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[BIO44] Cloning and characterization of polyhydroxyalkanoate (PHA) genes from *Pseudomonas* sp. isolated from Antarctica

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Introduction

Pseudomonas strains accumulate medium-chain-length poly(*R*)-3-hydroxyalkanoate (PHA) as carbon and energy source under conditions of limiting nutrients in the presence of an excess of carbon source (Fidler *et al*., 1992). This bacterial storage material, mainly formed of monomers of 6 to 14 carbon atoms, has potential as a renewable and biodegradable plastic (Prieto *et al*., 1999).

Most of the research on PHAs was done on well-known PHA-producing soil microorganisms. However, PHA-producing microorganisms from the Arctic or Antarctic regions are still unknown. Psychrophilic bacteria are bacteria normally found in temperate countries. They can survive at low temperature (below 10°C). The psychrophiles are subdivided into obligate and facultative groups. Obligate psychrophiles seldom grow above 22°C and facultative psychrophiles (psychrotrophs) grow very well above 25°C.

Several genes such as *phaC* (PHA synthase), *phaA* (β-ketothiolase), *phaB* (NADH-acetoacetyl-CoA reductase), *phaG* (3-hydroxyacyl-carrier protein-coenzyme A transferase) and *phaJ* (enoyl-CoA hydratase) are responsible in the accumulation of PHA. An enzyme known as PHA depolymerase coded by *phaZ* gene will then degrade these PHA compounds (Bernd *et al*., 1999). The PHA biosynthesis genes are often clustered in the bacterial genome as an operon called *phaCAB* (Steinbüchel *et al*., 1992). PHA synthase enzymes can be divided into three types; Type I PHA synthases (R. *eutropha*) preferentially uses coenzyme A thioesters of various short chain length ($3HA_{SCL}$) comprising three to five carbon atoms, whereas type II PHA synthases (P. *aeruginosa*) preferentially utilize coenzyme A thioesters of various medium chain length with at least five carbon atoms ($3HA_{MCL}$). Type III PHA synthases (*C. vinosum*) prefer coenzyme A thioester of $3HA_{SCL}$. In this paper, we describe the isolation of a PHA-producing bacterium from Antarctica for *pha* Synthase gene characterization.

Materials and methods

Bacteria were isolated from Antarctica seawater sample using different medium and cultured in 4°C. Isolated bacteria were stained with Nile blue A and Nile red for the detection of PHA (Spiekermann *et al*., 1999). One of the PHA-producing bacteria was chosen and its PHA composition was tested using gas chromatography. The 16S ribosomal DNA was amplified using universal primers and was cloned into pGEM-T vector (Promega). Sequencing result was aligned with sequences in Genbank database.

A fragment of the PHA synthase gene was amplified with a pair of specific primer. Primers with the following sequences were used: 5' GTAGACCTTCATCRTCAGYTAGGAGTA ACC 3' and 5' ACAGACCTTCATCRTCAGYTAGAGTAA CC 3'. Sequencing result were compared with the sequenses available in Genbank database.

Genomic library was constructed, utilizing λGEM as its cloning vector (Promega). Positively hybridized clones were screened and its DNA fragment was subcloned into pGEM-T vector and sequenced. Standard molecular biology protocols used in this study were as described in Sambrook *et al*., 1989.

Results and Discussion

The bacteria of study is a psychrotroph (psychrotolerant). This bacterium grew in a wide range of temperature (4°C to 30°C) but not at 37°C. It grew well in both cold and room temperatures. It required about three to five days to grow at 4°C and two to four days at 30°C. Phase contrast microscopy revealed rod-shaped cells with Gram-negative characteristic.

Bacterial culture grown in limiting nitrogen and high level of carbon was

prepared in order to harvest the PHA components. PHA extracted from freeze dried and processed cells were then used for gas chromatography (GC) test. GC results indicated that this bacterium produce medium chain length PHA (PHA_{MCL}) comprising 6, 8, 10, 12 and 14 atom carbons. The total PHA content in the cells was about 17% of the cells dry weight. The 16S ribosomal DNA of this bacterium had been cloned and sequenced. The result of the sequencing showed that this sequence shared 99% identity to *Pseudomonas* species when compared with available sequences deposited in Genbank database.

A PCR fragment corresponding to the expected size of 500bp had been successfully cloned and sequenced. Sequencing results indicated that it shared a 92% identity with PHA synthases from several *Pseudomonas* species. Southern analysis also indicated that this bacterium contain PHA synthase gene in its genome.

Genomic library was constructed utilizing λGEM as its cloning vector. Six positive clones were selected in the primary screening. Southern analysis of phage DNA digested with restriction enzyme *Sph*I demonstrated two positively hybridized DNA fragments (1557 bp and 4kb). These fragments were succesfully cloned into pGEM-T vector.

Fragment 1557 bp possesses a putative truncated *phaC1* gene and a putative truncated *phaZ* gene. Meanwhile, fragment 4 kb possesses four putative genes, which are *phaC2*, *phaD*, *phaF* and *phaI* genes. Another fragment 890 bp was discovered which bridging the 1557 bp and 4 kb fragments together. The assembly of these 3 fragments turns out to be an operon of *pha* Synthase gene which is common in *Pseudomonad* famili (FIGURE 1).

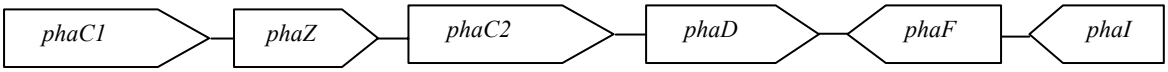

FIGURE 1 An operon of *pha* Synthase gene of *Pseudomonas* sp. was discovered by assembly of 3 subcloned fragments from genomic library.

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