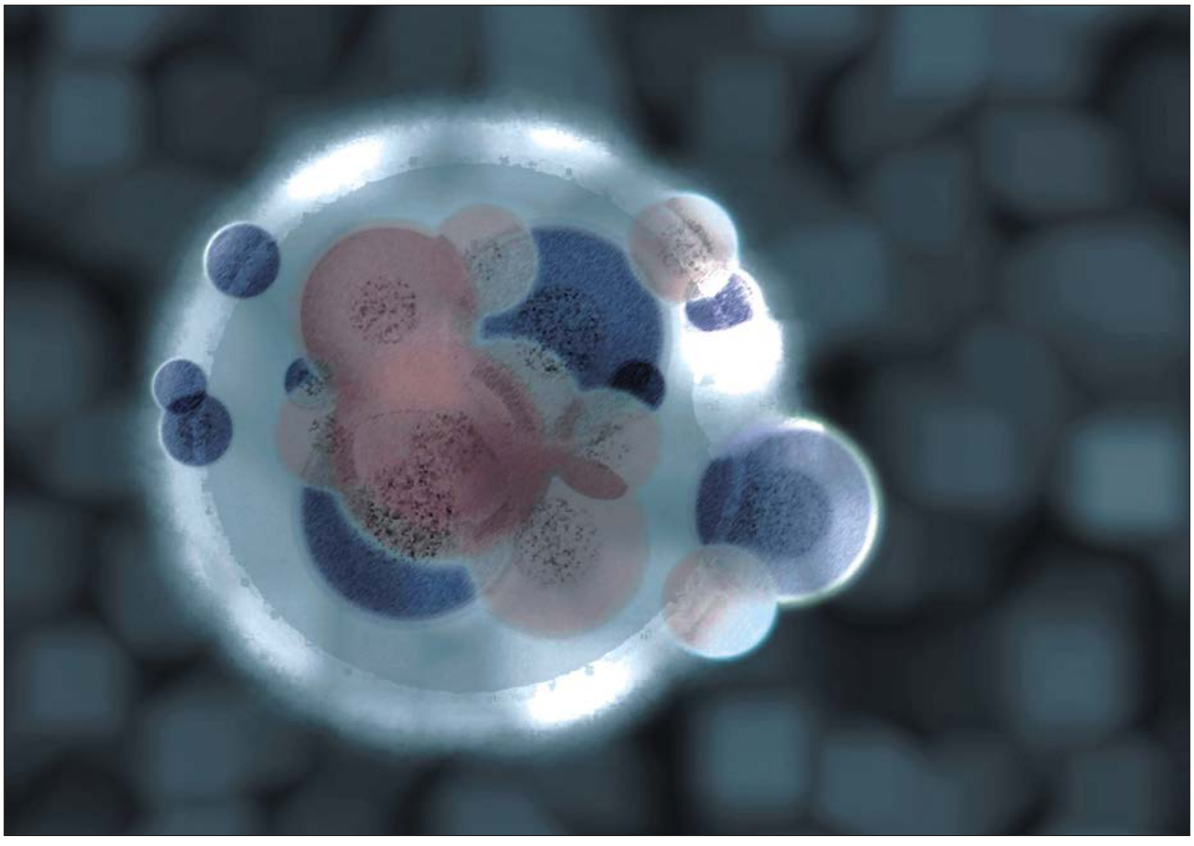

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Molecular Analysis of Dehalogenase Gene in Genomic DNA of *Bacillus megaterium* Strain GS1 Isolated from Volcanic Area Gunung Sibayak

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Abstract: A bacterial species identified as *Bacillus megaterium* GS1 was previously isolated from volcanic area of Gunung Sibayak, Indonesia. Therefore, the main aim of the present study was to identify the presence of dehalogenase gene in the microorganism. To achieve this, is to apply basic molecular techniques that include the use of oligonucleotide primers specific to microorganism that can grow in halogenated compound. A putative dehalogenase gene was determined by direct sequencing and analysis of the PCR-amplified genomic DNA of the bacterium. A comparative analysis of the sequence data revealed that, DehGS1 amino acid sequence is related to L-specific dehalogenase or group II αHA with an overall of 25% amino acids identity. This investigation is useful in studying the microbial populations in order to monitor the presence of specific or novel type dehalogenase genes. As a result, it will provide better understanding of the microbial populations that present in soil or in water systems treating halogenated compounds.

