The detection of insertion sequences within the human pathogen *Burkholderia pseudomallei* which have been identified previously in *Burkholderia cepacia*

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Abstract

Using primers designed from the nucleotide sequences of five insertion elements identified previously in *Burkholderia cepacia*, the presence of two insertion sequences (IS406 and IS407) was detected in chromosomal DNA isolated from strains of the human pathogen *Burkholderia pseudomallei*. The IS407 homologue was cloned from *B. pseudomallei* NCTC 4845 and nucleotide sequenced to confirm its identity and degree of homology with *B. cepacia* IS407. A PCR amplification product from *B. pseudomallei* NCTC 4845 DNA provided an IS407 probe which was used to determine, by Southern blotting, the number and location of copies of IS407 in ten strains of *B. pseudomallei* and four representatives from three of the five genomovars of *B. cepacia*. © 1998 Published by Elsevier Science B.V.

Keywords: *Burkholderia pseudomallei*; *Burkholderia cepacia*; Insertion sequence; Detection; Strain variation

1. Introduction

*Burkholderia cepacia* and *Burkholderia pseudomallei* were formally considered to belong to the genus *Pseudomonas* in the γ-subclass of the proteobacteria. More recently they were re-assigned to the β-subclass of the proteobacteria on the basis of ribosomal ribonucleotide gene sequence data, and other phenotypic characteristics. On the basis of the 16S rRNA sequence comparisons they were moved into the separate genus *Burkholderia* [1]. New molecular taxonomic evidence has revealed that environmental and clinical isolates presently identified as *B. cepacia* comprise at least five distinct species or genomovars. Genomovars II and V have recently been renamed *B. multivorans* and *B. vietnamiensis* respectively [2]. *B. cepacia* is described as an opportunistic pathogen of immunocompromised hosts, particularly those with chronic granulomatous disease and cystic fibrosis [3]. Although *B. cepacia* is generally considered to be ubiquitous and non-pathogenic, culture of *B. cepacia* from the environment is surprisingly difficult [4] and virulent, multiresistant epidemic strains of *B. cepacia*, particularly isolates such as J2315 and other genomovar III strains, are a major concern to individuals with cystic fibrosis [3].

*B. pseudomallei* is the causative agent of meliodosis and is particularly noted for its ability to cause either an acute, often fatal, septicaemia [5,6]; or re-
maining dormant in the body for many years apparently asymptomatically, yet retaining its ability to flare into an acute septicemia at any time [7–10]. As with B. cepacia, B. pseudomallei taxonomy is still in the process of being established and a new avirulent biotype of this organism has only recently been described [11]. No vaccine exists for either of these organisms and treatment with antimicrobials is protracted for both diseases because of the natural resistance of both B. pseudomallei and B. cepacia to many of the commonly used antibiotics. Although B. pseudomallei and B. cepacia cause very different diseases in man, they are nevertheless taxonomically closely related.

Insertion sequences (IS) have been defined as 'genetic entities which are capable of inserting as discrete, non-permuted DNA segments at many different sites in prokaryotic genomes' [12]. Their small size means that their structural, regulatory and transposition genes are well organised and may even overlap. IS elements usually integrate into the genome by mechanisms essentially independent of DNA sequence homology [13] and are known to inactivate not only the gene into which they have inserted but also genes downstream in the same operon. The plasticity of the B. cepacia genome is now well established [14] and much of this plasticity has been attributed to the high number and diversity of insertion sequences present in the genome, and the presence of multiple copies of many of the elements. It has been suggested that the biological function of insertion sequences, which lack a phenotypic manifestation, is the generation of a genetic diversity that helps drive evolution and that the capacity to introduce a complex regulatory function in a single mutational step is a feature that clearly would increase the evolutionary potential of any cell containing such elements [15]. This research may indicate that the genome of B. pseudomallei also has the potential to rearrange and evolve in this manner. No IS elements have previously been identified in B. pseudomallei.

