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Construction of *Bacillus subtilis* Spizizenii W23 Mutant Transposon for Biohydrogen Production

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Abstract. Several bacteria, such as *Bacillus sp.* were reported can produce biohydrogen, the environmentally-friendly-renewable energy. However, not all of the species belong to the genus *Bacillus* is a bio-hydrogen producers. *B. subtilis* is one of the non-hydrogen-producer bacilli although there are several genes on its genomic sequence that are predicted involved in the pathways. In this study, we improve the ability of *B. subtilis* W23 to produce biohydrogen through transposon mutagenesis. The *B. subtilis* W23 strain mutagenesis was performed through genomic insertion with a Tn5-transposon derived from pUTmini-Tn5-luxCDABE-Km plasmid. Transformants were screened on Luria-Bertani plates containing kanamycin 30 µg/ml and then were selected based on the lowering acid production, using pH indicator phenol red on the medium, spectrophotometrically and acid probe based assays. From 133 transformants grown on LB-kanamycin plates, 131 of them showed decreased in its acid productions. There were two mutants; number 99 and 126 that produced biohydrogen in mineral-glucose medium. Transposon positions of both mutants were determined.

Keywords: *Bacillus subtilis* spizizenii W23, transposon mutagenesis, pUTmini-Tn5-luxCDABE-Km, biogas

Introduction

The requirements of energy now a days are increased 7% each year. This demand rate is not equivalent to the availability of conventional energy stock in the world (Kementerian Energi dan Sumber Daya Mineral, 2012). If there is no effort to halt, it was predicted that this energy sources will be deprived in 18 years. The global warming and energy deprivation issues drive the experts to seek the alternative, renewable and eco-friendly energy.

Hydrogen (H₂) is one of the alternative energy sources. The by-product of the hydrogen during energy creation is merely water (Vazquez, et al., 2008). Bio-hydrogen is commonly produced by obligate anaerobes and facultative anaerobes, such as *Clostridium*, and aerobic bacteria, such as *Alcaligenes* and *Bacillus* (Vazquez, et al., 2008). Main carbon sources for hydrogen producer bacteria are simple sugars, rarely from complex compounds such amyllum, celluloses or others big polymers.

One of the well-known bio-hydrogen producers is *Enterobacter aerogenes*. Bio-hydrogen production was much increased in *E. aerogenes* ADH43 by double mutated this bacterium (Said et al., 2007). However, this bacterium generates hydrogen from simple sugars, not from lignocellulose because of its inability to hydrolyse the lignocellulose. Commonly, the limitation of the usage of waste-containing-lignocellulose is in pre-treatment process. Few subsequent degradation steps are needed before lignocelluloses be ready as substrates.

Bacillus are Gram-positive bacilli which have been known as lignocellulose degrader. Many lignocellulases enzymes are commercially produced from this bacterium. Instead of the advantages, little information about hydrogen production by this genus. Although *Bacillus* genome was predicted contains genes involved in bio-hydrogen production, *Bacillus* were rarely exploited maximally in hydrogen production. Based on the facts, we constructed the *B. subtilis* mutants by transposon mutagenesis (Lewenza et al, 2005; De Lorenzo et al., 1990), which can used lignocelluloses waste as substrate. We chose *B. subtilis* subsp. *spizizenii* W23, because its complete genome sequence is available at National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov/>).

The aims of this research were to construct *B. subtilis* subsp. *spizizenii* W23 transposon mutants that were able to produce more biogas than the wild type strain.