

Short Communication

Application of genefishing discovery system on differential gene expression study for prokaryotic system

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GeneFishing Discovery is a system applied on disease and gene related research for eukaryotes and the starting material for the study could be either total RNA or mRNA. In this study we demonstrate the use of this discovery system for a prokaryotic system by modifying the eukaryotic protocol using the poly (A)-tailing approach. As an example we compared the gene expression pattern of two closely related species of bacteria under the same family (*Burkholderia thailandensis* and *Burkholderia cepacia*) with satisfactory results.

Key words: Genefishing, differential expression, prokaryotic system.

INTRODUCTION

GeneFishing Differentially Expressed Gene (DEG) Discovery system (Seegene) is a system for identifying differentially expressed genes in two RNA samples. Applications of this such as the study of genes which express differentially at each developmental stage in a eukaryotic system mainly in humans, screening of genetic markers or inhibitors in cancer samples (Lee et al., 2009; Liao et al., 2009) and the study of genes which express differentially due to biological or environmental changes can be carried out. Overall, this system is simpler to use than the conventional mRNA differential display method which is laborious and application of polyacrylamide gel electrophoresis is necessary and requires radioactive labeling for the experiment. Only a few studies have been reported on the use of this approach on prokaryotic systems (Asakura et al., 2007; Kang et al., 2007; Shin et al., 2007).

Two *Burkholderia* species were chosen as the prokaryotic

system to carry out this study. *Burkholderia* is a genus of proteobacteria commonly found in the environment consisting of several species. Most of the species may be considered as human pathogens, for example *Burkholderia pseudomallei* that causes melioidosis in humans (Puthuchery et al., 1992 and *Burkholderia cepacia* that can cause pulmonary infection in cystic fibrosis patients (Lipuma, 2005). Species such as *Burkholderia thailandensis* is considered as avirulent and rarely cause disease in humans (Glass et al., 2006). In this study, *B. cepacia* (virulent strain) and *B. thailandensis* (avirulent) have been selected to carry out this experiment. Although they are from the same genus, these two bacteria are expected to have some differences in terms of gene expression. Here, we are delineating the usefulness of this approach in recognizing the differentially expressed genes between two *Burkholderia* species, which were chosen as the prokaryotic system in our study.

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Abbreviations: DEG, GeneFishing Differentially Expressed Gene; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; mRNA, messenger RNA; rRNA, ribosomal RNA.

MATERIALS AND METHODS

Bacterial strains

B. thailandensis (ATCC 700388), and *B. cepacia* (ATCC 25416) were cultured and incubated overnight at 37°C in 10 ml of Luria Bertani broth (Sigma-Aldrich).

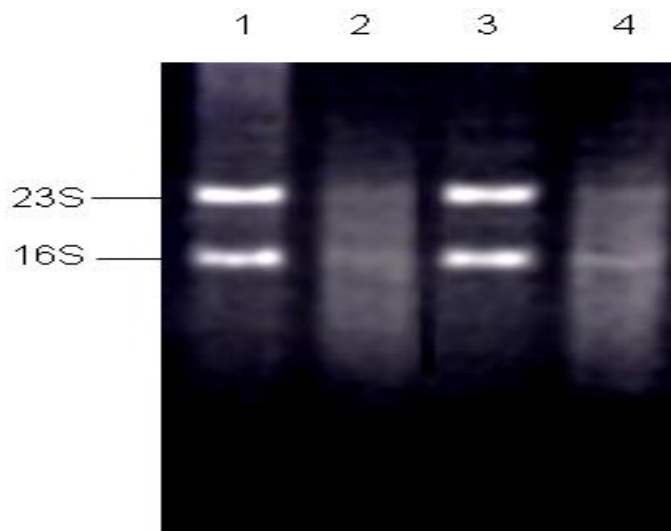


Figure 1. Poly(A)-tailing reaction of RNA. **Lane 1:** *B. thailandensis* total RNA; **Lane 2:** *B. thailandensis* poly(A)-tailed RNA; **Lane 3:** *B. cepacia* total RNA; and **Lane 4:** *B. cepacia* poly(A)-tailed RNA. Electrophoresis of RNA was run on a native agarose gel.

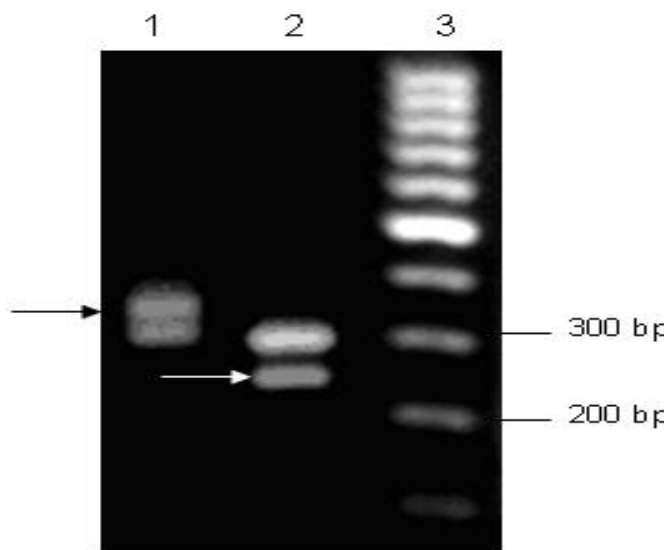


Figure 2. Differential gene expression patterns of *B. thailandensis* and *B. cepacia* following amplification with arbitrary primers ACP12 from Seegene discovery system. **Lane 1:** *B. thailandensis*; **Lane 2:** *B. cepacia*; and **Lane 3:** 100 bp DNA marker. Differential bands are indicated by arrows.