2. Materials and methods

2.1. Bacterial strains and culture

Escherichia coli JM109 (Stratagene LTD, UK); B. pseudomallei NCTC 4845, 708A and E38 (Microbiology culture collection, CBD, Porton Down) 204, 576, E25, E27, E82 and E8 (courtesy of T. Pitt, PHLS, Colindale Avenue, London). B. cepacia J2315 (Edinburgh/Toronto epidemic strain [16], genomovar III), C1964 (Edinburgh cystic fibrosis isolate, genomovar I, courtesy of J. Govan, University Edinburgh, UK), C1576 (Glasgow cystic fibrosis isolate, epidemic strain, described by Whiteford et al. [17], genomovar II, recently renamed B. multivorans [2]) and C1962 (non-cystic fibrosis isolate from a brain abscess [18], genomovar II, courtesy of J. Govan, University Edinburgh, UK). All organisms were cultured on LB medium.

2.2. PCR

All PCR reactions were performed in a Perkin Elmer Cetus Ltd. GeneAmp PCR system 9600 (Buckinghamshire, UK) according to manufacturer’s instructions. Primer pairs and reaction conditions used for the amplification of IS elements were designed by Tyler et al. [19].

2.3. Standard genetic techniques

Chromosomal DNA was prepared by centrifuging 30 ml of a 12 h broth culture at 3000×g for 10 min. The pellet was resuspended in 15 ml of lysing solution (10 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1 mM EDTA and 0.5% (w/v) SDS) containing proteinase K at a final concentration of 100 µg/ml and incubated for 15 h at 50°C. The lysate was washed with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) with centrifugation at 5000 rpm to separate the phases. The top phase was transferred to a separate tube and the DNA precipitated by the addition of 2 volumes of ice-cold 95% ethanol. The DNA was spoiled onto a glass rod and transferred to a suitable volume of TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0).

Chromosomal DNA was prepared from each of the bacterial species and digested with BglII restriction endonuclease and with BamHI and HindIII restriction endonucleases. None of these enzymes cuts within the B. cepacia IS407 encoding DNA. Digestion with restriction enzymes and ligations were performed as described by Sambrook et al. [20]. Di-
gested chromosomal DNA run on a 0.7% TAE (40 mM Tris-acetate, pH 8.3, 1 mM EDTA) agarose gel was transferred onto a positively charged nylon membrane (Boehringer Mannheim, UK) by capillary transfer as described by Southern [21]. Specific sequences were detected using a dioxygenin labelled IS407 PCR product probe, produced according to manufacturer’s instructions (PCR DIG labelling mix, Boehringer Mannheim, UK).

2.4. Nucleotide sequencing

Nucleotide sequencing was performed using an automated DNA sequencer (Model 373A, Applied Biosystems Ltd., UK) according to manufacturer’s instructions. The gels were analysed using the 373A sequencing software supplied by Applied Biosystems Ltd., UK. All sequence data were obtained in triplicate, in both the 5’-3’ and the 3’-5’ direction.

3. Results and discussion

3.1. Identification of IS elements in B. pseudomallei

The PCR was used to determine whether IS402, IS406, IS407, IS408 or IS1356 elements, isolated previously from B. cepacia [19], could be detected in DNA isolated from strains of B. pseudomallei. The insertion element IS407 could be amplified from all strains of B. cepacia tested; however, it could not be detected in two of the ten strains of B. pseudomallei (E27 and E82). Similarly, IS407 could not be amplified from the E27 and E82 strains of B. pseudomallei, although additionally it could not be amplified from two out of four B. cepacia strains tested (Table 1). The apparent absence of IS407 from the E27 and E82 strains of B. pseudomallei, both of which are considered to be avirulent in man, is particularly interesting in light of their recent differentiation from virulent B. pseudomallei strains into a new bioype on the basis of an arabinose assimilation phenotype [11]. The two strains of B. cepacia which contained IS407 (C1576 and J2315) have been associated with epidemic outbreak [16,17]. IS402, IS408 and IS1356 (which has also been associated with epidemic strains of B. cepacia [19]) were not amplified from the extracted DNA of any of the B. pseudomallei strains which were tested. The question of whether these two Burkholderia strains share IS406 and IS407 by horizontal gene transfer, or whether they descend from a single microbial ancestor [22], is an important one considering the current debate on the human hazards and ecological consequences of using strains belonging to B. cepacia genomovar V (B. vietnamiensis) as soil decontaminants and agricultural biopesticides [3].