Key words: *Bacillus megaterium* GS1, dehalogenase, 2,2-dichloropropionic acid, degradation, dehalogenase gene

INTRODUCTION

Halogenated organic compounds are produced by chemical synthesis and are very toxic. These compounds are widely used as herbicides, fungicides and insecticides throughout the environment. Investigations of microbial degradation of haloaliphatic and haloaromatic compounds led to the identification of a variety of dehalogenases and dehalogenation mechanisms (Janssen *et al*., 1994; Fetzner, 1998). Nowadays, dehalogenases not only have potential applications in environmental technologies but also in chemical industry (Kurihara, 2011; Swanson, 1999). Dehalogenase enzymes were classified according to their substrate specificities and the putative reaction mechanisms (Slater *et al*., 1995). In literature, various names were given to these enzymes for example, haloacid dehalogenase (HAD), 2-haloacid dehalogenase, 2-haloacid halidohydrolase, 2-haloalkanoic acid dehalogenase and 2-haloalkanoic acid halidohydrolase (Fetzner and Lingens, 1994). Haloalkanoic acid hydrolytic dehalogenases act on carbon No. 2 or α-carbon of halogenated short chain aliphatic acids for example L-isomer specific-, D-isomer specific- and D, L-isomer non-stereo specific classes. Most of the well-known dehalogenases are L-isomer specific and ten genes of this type are sequenced so far. Hill *et al*. (1999) reported that α-haloalkanoic acid (αHA) can be grouped into two, group I αHA and group II αHA. Most of L-isomer specific are in group II αHA.

In Rhizobial system, production of more than one dehalogenases was reported based on substrates specificities (Cairns *et al*., 1996; Stringfellow *et al*., 1997). It was curious, therefore, that some organisms had more than one dehalogenases and a possible explanation of this phenomenon was that dehalogenases could evolved and gained additional ability to degrade many type of halogenated substrates (Stringfellow *et al*., 1997). The discovery of new dehalogenases is still the highlighted area of research (Huang *et al*., 2011).

Recently, a bacterium isolated from volcanic soil by elective culture on 2, 2-dichloropropionic acid (2, 2DCP) and identified as a *Bacillus megaterium* strain GS1 by 16S-rDNA analysis. A better understanding of these microorganisms may reveal novel enzymes to stimulate better biodegradation activity in future. One approach is to study the isolated microbial specie(s) from its populations in soil by applying molecular tools and techniques to probe specific microorganism possessing dehalogenase genes.

In present research, using PCR technique is to identify a potential new kind of dehalogenase gene(s) that may responsible for bacterial growth on 2, 2DCP as sole source of carbon and energy.

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MATERIALS AND METHODS

Bacteria cultivation: The soil sample was taken from volcanic area of Gunung Sibayak, Indonesia. All strains were cultivated aerobically at 37°C on solid minimal media containing 20 mM of 2,2-DCP using streak plate method. The sample was repeatedly streaked on the same type of medium to obtain a pure colony.

The liquid minimal media was prepared as 10X concentrated basal salts containing K_2HPO_4. $3H_2O$ (42.5 g L^{-1}), $NaH_2PO_4.2H_2O$ (10.0 g L^{-1}) and $(NH_4)2SO_4$ (25.0 g L^{-1}). The trace metal salts solution was a 10X concentrate that contained nitriloacetic acid (NTA) (1.0 g L^{-1}), $MgSO_4$ (2.0 g L^{-1}), $FeSO_4$. $7H_2O$ (120.0 mg L^{-1}), $MnSO_4$. $4H_2O$ (30.0 mg L^{-1}), $ZnSO_4.H_2O$ (30 mg L^{-1}) and $COCl_2$ (10.0 mg L^{-1}) in distilled water (Hareland *et al*., 1975). Minimal media for growing bacteria contained 10 mL of 10X basal salts and 10 mL of 10X trace metal salts per 100 mL of distilled water and were autoclaved. The sterilized 2,2-DCP carbon source was added to the autoclaved salts medium to a final concentration of 20 mM. The extent of growth determined by measuring the absorbance at A_{680nm}.

Molecular analysis

PCR and dehalogenase gene identification: The PCR primers were described according to Fortin *et al*., (1998), from *Xanthobacter autotrophicus* dhlB 314 5′-TCT GGC GGC AGA AGC AGC TGG- 3' dhlB 637 5′- CGC GCT TGG CAT CGA CGC TGA TG- 3′ (Van Der Ploeg *et al*., 1991). The PCR conditions were set at 30 cycles of the following parameters: Denaturation, 95°C for 1 min; annealing, 55°C for 1 min; extension, 72°C for 2 min. The reaction mixture was electrophoresed on a 1% agarose gel and purified using ExoSAP-IT PCR Clean-up Kit (GE Healthcare Bio-Sciences Corp. USA) for sequencing at 1st Base Laboratory, Malaysia. The DNA sequencing results were converted into amino acids. The amino acid sequence was analysed by sequence comparison in the public databases using BLASTp search program on the National Center for Biotechnology Information (NCBI) website (http://www .ncbi.nlm.nih.gov/).

