NOTE

***Burkholderia thailandensis* sp. nov., a *Burkholderia pseudomallei*-like species**

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Alberta, Canada T2N 4N1**The presence of a *Burkholderia pseudomallei*-like species based upon the significant genotypic and phenotypic dissimilarities exhibited between these organisms and true *B. pseudomallei* strains has been reported previously. In this study, a comprehensive 16S rDNA-based phylogenetic analysis further supports the existence of this newly described *Burkholderia* species for which the name *Burkholderia thailandensis* sp. nov. is proposed.****Keywords:** *Burkholderia thailandensis* sp. nov., *Burkholderia pseudomallei*, 16S rDNA

Burkholderia pseudomallei, the aetiological agent of melioidosis, is responsible for disease in both humans and animals (8, 13). While this organism is endemic to a number of tropical regions, the incidence of melioidosis is particularly high in South-East Asia and Northern Australia. The bacterium is capable of causing a broad spectrum of diseases with protean clinical manifestations. However, the acute pulmonary and septicaemic forms are of particular concern due to the high incidence of mortality associated with these cases even when vigorous chemotherapeutic intervention is implemented (8, 13, 14). *B. pseudomallei* is especially problematic in North-East Thailand where approximately 20% of community-acquired septicaemias can be attributed to this bacterial pathogen (3).

The major focus of our laboratory has been to investigate the pathogenesis of disease caused by *B. pseudomallei*. To facilitate these studies we have utilized clinical isolates and a variety of environmental isolates acquired from Central and North-East Thailand (16, 21). The environmental isolates have been tentatively identified as *B. pseudomallei* strains based upon growth on selective media, biochemical profiling (API 20NE) and latex agglutination assays (16, 21). Recently, while attempting to develop a transposon-based mutagenesis system for *B. pseudomallei*, we observed a number of phenotypic and genotypic dissimilarities between clinical isolates and some of the environmental isolates (2, 4). One of the most significant differences associated with these particular environmental isolates was their decreased virulence

for Syrian golden hamsters in comparison to the true *B. pseudomallei* strains. These *B. pseudomallei*-like strains, as they were termed, demonstrated a > 10⁵-fold decrease in virulence relative to *B. pseudomallei* strains in this animal model of acute melioidosis (2). Wuthiekanun *et al.* have recently demonstrated differences in the biochemical characteristics of *B. pseudomallei*-like isolates and true *B. pseudomallei* isolates (22). It has been suggested that the *B. pseudomallei*-like environmental isolates may represent a new species of *Burkholderia* (2, 22).

In this study, we have cloned and sequenced > 95% of the 16S rDNA from *B. pseudomallei* 1026b (a virulent clinical isolate) and *B. pseudomallei*-like E264 to definitively assess the relatedness of these organisms to each other. The results of these studies, based upon a 16S rDNA phylogenetic analysis, confirm the presence of a new *Burkholderia* species for which we propose the name *Burkholderia thailandensis*.

Cloning and sequencing of the 16S rRNA genes

The origin of *B. pseudomallei* and *B. pseudomallei*-like strains has been described previously (2). Modified Ashdown's selective medium was used to maintain and differentiate the bacterial strains in routine laboratory use (2). The oligodeoxyribonucleotide primers used for the PCR were designed based on published *B. pseudomallei* 16S rDNA sequences (25) and are shown in Table 1. The isolation of chromosomal DNA and the amplification of the 16S rRNA gene via PCR was described previously except that the cycling protocol used here was 97 °C for 5 min followed by 30 cycles of 97 °C for 45 s, 55 °C for 45 s and 72 °C for 90 s (2).

The GenBank accession numbers for the sequences reported in this paper are U91838 (*B. thailandensis* E264) and U91839 (*B. pseudomallei* 1026b).

Table 1. *B. pseudomallei* and *B. thailandensis* PCR amplification and sequencing primers

Primer	Sequence (5'-3')*	Position
16S-5'	AGTTTGATCCTGGCTC*	10-25†
16S-FA	GATTAGCTAGTTGGTGGG	237-254‡
16S-FB	GGAAGTGCATTGGTGACT	622-639‡
16S-FC	GTGAGATGTTGGGTAAAG	1073-1090‡
16S-3'	AAGGAGGTGATCCAGCC*	1541-1525†
16S-RA	GTATGACGTGTGAAGCCC	1227-1210‡
16S-RB	TTTGCTCCCCACGCTTTC	777-760‡
16S-RC	CCAAATTCCCCACTGCT	365-348‡

* PCR primers are as described by Yabuuchi *et al.* (25).

