to Rouchka and Cha (2009), pseudogenes can be classified as processed and unprocessed pseudogenes. Processed pseudogenes are from retrotransposable events from mRNA and lack of intronic regions, flanking repeat regions and poly-A tracts at the carboxyl 3' end (Pavlicek *et al.,* 2006). This type of pseudogene can be identified by its 3' end by the presence of polyadenine (Esnault *et al.,* 2000). Unprocessed pseudogenes emerge during the gene duplication process and the genetic sequences can go through several changes such as

mutations, frame shifts, insertions and deletions (Rouchka and Cha, 2009). Any obvious changes during the transcriptional or translational process can affect the functionality of the gene. Failure of duplication of the genes resulted in a new sequence that could be a pseudogenes (Vanin, 1985). In addition, functional genes have high possibilities to turn into pseudogenes without duplication process when their selective constraints are rapidly removed or diminished due to the changes of genetic background or environment (Sakai *et al.,* 2008).

The uncorrupted *yqiG* encodes for an 821 amino acid (aa) protein (Keseler *et al.*, 2010). However, in *E. coli, yqiG* is characterized as a pseudogene due to internal insertion-sequences (IS) insC9 and insD9 (Zhou and Rudd, 2013; Hayashi *et al.,* 2006). IS elements being a segment of bacterial DNA that can be shifted from one part of the chromosome to other parts of the same chromosome or another chromosome. The presence of IS elements in the middle of a gene interrupts the coding sequence, thus inactivating the protein product. Furthermore, the presence of an IS element near the promoter of an operon can block the expression of other genes in the same operon (Griffiths *et al.*, 2000). Hence, the presence of IS elements suggests that *yqiG* is a non-functional gene in *E. coli* and results its classification as a pseudogene (Korea *et al.*, 2010).

1.2 Problem statements

The function of the *yqiG* pseudogene in *E. coli* is unknown; however, it is necessary to consider pseudogenes in hydrogen metabolism since deleting some of pseudogenes in *E. coli* has delivered a positive response on the hydrogen production (Mohd Yusoff *et al.,* 2013). Most of the researchers conceive pseudogenes as junk or non-functional genes and almost no report has mentioned a relation of pseudogenes towards hydrogen metabolism. This consideration is supported since the metabolic pathway of biohydrogen production is well understood which neglect the presence of pseudogenes in *E. coli.* Other studies carried out by Watanabe *et al.* (2008) and Tam *et al.* (2008) found that pseudogenes involved in biological interaction especially in gene regulation. In this study, thorough investigations of *yqiG* characteristics were carried out to elucidate *yqiG* role during hydrogen metabolism. Hence, fermentation and molecular biotechnology approaches were considered to elaborate the role of *yqiG* protein towards biohydrogen production in *E. coli.*

1.3 Objectives

The objectives of this research are:

1. To confirm the presence of *yqiG* pseudogene in wild type *E. coli* BW25113 genome through molecular biotechnology technique.
2. To study the role of *yqiG* pseudogene in *E. coli* for biohydrogen production.

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