RESULTS

Growth experiment of strain GS1 on 2,2DCP: A pure colony of GS1 grew well on liquid and solid minimal media supplied with various concentrations of 2,2-DCP up to 40 mM at 37°C. However, no growth was detected at 50 mM 2,2-DCP suggesting 50 mM of 2,2-DCP is toxic to the cells. A control plate without 2,2-DCP showed no growth at all.

Molecular analysis of strain GS1 and the presence of putative dehalogeanse gene: Oligonucleotide primers (Van Der Ploeg *et al*., 1991) were used to perform PCR amplification using genomic DNA extracted from strain GS1 grown in 2,2DCP. PCR fragment of the expected size of 500-600 bp (Fig. 1) was generated and the negative control using *E. coli* genomic DNA did not show any amplification. The PCR fragment was sent for sequencing. The nucleotide and amino acid sequences were analyzed against EMBL and SWISSPROT databases, respectively. A FASTA search revealed that the PCR fragment was 69% homologous to the known coding region of the

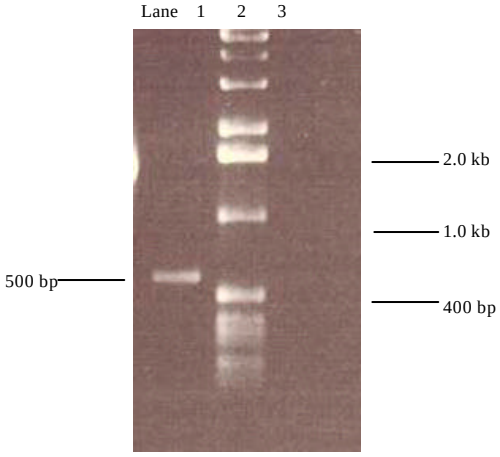

Fig. 1: PCR analysis of genomic DNA from strain GS1 using nucleotide primers derived from dhlB. Lane 1: GS1 PCR product (Approximately 500 bp); Lane 2. 21 kb DNA ladder; Lane 3; Negative control

```
DhlB          33 YITQVWRQKQLEYSWLRALMGRYADFWGVTREALAYTLGTLGLEPDESFLAGMAQAYNRL 92
DehGS1         1 ----VWRQKQL-YKKLR-LQ-RYAFFWGVTHCALAYTLEPLGLEPDESFLAGQYQAENR- 60
                 ******* *  *  *  **   ****  ******  ************  ** **

DhlB          93 TPYPDAAQCLAELAPLKRAILSNGAPDMLQALVANAGLTDSFDAVISVDAKRVFKPHDER 149
DehGS1        61 KPYPDAAQCLKELPQAKRAILALGAVTRLQALVAPAG----PDAVIMVDAKRVFKP---- 120
                 ********* **   *****  **   ****** **     **** ****** **
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Fig. 2: Amino acid sequence comparison of *dehGS1* and the dehalogenase gene *dhlB*. The *dehGS1* PCR product is 69% homologous to *dhlB*, encoding the haloalkanoic acid dehalogenase from *X. autotrophicus*. Identical amino acid residues are asterisks. The GeneBank accession number for *dhlB* is M81691

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HadL           -MKNIQGIVFDLYGTLYDVHSVVQACEEVYPGQGDAISRLWRQKQLEYTWLRSLMGRYVN 59
DehH109        -MQPIEGIVFDLYGTLYDVHSVVQACESAYPGQGEAISRLWRQKQLEYTWLSSLMGRYAS 59
HdlIV          MVDSLRACVFDAYGTLLDVHSAVMRNADEVGASAEALSMLWRQRQLEYSWTRTLMHQYAD 60
DehGS1         ----------------------------------------VWRQKQLYKKLR---LQRYAF 18
                                                        :***:**        : :*.

HadL           FEKATEDALRFTCTHLGLSLDDETHQRLSDAYLHLTPYADTADAVRRLKAAGLPLGIISN 119
DehH109        FEQRTEEALRYTCKHLGLATDETTLRQLGQAYLHLAPHPDTTAALRRLKASGLPMAIASN 119
HdlIV          FWQLTDEALTFALRTYHLEDRKGLKDRLMSAYKELSAYPDAAETLEKLKSAGYIVAILSN 120
DehGS1         FWGVTHCALAYTLEPLGLEPDESFLAGQYQAENRK-PYPDAAQCLKELPQAKR--AILAL 75
               *   *  ** ::     *            *      : *::  :  *  :     * :

HadL           GSHCSIEQVVTNSEMNWAFDQLISVEDVQVFKPDSRVYSLAEKRMGFPKENILFVSSNAW 179
DehH109        GSHHSIEQVVSHSDMGWAFDHLISVETVKVFKPDNRVYSLAEQTMAIPRDRLLFVSSNSW 179
HdlIV          GNDEMLQAALKASKLDRVLDSCLSADDLKIYKPDPRIYQFACDRLGVNPNEVCFVSSNAW 180
DehGS1         GAVTRLQALVAPAGP----DAVIMVDAKRVFKP---------------------------- 104
               *    ::  :  :      *  :  :  :::**

HadL           DASAASNFGFPVCWINRQNGAFDELDAKPTHVVRNLAEMSNWLVNSLD--- 227
DehH109        DATGARHFGFPVCWVNRQGAVFDELGATPTREVRDLGEMSDWLLD------ 224