3.2. Nucleotide sequence of IS elements

The PCR products (Fig. 1) from the amplification of the IS407 elements from B. pseudomallei NCTC

Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>IS406</th>
<th>IS407</th>
</tr>
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<tbody>
<tr>
<td>B. pseudomallei NCTC 4845</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. pseudomallei 708A</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. pseudomallei E38</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. pseudomallei MAL6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. pseudomallei 204</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. pseudomallei 576</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. pseudomallei E25</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. pseudomallei E27</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>B. pseudomallei E82</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>B. pseudomallei E8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. cepacia C1962 (genomovar II)</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>B. cepacia C1964 (genomovar I)</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>B. cepacia C1576 (genomovar II)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. cepacia J2315 (genomovar III)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

A ‘+’ indicated a positive PCR amplification and a ‘−’ a negative PCR amplification.
4845 and *B. cepacia* J2315 were sequenced using the amplification primers as sequencing primers. The nucleotide sequence showed between 95.7% identity (*B. cepacia* J2315) and 95.0% identity (*B. pseudomallei* NCTC 4845) with the published sequence of IS407 isolated from *B. cepacia* 17616 (Genbank NID G151305) (Fig. 2). The sequences of the IS407 homologs from *B. pseudomallei* NCTC 4845, *B. cepacia* ATCC 17616 and *B. cepacia* J2315 indicate heterogeneity of IS407.
3.3. Strain identification by Southern blotting

A Southern blot using labelled IS407 as a probe was used to determine the number and location of copies of the IS in each bacterial chromosome (Figs. 3 and 4). This probe showed clearly defined bands and the patterns obtained could be used to distinguish one strain from another. Using DNA digested with two separate restriction endonucleases demonstrated that the number of IS407 elements present within the genomes of different bacterial strains varies. The numbers of copies of the element IS407 ranged between 2 and 4 for the *B. pseudomallei* strains, and between 8 and 9 for the strains of *B. cepacia* tested.

The difference in intensity seen between some of the bands in the Southern blot cannot easily be explained. It could suggest that there are differences in the sequences between copies of IS407 with a genome, or it may suggest that more than one copy of the insertion element lies on each restriction fragment. The use of two separate restriction endonucleases did not resolve this issue; although it has been reported previously that some insertion sequen-
ces can be present as multiple, but non-identical copies within a genome [15]. Interestingly, the two strains of *B. pseudomallei* which were negative for both IS406 and IS407 by PCR showed extremely faint bands present by Southern blot using the IS407 probe. This could suggest that the IS407 probe is binding to a fragment of DNA which has some identity to IS407 – perhaps even a distant relative of IS407. The two strains of *B. cepacia* were negative for IS407 by PCR were also clearly negative by Southern blot.

Although insertion of either IS406 or IS407 into a gene would effectively interrupt the function of that gene, both IS elements have been shown to contain an outward facing σ^0^ like promoter [14,23]. This may serve to increase the expression of genes downstream of the site into which the IS has inserted. "As a result of this work, *B. pseudomallei* has now been shown to contain mobile genetic elements. These insertion sequences may serve a role in the genetic diversity of this species, as they do for the *B. cepacia* species, perhaps by increasing the level of expression of genes downstream of their insertion site, or perhaps by direct insertional inactivation. The incidence of insertion sequences across the *B. pseudomallei* species, as well as their diversity and locations, would make interesting topics for the future, enabling a greater understanding of this species."

References


