CHAPTER 6.

IDENTIFICATION OF RESPONSIBLE GENE INVOLVED IN BENZOATE DEGRADATION OF Serratia marcescens DS-8 AND Rhodopseudomonas palustris DS-4

ABSTRACT

To analyse the responsible genes in benzoate degradation, transposition mutagenesis was conducted through triparental and diparental mating. Mutants of diparental mating using pTnMod-OGm as donor to an anoxygenic photosynthetic bacteria, Rhodopseudomonas palustris DS-4 could not be obtained. This might be due to the complexity of the cell membrane. Diparental mating of Serratia marcescens DS-8 using pJFF350 was successfully done (Omegon-Km). However, Southern hybridization analysis showed that benzoate minus mutants were not caused by this transposable element. Other Omegon-Km mutants have not been characterized yet.

Keywords: Omegon-Km, transposition mutagenesis, benzoate degradation, Serratia marcescens, and Rhodopseudomonas palustris
INTRODUCTION

Metabolic pathway of aerobic and anaerobic degradation of benzoate have been described (Altenschmidt et al., 1993; Williams and Sayers, 1994; Fuenmayor 1998; Pelletier and Harwood, 1998). Such efforts have been utilized to determine the responsible genes or enzymes in metabolizing benzoate. However, such pathways are still needed to be determined (Harwood and Gibson, 1988; Coschigano and Young, 1997; Pelletier and Harwood, 1998).

To characterize of bacteria, transposon mutagenesis is still one of the most extensively utilized techniques available. This technique is especially useful for bacterial species with poorly described genetic systems or when existing molecular techniques are insufficient (Dennis and Zylstra, 1998). Transposons are believed to insert at random location. However, transposon mutagenesis is not completely random. Many have specific sites of transposition either in Gram-negative or Gram-positive bacteria (Berg et al., 1983; Scott, 1991; Wall et al., 1996).

Mutants unable to completely degrade aromatic compounds has been derived from transposon. Civolani et al. (2000) derived mutants unable to further metabolize vanillic acid in Pseudomonas fluorescens BF 13 using TnMod-OKm. This transposon consists of the Tn5 inverted repeat, a conditional origin of replication, a rare restricted endonuclease multiple cloning sites, and exchangeable antibiotic resistance cassettes (Dennis and Zylstra, 1998). Insertion inactivation with Tn5 was used for constructing mutant unable to degrade 2,4-D from Burkholderia strain RASC (Suwa et al., 1996).

Conjugative transposons have a broad host range, since they are capable of transposition in both gram-positive and gram-negative bacteria (Scott, 1991).
In this study, conjugative transposons, TnMod-OGm and Omegon-Km, were used to determine and to locate genes that are responsible in metabolizing benzoate both in anoxygenic photosynthetic bacteria, \textit{Rs. palustris} DS-4, and \textit{S. mamcens} DS-8. Omegon-Km was designed to carry the artificial interposon flanked by two synthetic inverted 28-bp repeats of IS1. The reason using these transposons is that inserted fragment could be cloned derived plasmids were stable (Fellay et al., 1989; Dennis and Zylstra, 1998; Civolani et al. 2000; Downing et al., 2000).

\section*{RESULTS AND DISCUSSION}

Triparental mating of DS-4, \textit{E. coli} HB101 (pRK2013), and \textit{E. coli} M109 (pTnMod-OGm) was done with no conjugants were observed. Dennis and Zylstra (1998) reported a good mating of gram-negative bacteria like \textit{Burkholderia}, \textit{Escherichia}, \textit{Pseudomonas}, and \textit{Sphingomonas} using this plasposon. Civolani et al. (2000) utilizing pTnMod-OKm for transposition mutagenesis of gene for bioconversion of ferulic acid into vanillic acid of \textit{P. fluorescens} strain BF13. However, no works in transposition mutagenesis were reported on anoxygenic photosynthetic bacteria using the plasposon. It is unknown why this bacteria was unsuccessfully conjugated. Culture and physiological conditions might cause a complexity of the cell membrane of the APB. The delicate conjugation pili probably might also contribute in unsucceeded conjugation. Among APB, \textit{Rhodobacter sphaeroides} 2.4.1 was one that was successfully conjugated (Suwanto and Kaplan, 1992).
The designated primers of \textit{benA} were not able to amplify suspected \textit{benA} in DS-8. The \textit{benA} with \textit{benBC} code for benzoate dioxygenase (BenABC). One of the possibility is that no homology region of this gene is in the chromosome of DS-8, in other word, this gene might not be in the strain. In addition, the catechol test indicated that the colony of this strain did not produce yellow color as a product of metabolizing catechol to 2-hydroxymuconic semialdehyde (HMSA). These results imply that another degradation pathway might take place in this strain rather than through intermediate catechol. No test was conducted weather the strain degraded benzoate via protocatechuet, gentisate, or benzoyl Coenzyme.

Differential mating of \textit{S. marcescens} DS-8 with \textit{E. coli} (pJFF350) was obtained at a frequency of $5 \times 10^7$ to $2 \times 10^6$. Several benzoate minus mutants with color alteration were observed. However, Southern blot analysis (Figure 1) showed that benzoate minus mutants were not actually caused by Omegon-Km insertion. These were probably due to pleiotrophic mutation affected other traits. Some other mutants might be caused by the Omegon-Km insertion. These mutants have not been evaluated yet.

Transformation of total genome of benzoate minus mutants digested with \textit{KpnI} to \textit{E. coli} DH5\textalpha{} was failed. No colonies were observed in media with appropriate antibiotic. This result indicated that pJFF350 was not incorporated into the chromosome. Some other mutants might caused by Omegon-Km insertion as showed in Southern hybridization analysis (Figure 1). The insertion was detected at approximately 4.8 kb fragments.
2. During maintenance of integration, question adhesion to cellular DNA. How likely is it that DNA integrates properly?

b. The integration of the myc gene is regulated by a specific promoter. How does this affect the expression of the gene?

c. The expression of the myc gene is under the control of the thymidine kinase promoter. How does this affect the expression of the gene?

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Part 1. Southern blot analysis of total cellular DNA and its Omegom-Km has specific site preferences.

Although the DNA of line 1 was marker, lanes 2-5 were EcoRI, lane 6 was EcoRI, lane 10 was EcoRI, lane 10 was EcoRI.