† *Escherichia coli* numbering system.

‡ Primer positions based upon numbering used in Fig. 1.

PCR products were cloned into the *EcoRV* site of pZero-2.1 (Invitrogen) and the sequencing of insert DNA was performed on both strands by Genomix (Augusta, GA, USA). The oligodeoxyribonucleotide primers used for sequencing the 16S rRNA genes are shown in Table 1.

In all, 1488 bp of the 16S rRNA genes from both *B. pseudomallei*-like E264 and *B. pseudomallei* 1026b were sequenced, with a total of 15 nucleotide dissimilarities observed upon alignment of the two alleles (Fig. 1). The majority of the differences (11/15) were clustered into three distinct hypervariable domains (Fig. 1), two of which have been exploited previously to design PCR amplification primers for the characterization of suspected *B. pseudomallei* isolates from clinical samples (6).

It is worth noting that the 16S rDNA sequence obtained for *B. pseudomallei* 1026b is the first complete nucleotide sequence for this particular species since previous studies have failed to obtain sequence information regarding positions 992-1138 (25). A comparison of the previously published *B. pseudomallei* ATCC 23343 sequence fragments (25) with the sequence of *B. pseudomallei* 1026b demonstrates five differences between the two 16S rRNA genes. The *B. pseudomallei* 1026b 16S rDNA contains C residues at positions 87 and 843 that are not present in the *B. pseudomallei* ATCC 23343 16S rDNA (Fig. 1). There are also nucleotide differences at residues 254, 1142 and 1318 (Fig. 1). Interestingly, identical differences that support the *B. pseudomallei* 1026b sequence data at residue 254 have been reported for several *B. pseudomallei* clinical isolates by Dharakul *et al.* (6).

Phylogenetic analysis

The phylogenetic analysis in Fig. 2 is based on the 16S rDNA sequences presented here and the 16S rDNA sequences from closely related bacterial species in the

genera *Burkholderia* and *Ralstonia*. Based on its location on the phylogenetic tree, *B. pseudomallei*-like E264 can clearly be distinguished as a distinct *Burkholderia* species (Fig. 2). Since *B. pseudomallei*-like organisms have been isolated from multiple sites in Central and North-East Thailand, we propose the name *Burkholderia thailandensis* for this new species. *B. thailandensis* is more closely related to *B. pseudomallei* than to any other *Burkholderia* species with the exception of *Burkholderia mallei* (25), which reportedly has an identical 16S rRNA gene (Fig. 2). This may explain the antigenic and morphologic similarities of these two species (2, 16, 22). The results from a spectroscopic analysis of purified O polysaccharide from *B. thailandensis* E264 (11) has demonstrated that this organism synthesizes a repeating disaccharide having the structure $\rightarrow 3\text{-}\beta\text{-D-glucopyranose-(1}\rightarrow 3\text{)-6-deoxy-}\alpha\text{-L-talopyranosyl-(1}\rightarrow$. *B. pseudomallei* strains also produce this repeating disaccharide as well as a 1,3-linked homopolymer of 2-O-acetylated 6-deoxy- $\beta\text{-D-manno-heptopyranosyl}$ residues (7, 12). Cross-reactive antibodies to the common O polysaccharide may be part of the reason why *B. thailandensis* strains were initially identified as *B. pseudomallei* strains by latex agglutination tests (15, 16).

Description of *Burkholderia thailandensis* sp. nov.

Burkholderia thailandensis (thai.lan.den'sis. M.L. adj. *thailandensis* pertaining to Thailand from where the organism was originally isolated).

Cells are Gram-negative and motile due to the presence of a polar tuft of two to four flagella. The organism is nutritionally diverse and grows at temperatures ranging from 25 to 42 °C. The production of siderophore, lipase, lecithinase and protease are apparent at both 25 and 37 °C (2). *B. thailandensis* colonies are smooth and glossy with a pink pigmentation on modified Ashdown's selective medium while *B. pseudomallei* colonies are rough and wrinkled with a dark purple pigmentation (2). The API 20NE and API 50CH biochemical profiles for *B. thailandensis* strains are similar to those for *B. pseudomallei* strains (22). Notable exceptions, however, include the capacity to assimilate L-arabinose, 5-keto-gluconate and adonitol and the inability to utilize erythritol and dulcitol as sole carbon sources. Previous studies describing the presence of *B. thailandensis* during epidemiological surveys in Thailand have been unable to correlate human disease with this organism (22). These findings support our animal studies which clearly demonstrate that *B. thailandensis* strains are avirulent for Syrian golden hamsters (2). *B. thailandensis* isolates are resistant to aminoglycosides but sensitive to tetracycline, ceftazidime and trimethoprim. The type strain, E264, was isolated from a rice field soil sample in central Thailand (16). The type strain has been deposited in the American Type Culture Collection as ATCC 700388.