HdlIV          DLGGAGKFGFNTVRINRQGNPPEYEFAPLKHQVNSLSELWPLLAKNVTKAA 231
DehGS1         ---------------------------------------------------
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Fig. 3: Amino acid sequence comparison between DehGS1 and HadL, DehH109 and HdlIV. Identical amino acid residues are asterisks. Two dots indicate amino acid similarity

haloacid dehalogenase from *X. autotrophicus* (Fig. 2). This PCR product was named DehGS1 and has some homology with a variety of other haloalkanoic acid dehalogenases as shown in Fig. 3. The overall protein sequence identity was 25%. Examples are: The HadL from *Pseudomonas putida* AJ1- 25% (Jones *et al*., 1992); DehH109 dehalogenase from *P. putida* H109-26% (Kawasaki *et al*., 1994) and the HdlIV dehalogenase from *Pseudomonas cepacia* MBA4- 25% (Murdiyatmo *et al*., 1992). However, there was less than 5% homology with haloacetate dehalogenase and most of group 1 αHA. These results suggest current DehGS1 might be a novel group II αHA.

DISCUSSION

In previous study, a group of bacteria that can grow on halogenated compound as sole source of carbon were identified (Jing and Huyop, 2008; Ismail *et al*., 2008; Jing *et al*., 2008; Mesri *et al*., 2009; Thasif *et al*., 2009; Zulkifly *et al*., 2010). In addition, some of the dehalogenase producing microorganisms were intensively studied and their corresponding genes were identified (Thomas *et al*., 1992; Cairns *et al*., 1996; Stringfellow *et al*., 1997; Yusn and Huyop, 2009).

To the best of our knowledge, this is the first reported *Bacillus* sp. that can degrade 2,2DCP as sole source of carbon. There are very limited studies focused on chlorinated compounds degradation by *Bacillus* species. Other reported strains of the same genus are dichloromethane degrading bacteria (Wu *et al*., 2009) and degradation of low concentration of monochloroacetic acid by *Bacillus* sp. TW1 (Zulkifly *et al*., 2010).

Oligonucleotide primers for the dhlB gene were capable of detecting the presence of dehalogenase gene in a bacterium that could grow on 2,2DCP. In future using similar technique might be useful in detecting the presence of bacteria with the capacity to degrade

halogenated compounds by hydrolytic dehalogenation. In this study, the PCR product was identified to be a dehalogenase gene associated with L-haloacid dehalogenases.

DehL dehalogenase in *Rhizobium* sp. could not act on 2,2DCP (Leigh, 1986). However, growth of *Rhizobium* sp. on 2,2DCP could trigger production of DehL dehalogenase suggesting 2,2DCP is substrate-inducer. In current study might suggest that the identified gene might or might not be responsible for growth on 2,2DCP.

CONCLUSION

In conclusion, using molecular approach can be used in finding a novel gene of interest. This is the first report demonstrating the dehalogenase gene is present in *Bacillus* sp. associated with growth on halogenated substrate as sole source of carbon. This technique could also apply to monitor for microorganisms possessing specific dehalogenase gene(s) in soil community or in water systems. In future, it was hoped that such studies would be possible to identify new kind of dehalogenase from newly isolated microorganism. Therefore, it provides a better understanding of the microbial populations as a whole.

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