Short Communication

A transposase-like gene isolated from *Burkholderia pseudomallei* by DDRT-PCR technique

Amaraponchoeycharoen, Napaporn Vinichnaiyapark, Prachaya Vichchathorn, Suda Tunpiboonsak, and Sumalee Tungpradabkul

Department of Biochemistry, Faculty of Science, Mahidol University, Thailand

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*Burkholderia pseudomallei* is a motile gram-negative bacteria which causes melioidosis, a highly fatal tropical infectious disease endemic in Southeast Asia and Northern Australia. The organism is limited in its distribution to tropical and subtropical areas between 20°N and 20°S (Yabuuchi et al., 1992) and is commonly found in water and soil. The bacteria is usually transmitted to humans and also animals. Unlike, *B. mallei* is a gram-negative, non-motile bacteria and is considered to be an equine parasite, in which host it causes glands. The infection can be transmitted to a large variety of animals including humans (Goven, 1997). *B. mallei* is genetically related to *B. pseudomallei* and the 16S rRNA sequences have 100% homology (Yabuuchi et al., 1992). As such, *B. mallei* is similar to *B. pseudomallei* genetically, clinically and pathologically; however, epidemiologically they are dissimilar. In contrast, *B. pseudomallei* is epidemiologically similar to *B. thailandensis*, which is found in soil isolates from the northeast of Thailand. Significant differences between *B. pseudomallei* and *B. thailandensis* such as 16S rRNA gene sequence, exoenzyme production (Brett et al., 1998), the ability to assimilate arabinose and hamster virulence (Smith et al., 1997) have led to *B. thailandensis* being classified as a new species (Brett et al., 1998).

Since *B. thailandensis* is a non-virulent species in this genus, which is structurally and immunologically similar to *B. pseudomallei*, it was interesting to identify gene products that might be involved in the virulence of the bacteria, *B. pseudomallei*, based on differential display gene expression RT-PCR or DDRT-PCR. The concept of DDRT-PCR was first described and applicable in eukaryotic cells (Liang and Pardee, 1992). Recently, it was modified and succeeded in prokaryotic organisms, the *Enterobacteriaceae* group (Fislage et al., 1997).

Under a DDRT-PCR technique, an entire gene encoding for a transposase was identified from *B. pseudomallei* NF 10/38. In addition, we have compared the localization of the transposase in the genomes among *B. pseudomallei, B. thailandensis* and *B. mallei* using Southern blot analysis.

The primers for DDRT-PCR were designed by modifying the method of R. Fislage et al. (Fislage et al., 1997) and RNA arbitrarily primed PCR-based method (Welsh and McClelland, 1990; Williams et al., 1990). A forward primer was 5'-TGGCCATGAA-3' and a reverse primer was 5'-CCCGTCAGCCC-3'. Total RNA was isolated from *B. pseudomallei* and *B. thailandensis* with an RNeasy Minikit (QIAGEN GmbH, Hilden, Germany).
Germany) as the manufacturer’s protocol recommended, based on guanidium isothiocyanate disruption. Bacterial DNA contamination was removed by RNase free DNase I digestion as described in the manufacturer’s protocol. Synthesis of cDNA was carried out using 6–10 μg of total RNA after denaturation for 10 min at 70°C and cooling to 30°C. The reaction mix contained 200 U M-MLV reverse transcriptase (Gibco BRL, Gaithersburg, MD, USA), 0.5 mM dNTPs, 10 mM DTT, 40 U RnaseInβ, 1× first-strand buffer and 6.3 μM reverse primer in a final volume of 30 μl. The reaction was elongated at 30°C for 5 min followed by an hour’s synthesis at 37°C and denaturation at 95°C for 10 min. A total of 2 μl cDNA was used for differential display PCR in a volume of 20 μl containing 200 μM dNTPs, 5 μM reverse primer, 0.5 μM forward primer, 1 μM betaine and 2.5 U Taq DNA polymerase (Boehringer Mannheim, Mannheim, Germany) in 1× buffer reaction. The reaction was run in a thermocycler (model 480, Perkin Elmer Cetus, Perkin Elmer Cetus, NJ, USA) for 40 cycles using the annealing temperature. The PCR products were analyzed on a 1% agarose gel with ethidium bromide staining and the differential products approximately between 0.5–1.0 kb in size were purified and cloned into pUC19 at SmaI site according to standard cloning protocols. The recombinant clones were sequenced in both strands using an automated DNA sequencer (model 377, Applied Biosystem model 377, Applied Biosystem, Foster City, CA, USA). The resulting sequences were used to search for sequence similarity amongst known sequences using the BLAST database search program (Altschul et al., 1997). One of these clones desig-nated pIS1 was interesting when compared with the GenBank database. The result of BLAST showed 99% of the nucleotide or 100% of amino acid homology to the transposase (Tn10) of Salmonella typhi (accession number AF223162). The nucleotide sequence and the reduced amino acid sequence of pIS1 was submitted to the GenBank with accession number AF301536. The presence of a potential Shine-Dalgarno sequence at the 5′ end of this gene, a starting codon, and a stop codon with an open reading frame showed an entire coding sequence able to be expressed as shown in Fig. 1.

In order to demonstrate a transposase containing

![Fig. 2. An ethidium bromide staining of genomic DNA digested pattern (a) and Southern blot hybridization pattern (b) of B. pseudomallei, B. thailandensis and B. mallei digested with Avell (lanes 2, 3, 4), with EcoRII (lanes 5, 6, 7) and EcoRI plus PstI (lanes 8, 9, 10), respectively. Lane 1 is the positive control and lane S is a standard size marker. The transposase-like gene from B. pseudomallei was used as a probe that was nonradioactively labeled according to Digoxigenin protocol (Boehringer Mannheim). The hybridization condition used the high stringency of 0.1% SDS, 0.1× SSC at 65°C.](image)
transposable element localization in the genome of the bacteria, Southern blot analysis was performed (Southern, 1975). Figure 2a was a 1% agarose gel electrophoresis of restricted genomic DNA of *B. pseudomallei*, *B. thailandensis* and *B. mallei*. The same DNA pattern from 2a was transferred onto nylon membrane and the hybridization pattern was determined using a transposase-like gene as a probe as shown in Fig. 2b. The hybridization signals were detected in restricted genomic DNA of *B. pseudomallei* and *B. mallei* whereas in *B. thailandensis* they were not able to detect. Moreover, the localization of the gene in *B. pseudomallei* and *B. mallei* was found in more than one copy and the gene organization pattern found in *B. pseudomallei* was basically similar to that in *B. mallei*. However, the signal intensity found in *B. mallei* was approximately 5 times higher than in *B. pseudomallei*. Our finding demonstrated that DDRT-PCR in the identification of differentially expressed mRNAs products between *B. thailandensis* and *B. pseudomallei* gave a desirable result. The technique has more advantage than DNA subtractive hybridization technique (Brown et al., 2000) in that a target product could be focused on expressed genes or functional genes. Obviously, our result also supported a close genetic relationship between *B. pseudomallei* and *B. mallei* based on hybridization pattern, although a transposase gene in *B. mallei* was found in higher copy than in *B. pseudomallei*.

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**References**


