PCR cloning of polyhydroxybutyrate synthase gene (*phbC*) from *Aeromonas hydrophila*

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ABSTRACT

Plastic wastes are considered to be severe environmental contaminants causing waste disposal problems. Widespread use of biodegradable plastics is one of the solutions, but it is limited by a high production cost. A Polymerase Chain Reaction (PCR) protocol was developed for the specific detection and isolation of full-length gene coding for polyhydroxybutyrate. PCR strategy using PHB primers resulted in the amplification of DNA fragments with the expected size from all isolated bacteria.

PHB synthase gene was cloned directly from Aeromonas hydrophila genome for the first time from this bacteria. The cloned fragment was named $phbC_{Ah}$. This fragment was partially sequenced and open reading frame was found representing the PHB synthase gene. The nucleotide sequence of $phbC_{Ah}$ gene exhibits similarity to Caulobacter crescentus phbC (40.3%), Pseudomonas oleovorans phbC (34.3%), and Rhodobacter sphaeroides phbC (40.6 %).

Keywords: *PCR, Polyhydroxybutyrate, Cloning, Aeromonas hydrophila, Biodegradable thermoplastic.*

INTRODUCTION

Poly(3-hydroxybutyrate) (PHB) is a carbon and energy reserve accumulated by several kinds of bacteria under conditions of nutrient stress, e.g. when an external carbon source is available but the concentration of nutrients such as nitrogen, phosphorus, or oxygen are limiting the growth (Senior and Dawes, 1973). Poly (3-hydroxybutyrate) biosynthesis genes are phbA (for 3-ketothiolase), phbB (NADPH-dependent acetoacetyl-coA reductase), and phbC (PHB synthase); these genes have been cloned recently and expressed in *E. coli* (Slater *et al.*, 1988).

Most *Pseudomonas* strains are able to accumulate polyhydroxyalkanoic acids (PHA) as carbon energy storage compound, consisting of saturated and unsaturated 3-hydroxy fatty acids with carbon chain lengths ranging from six to fourteen carbon atoms (Anderson and Dawes, 1990). *Alcaligenes eutrophus* is now used for commercial PHA production, but many other microorganisms accumulate PHB and can grow on more or different carbon sources than *A. eutrophus* (Page, 1992).

Takeda *et al.* (1995) reported that Poly-3-hydroxybutyrate (PHB) has potential applications for biodegradable and safe thermophilic plastics with much less environmental impact than many other