E264	AGATTGAACGCTGGCGCATGCTTACACATGCAAGTCGAACGGCAGCAGGGCTTCGGCTGGTGGCA	96
1026b	AGATTGAACGCTGGCGCATGCTTACACATGCAAGTCGAACGGCAGCAGGGCTTCGGCTGGTGGCA	96
E264	GTGGCGAACGGGTGAGTAATACATCGGAACATGCTCTAGTGGGGGATAGCCGGCGAAAGCCGGATTA	166
1026b	GTGGCGAACGGGTGAGTAATACATCGGAACATGCTCTAGTGGGGGATAGCCGGCGAAAGCCGGATTA	166
E264	ATACCGCATACATGAGGATGAAGCGGGGACCTTCGGCTTCGCGCTATAGGGTTGGCCATATGCT	236
1026b	ATACCGCATACATGAGGATGAAGCGGGGACCTTCGGCTTCGCGCTATAGGGTTGGCCATATGCT	236
E264	GATTAGCTAGTTGGTGGGTAAAGGCTTACCAAGGCGACGATCAGTGGTCTGAGAGGACACACG	306
1026b	GATTAGCTAGTTGGTGGGTAAAGGCTTACCAAGGCGACGATCAGTGGTCTGAGAGGACACACG	306
E264	CACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCGACAGTGGGAAATTTGGACAATGGCGC	376
1026b	CACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCGACAGTGGGAAATTTGGACAATGGCGC	376
E264	AAGCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGCTTCGGGTGTAAAGCACTTTTTCGGGAAAGA	446
1026b	AAGCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGCTTCGGGTGTAAAGCACTTTTTCGGGAAAGA	446
E264	AATCATTCTGGCTAATACCCGAGTGGATGACGGTACCGGAGGAATAAGCAGCGCTAACTAGTGGCAG	516
1026b	AATCATTCTGGCTAATACCCGAGTGGATGACGGTACCGGAGGAATAAGCAGCGCTAACTAGTGGCAG	516
E264	CAGCGCGGTAAATACGTAGGTTGCGAGCGTTAATCGGAATTACTGGCGTAAAGCGTGCAGCGCGGT	586
1026b	CAGCGCGGTAAATACGTAGGTTGCGAGCGTTAATCGGAATTACTGGCGTAAAGCGTGCAGCGCGGT	586
E264	GCTAAGACCGATGTGAATCCCGGGCTCAACCTGGGAAGTGCATTGGTACTGGCAGGCTAGAGTATGG	656
1026b	GCTAAGACCGATGTGAATCCCGGGCTCAACCTGGGAAGTGCATTGGTACTGGCAGGCTAGAGTATGG	656
E264	CAGAGGGGGTAGAATTCACGCTAGCAGTGAATGCGTAGAGATGTGGAGGAATACCGATGGCAGAG	726
1026b	CAGAGGGGGTAGAATTCACGCTAGCAGTGAATGCGTAGAGATGTGGAGGAATACCGATGGCAGAG	726
E264	CAGCCCCCTGGCCAATACGACGCTCATGCACGAAGCGTGGGAGCAACAGGATTAGATACCTGGT	796
1026b	CAGCCCCCTGGCCAATACGACGCTCATGCACGAAGCGTGGGAGCAACAGGATTAGATACCTGGT	796
E264	AGTCCACGCCCTAAACGATGCACTAGTTGTTGGGATTCTTCCTTAGTAACGATGCTAACGCGTGA	866
1026b	AGTCCACGCCCTAAACGATGCACTAGTTGTTGGGATTCTTCCTTAGTAACGATGCTAACGCGTGA	866
E264	AGTTGACCGCTGGGAGTACGTCGCAAGATTAAAGTCAAGGAATTGACGGGACCCGCAACAAGCG	936
1026b	AGTTGACCGCTGGGAGTACGTCGCAAGATTAAAGTCAAGGAATTGACGGGACCCGCAACAAGCG	936
E264	TGGATGATGTGGATTAAATTCGATGCAACGCGAAAAACCTTACCTACCTTGCATGGTGGAGGCGCAT	1006
1026b	TGGATGATGTGGATTAAATTCGATGCAACGCGAAAAACCTTACCTACCTTGCATGGTGGAGGCGCAT	1006
E264	GAGGTTGGGCTGCTCGAAGAGAACCGGCGACAGTCTGCTGATGGCTCTGTCAGCTGCTGCTG	1076
1026b	GAGGTTGGGCTGCTCGAAGAGAACCGGCGACAGTCTGCTGATGGCTCTGTCAGCTGCTGCTG	1076
E264	GATGTGGGTTAAGTCCCGCAACGAGCGACACCTTGTCTTAGTTGCTACGCAAGAGCACTTAAGGAG	1146
1026b	GATGTGGGTTAAGTCCCGCAACGAGCGACACCTTGTCTTAGTTGCTACGCAAGAGCACTTAAGGAG	1146
E264	ACTGCCGCTGACAAACCGGAGGAGGTGGGGATGACGTCAGTCTCATGGCCCTTATGGGTAGGGCTTC	1216
1026b	ACTGCCGCTGACAAACCGGAGGAGGTGGGGATGACGTCAGTCTCATGGCCCTTATGGGTAGGGCTTC	1216
E264	ACACGTCATACATGGTGGCAAGAGGCTGCCAACCCGCGAGGGGGAGCCAAATCCAGAAACCGATC	1286
1026b	ACACGTCATACATGGTGGCAAGAGGCTGCCAACCCGCGAGGGGGAGCCAAATCCAGAAACCGATC	1286
E264	GTAGTCCGATTGCACTCTGCACTCGAGTGTATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGC	1356
1026b	GTAGTCCGATTGCACTCTGCACTCGAGTGTATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGC	1356
E264	CGCGGTGAATACGTTCCCGGCTTGTACACACCGCCGCTACACCATGGGAGTGGGTTTACCAGAGT	1426
1026b	CGCGGTGAATACGTTCCCGGCTTGTACACACCGCCGCTACACCATGGGAGTGGGTTTACCAGAGT	1426
E264	GGCTAGTCTAACCCGAGGAGGAGGCTACACCGGTAGGATTCATGACTGGGTTGAAGTCTGAACAGGT	1496
1026b	GGCTAGTCTAACCCGAGGAGGAGGCTACACCGGTAGGATTCATGACTGGGTTGAAGTCTGAACAGGT	1496
E264	AGCCGTATCGGAAGGTGC	1514
1026b	AGCCGTATCGGAAGGTGC	1514

