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[BIO23] Construction of integration vector for nitrogen fixing genes (*nif*) regulation studies of *Paenibacillus macerans*

Goh Boon Poh, Zafarina Zainuddin, Nazalan Najimudin

School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang

Introduction

Nitrogen is very important to living organism because nitrogen is one of the components for proteins and nucleic acids. Nitrogen is one of the limiting factors in agriculture. The used of fertilizer for agriculture was estimated at 40 millions tons in 1975 (Hardy et al., 1977). The world population is going to increase to 8 billions and the need of nitrogen for agriculture is estimated to increase at least two folds (Sanchez et al., 1999).

Fixing of nitrogen by nitrogen fixing organisms is being catalyse by nitrogenase enzyme (Eady, 1991). Nitrogenase from all diazotrophs studied to date can be separated into two oxygen sensitive redox proteins, one of which contains molybdenum and iron (MoFe protein), and the other of which contains iron (Fe protein). The MoFe protein is encoded by *nif*D and *nif*K genes while Fe protein is encoded by *nif*H gene (Jacobson et al., 1989).

Most of the structure and regulation studies of *nif* genes have been done on Gram negative nitrogen fixing bacteria. Little knowledge about regulation of *nif* genes from Gram positive nitrogen fixing bacteria has been published. Thus, the aim of this study is to construct an integration vector to study the *nif* genes of Gram positive bacteria *Paenibacillus macerans* (ATCC 8244).

Materials and methods

Primers for polymerase chain reaction (PCR)

The oligonucleotides enlisted below amplified the coding region of Green Fluorescent Protein (GFP) without the promoter region:

5'-AAAAAGCGGCCGCAAGGATAAATA GGAGGGGATAGCGTAGAAAAAATGAG TAAAGGAGAAGAA-3'(including *Not*I site and ribosome binding site of *Paenibacillus macerans*).

5'-AAGGGTAACTATTGCCGTCATTATTT GTAGAGCTC-3'(including complementary site of chloroamphenical oligonucleotides 5' region)

The oligonucleotides enlisted below amplified the coding region of chloroamphenical:

5'-AGCTCTACAAATAATGACGGCAATA GTTACCCTTA-3'(including complementary site of GFP oligonucleotides 3' region)

5'AAAAAAGCGGCCGCATGGATCTGGA GCTGTAATATA-3'(including *Not*I site)

Polymerase Chain Reaction (PCR)

Both of the GFP and chloroamphenical genes were amplified according to PROMEGA protocols respectively. Amplified GFP and chloroamphenical gene fragments from the first PCR were assembled to form GFP-chloroamphenical gene.

Ligation, transformation and extraction

GFP-chloroamphenical that has been assembled together was then digested with *Not*I enzyme from PROMEGA. Then the digested GFP-chloroamphenical was ligated into recombinant plasmid pmc2.5 (Zafarina, 2001) and transformed into *E.coli* JM109. The ligated plasmids were then extracted and screened for the insert of GFP-chloroamphenical.

Results and discussion

Both of the GFP and chloroamphenical gene fragments were successfully amplified producing fragments of sizes 780bp (FIGURE 1) and 1070bp (FIGURE 2) respectively. GFP-chloroamphenical gene fragment was also successfully amplified producing fragment of

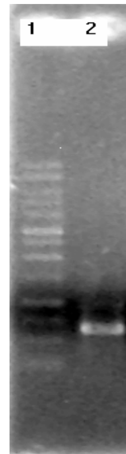

FIGURE 1 PCR amplified GFP fragment. Lane 1: 1kb DNA Ladder marker Lane 2: Amplified GFP at 780bp

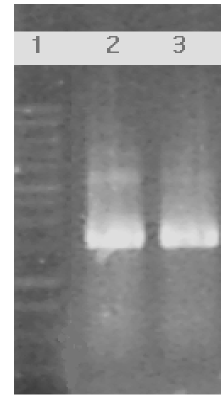

FIGURE 3 PCR assembled of GFP-chloroamphenical fragments. Lane 1: 1kb DNA Ladder marker Lane 2 and 3: GFP-chloroamphenical amplified at 1850bp

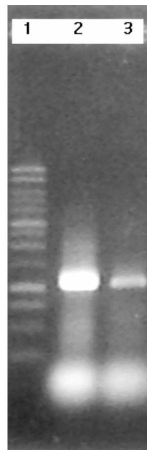

FIGURE 2 PCR amplified chloroamphenical fragments. Lane 1: 1kb DNA Ladder marker Lane 2 and 3: chloroamphenical amplified at 1070bp

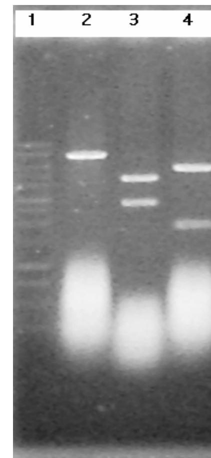

FIGURE 4 Recombinant plasmid with GFP-chloroamphenical inserted. Lane 1: 1kb DNA Ladder marker Lane 2: undigested pGBP301, 7036kb Lane 3: pGBP301 digested with *Hind*III with 4.3kb and 2.6kb fragments Lane 4: pGBP301 digested with *Not*I with 1.8kb and 5.0kb fragments

size 1850bp (FIGURE 3). PCR product comprising the coding region of GFP-chloroamphenical was then inserted in the *Not*I
site of pmc2.5. The successfully cloned plasmid was name pGBP301. The size of this vector is 7036bp with GFP as reporter gene and chloroamphenical as selection gene (FIGURE 4).

These vector can be use for further study of *nif* regulation of *Paenibacillus macerans* by letting the vector double recombinant with the chromosomal DNA of *Paenibacillus macerans*.

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