RNA isolation

Total RNA extraction from the bacterial cells was performed at room temperature using RNeasy[®] Mini Kit (Qiagen). Initially, the cells were lysed in 200 μ l TE buffer (30 mM Tris-Cl, 1 mM EDTA, pH 8.0) containing 10 mAU of proteinase K (Qiagen) and 15 mg/ml lysozyme (Amresco). Buffer RLT with absolute ethanol was added thereafter and 700 μ l of the lysate was transferred to an RNeasy

Mini spin column followed by a short centrifugation at 10,000 \times g. Washing and centrifugation steps were repeated 3 times and RNA was eluted from the spin column with 30 μ l RNase-free water by short centrifugation at 10,000 \times g.

Poly (A)-tailing reaction

Poly (A) tailing kit (Ambion) was used in this study to add poly (A)-tails to the 3'-end of the extracted RNA. Basically, 60 μ g of the RNA was added to the tailing reagents (1X *Escherichia coli* Poly (A) polymerase (*E*-PAP) buffer, 2.5 mM MnCl₂, 1 mM ATP and 1 U *E*-PAP) to a final volume of 100 μ l and incubated for 1 h at 37°C. Electrophoresis was carried out to confirm the resulting poly (A)-tailed RNA with 1% native agarose gel.

Reverse transcription polymerase chain reaction (RT-PCR)

The poly (A)-tailed RNA from the bacteria was ready to be used to synthesize first strand cDNA. Three μ g of the RNA was mixed with 1 μ M dT-ACP1 (Seegene) and reagents from RevertAid[™] H Minus First Strand cDNA Synthesis Kit (Fermentas), containing 1X reaction buffer, 20 units RiboLock[™] Ribonuclease inhibitor, 1 mM dNTP mix and 200 unit of RevertAid[™] H Minus M-MuLV reverse transcriptase. The mixture was incubated at 42°C for 90 min and heat deactivated. The final mixture was further diluted with 80 μ l of DNase-free water.

Fifty nanograms (50 ng) of the diluted first-strand cDNA was added to PCR reagents from GeneFishing[™] DEG Premix Kit (Seegene) (0.5 μ M of one of the 20 arbitrary ACPs, 0.5 μ M dT-ACP2 and 1X SeeAmp[™] ACP[™] Master Mix) and followed by 40 cycles of denaturation at 94°C for 40 sec and annealing at 65°C for 40 sec. Finally, 5 μ l of the PCR product was electrophoresed on a 2% agarose gel containing ethidium bromide.

RESULTS AND DISCUSSION

Figure 1 shows the results of the extracted total RNA and tailed-RNA after poly(A) tailing reaction. The tailed-RNA was further used in PCR reactions using primers from the GeneFishing DEG Discovery System. An obviously differentially expressed gene pattern was observed between *B. thailandensis* and *B. cepacia* when primer ACP12 was used (Figure 2). This is one of the arbitrary primer sets obtained from the GeneFishing DEG Discovery system. The differential bands shown by arrows (Figure 2) were further extracted from *B. thailandensis* and *B. cepacia* and proceeded to cloning prior to sequencing to obtain the gene information.

GeneFishing DEG Discovery System (Seegene) is specifically designed to study differential gene expression mainly for eukaryotic systems. This is because the system uses oligo dT-ACP to synthesize first-strand cDNAs from the mRNA samples, where the 3' termini of the oligo dT-ACP contains a complementary sequence to a poly(A) region of eukaryotic mRNA transcripts and allows annealing during reverse transcription PCR. Due to this reason and the fact that poly(A) tail does not exist in RNA prokaryotic bacteria, the system is seldom used for gene expression study in bacteria or prokaryotic system. We have demonstrated the use of this discovery system in a

in a bacterial system by a small modification on the bacterial RNA. Figure 1 shows poly(A)-tailing reaction of *B. thailandensis* and *B. cepacia* total RNA. rRNAs appeared as two distinct bands, 16S and 23S, whereas mRNAs appeared as smear. Upon poly(A)-tailing reaction, the samples did not contain strong rRNA bands and appeared as a smear ranging from approximately 6 to 0.5 kb, resulting from the population of mRNAs and depending on exposure times and conditions. Following this, reverse transcription was conducted using dT-ACP1 primer from the genefishing system to synthesize the first-strand cDNAs from the samples, whereby the 3'-end core portion of dT-ACP1 would anneal to the poly(A) region of mRNA transcripts. A sequential PCR can be carried out using arbitrary primers from the genefishing system, resulting in the amplification of the cDNAs that carry the complementary sequences of the arbitrary primers. In a previous study, genes expressing lytictransglycosylase, porin, outer membrane autotransporter barrel domain and the two component transcriptional regulator of the LuxR family were able to be picked up by arbitrary primers ACP5, ACP9, ACP10 and ACP12, respectively, in *B. cepacia* but not in the other *Burkholderia* species (Puthuchery et al., 2008).

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