Fig. 1. Comparison of the 16S rRNA gene sequences from *B. pseudomallei*-like E264 and *B. pseudomallei* 1026b. Bold letters show the positions of the 15 nucleotide dissimilarities between *B. pseudomallei*-like E264 and *B. pseudomallei* 1026b. Triangles indicate nucleotides present in the *B. pseudomallei* 1026b sequence but absent from the *B. pseudomallei* ATCC 23343 sequence (25); filled circles indicate nucleotide differences between these sequences. The underlined sequence (992–1138) marks the position of the previously uncharacterized *B. pseudomallei* ATCC 23343 16S rRNA gene.

In conclusion, although *B. thailandensis* and *B. pseudomallei* strains are structurally and immunologically similar to one another, we believe that the differences demonstrated between the 16S rDNA nucleotide sequences, the biochemical profiles and virulence traits associated with these organisms are sufficient grounds to propose the existence of the newly described species *B. thailandensis*. It is interesting that *B. thailandensis* and *B. pseudomallei* are closely related yet *B. thailandensis* is relatively avirulent (2). Further studies with both *B. thailandensis* and *B. pseudomallei* may aid in the identification of genetic factors responsible for the enhanced virulence of *B. pseudomallei*.

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Fig. 2. Phylogenetic analysis of 16 bacterial species based on 16S rRNA gene sequences. DNA sequences were analysed with GeneJockey version 1.20 software for the Macintosh and the University of Wisconsin Genetics Computer Group package (5). The nucleotide sequences of the 16S rRNA genes (1, 9, 10, 17–20, 23, 24) were aligned with the program PILEUP using the default parameters. The aligned sequences were edited so that there were no gaps and the program DISTANCES was used to calculate a matrix of the pairwise evolutionary distances between the aligned sequences. The Kimura method was used to correct the distances for multiple substitutions at a site. GROWTREE was used to reconstruct a phylogenetic tree from the distance matrix using the neighbour-joining method. The scale bar indicates distance in substitutions per nucleotide.

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