Genome Sequence Survey of Local Isolated Alkaliphilic *Bacillus* sp. G1

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Bacillus sp. G1 is an alkaliphilic microorganism which cannot grow or grow poorly under neutral pH conditions, but grow very well at pH higher than 9.5. Since 1969, a great number of alkaliphilic *Bacillus* strains from various environments were isolated and a variety of alkaline enzymes were obtained and successfully purified. Therefore, Genome Sequence Survey (GSS), an efficient procedure for gene discovery and genome characterization was used to discover novel functional gene and applicable industrial enzyme. GSS was carried out by determining and publicly distributing 300 – 1000bp of single-pass nucleotide sequence from both ends of thousands of randomly genomic-DNA clones. In total, approximately 400,000 nucleotides (613 sequence tags) of non-redundant, high-quality *Bacillus* sp G1 genome sequence were obtained. Sequence tags were searched against the GBBCT (Genbank Bacterial Sequences), *Bacillus subtilis* and *Bacillus halodurans* databases. Results showed that, *Bacillus* sp. G1 possessed a few useful and applicable industrial enzymes, such as protease, lipase, xylanase, glucanases and CGTase. Besides, a gene encoded for Na^+/H^+ antiporter membrane protein, a major component in alkaliphile Na^+ cycle, was also found in the *Bacillus* sp. G1 genome. The Na^+/H^+ antiporter activity plays an important role in pH homeostasis and maintaining low intracellular sodium concentrations in halophilic or halotolerant aerobic bacteria. This explained how this microorganism is able to survive in this harsh environment. Other major components in alkaliphile Na^+ cycle, such as Na^+/solute symporter, flagella, ABC transporters, and Teichuronopeptide (TUP), were also found in *Bacillus* sp. G1.

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1.0 Introduction

Generally, alkaliphilic *Bacillus* strains cannot grow or grow poorly under neutral pH conditions, but grow well at pH higher than 9.5. Most of the alkaliphilic *Bacillus* strain (such as *Bacillus halodurans* C-125) require Na^+ for growth under alkaline conditions (Horikoshi, 1971a and 1971b). The presence of sodium ions in the surrounding environment has been proved to be essential for effective solute transport through the cytoplasmic membrane of cells (Takami, 2000).

Since 1969, a great number of alkaliphilic *Bacillus* strains from various environments were isolated and a variety of alkaline enzymes were obtained and successfully purified (Horikoshi, 1991). During the past two decades, many studies have focused on the enzymology, physiology and molecular genetics of alkaliphilic *Bacillus* to elucidate their mechanisms of adaptation to alkaline environments (Horikoshi, 1991).

Therefore, Genome Sequence Survey (GSS), an efficient procedure for gene discovery and genome characterization was used to discover novel functional gene and some applicable enzyme. GSS was carried out by determining and publicly distributing 300 – 1000bp of single-pass nucleotide sequence from both ends of thousands of randomly genomic-DNA clones.

2.0 Materials and method

2.1. Selection of the alkaliphilic *Bacillus* sp. G1

Alkaliphilic *Bacillus* sp. G1 was selected for genome sequencing. *Bacillus* sp. G1 in glycerol stock was streaked on Horikoshi Broth (HB) plate and propagated in HB medium (0.2 g/L $MgSO_4$, 1.0 g/L KH_2PO_4, 5.0 g/L yeast extract, 5.0 g/L peptone, 1.0 g/L soluble starch and 10.0 g/L Sodium Bicarbonate) at 37 °C, overnight with agitation (~150 – 180 rpm).

2.2 Construction of Genomic DNA Library

Overnight culture of alkaliphilic *Bacillus* sp. G1 was used to isolate genomic DNA by using SDS-phenol extraction method. The isolated genomic DNA was used to construct genomic DNA library by using two different methods which are enzymatic method and mechanical method.

For the enzymatic method, genomic DNA was completely digested by *EcoR*I and *Hind*III. The digested genomic DNA from each restriction enzyme was ligated into appropriately digested, phosphatase-treated pUC 19 and transformed into *E.coli* JM109.

For construction of a random sheared DNA library, DNA was fragmented with sonicator (750-watt Ultrasonic Processor with Temperature Probe) into pieces with a distribution centered on 1.5 – 2kb. Blunt-ended DNA fragment was ligated into pUC19 (with *Sma*I arm) and transformed into *E.coli* JM109.

The insert-carrying recombinant plasmids will be identified in transformants by detecting white colonies after overnight incubation on LB/Ampicilin/X-Gal/IPTG plate at 37°C.

2.3 Isolation of Recombinant Plasmid

Only white colonies were selected for the recombinant plasmid isolation. Colonies were grown overnight in LB/Amp medium at 37°C. Alkaline lysis (with SDS) method was used to isolate the recombinant plasmid.

2.4 Analyzing Sequencing Trace Files with PHRED and CROSS_MATCH

Trace files were analyzed with PHRED and CROSS_MATCH algorithm of MacPhred/Phrap to translate the ABI377 chromatogram data of the sequence files into accurate quality information for each base and detection of plasmid sequences, respectively. Plasmid sequences were removed from each sequence tag and high quality sequence data (> 20 phred score) were collected into a database and searched against the non-redundant (nr) GBBCT (Genbank Bacterial Sequences), *Bacillus subtilis* and *Bacillus halodurans* database at National Center for Biotechnology Information (NCBI) or San Diego Super Computer Biology Workbench (SDSC Biology Workbench) by using BLAST (blastn) and BLAST (blastx) algorithms.

3.0 Results and Discussions

Insert sizes of clones from the sheared DNA library ranged from 0.6kb – 2kb and clones from the *Eco*RI or *Hind*III contained fragment sizes ranging from 150bp to 10kb. 384 inserts from *Eco*RI library and 341 inserts from *Hind*III library were sequenced from flanking primer sites, M13 forward and M13 reverse. While 128 inserts from the sheared DNA library were sequenced from flanking primer sites, SP6 and T7. Low quality and cloning vector sequences were removed from the database. The sequences were then searched against the complete NCBI nr database with the BLASTn and BLASTx algorithm. The result from the blasting showed highly repeatitive result. Therefore, only sequences with the high scores and low e-value were kept in the database. In total, approximately 400,000 nucleotides (613 sequence tags) of non-redundant, high-quality *Bacillus* sp. G1 genome sequence were obtained.

From the result gathered, a few useful and applicable industrial enzymes were discovered in alkaliphilic *Bacillus* sp. G1, such as lipase, protease, xylanase, glucanases and CGTase.

Besides, a gene fragment, Na^+/H^+ antiporter membrane protein gene, which is important in the ability to survive in alkaline environment was also found. The Na^+/H^+ antiporter is ubiquitous membrane protein found throughout the eukaryotic and prokaryotic kingdoms. Na^+/H^+ antiporter plays a major role in Na^+ cycle which transporting Na^+ across the cytoplasmic membrane of all living cells. It catalyze the electroneutral countertransport of Na^+ and H^+ across both cytoplasmic and organellar membranes, where their function is to maintain intracellular pH homeostasis, salt

concentration and osmolarity, detoxification of cells from Na^+, regulating of cell volume and establishment of an electrochemical potential of Na^+. Other major components in Na^+ cycle, such as Na^+/solute symporter, flagella, ABC transporters, and Teichuronopeptide (TUP), were also found in *Bacillus* sp. G1.

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