EPIDEMIOLOGY OF ARABINOSE ASSIMILATION IN *BURKHOLDERIA PSEUDOMALLEI* ISOLATED FROM PATIENTS AND SOIL IN THAILAND*

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Abstract. *Burkholderia pseudomallei* is an environmental saprophyte that has been isolated widely from soil in Southeast Asia and the relationship between environmental contamination and clinical melioidosis has been established. It has been shown that the arabinose assimilation property of *B. pseudomallei* is probably one of the determinants indicating virulence of this organism. Therefore, the distribution of arabinose assimilation biotypes of *B. pseudomallei* collected from four geographic regions of Thailand was studied in order to determine an association between arabinose assimilation of *B. pseudomallei* and the uneven distribution of melioidosis found among these four areas. A total of 830 isolates of *B. pseudomallei* (412 patient isolates and 418 soil isolates) collected from the patients and soil in four regions of Thailand in 1997 were tested for an ability to grow on a minimal agar medium supplemented with L-arabinose. All patient isolates except one could not utilise arabinose (Ara^-). For 418 soil isolates, 232 (55.5%) isolates were identified as Ara^- type. They comprised 180 (62.5%), 36 (46.8%), 6 (35.3%) and 10 (27.8%) isolates derived from northeastern, southern, northern and central regions respectively. The ratios of Ara^- to Ara^+ were 1.7, 0.9, 0.5 and 0.4 among isolates collected from northeastern, southern, northern and central regions respectively. The prevalence of Ara^- in soil isolates in northeast is significantly higher than those in other regions. This observation suggests that in addition to the presence of *B. pseudomallei* in soil which is one of the factors contributing to a burden of melioidosis in northeastern Thailand, the distribution of more virulent biotype (Ara^-) soil isolates is a factor contributing to a high prevalence of melioidosis in northeastern Thailand as well.

INTRODUCTION

Burkholderia pseudomallei is the causative bacteria of melioidosis, an infectious disease of human and animals, which is endemic in Southeast Asia, particularly in northeast Thailand and northern Australia (Dance, 1991). *B. pseudomallei* has been known as an environmental saprophyte widely isolated from soil in Southeast Asia. However *B. pseudomallei* isolates recovered from environment had distinct biochemical profiles from clinical isolates. In particular, there was frequent variation in arabinose assimilation among these isolates (Dance *et al*, 1989). Based on the ability to grow on a minimal salt agar supplemented with L-arabinose, *B. pseudomallei* isolates were differentiated into two biotypes, *ie* Ara^- and Ara^+ (those without and with an ability to utilize arabinose as a sole energy source respectively). This characteristics was examined further in animal experiments and at molecular levels. Based on the restriction fragment length polymorphisms of rRNA genes, two groups of ribotypes were found. These two ribotypes also differed in their ability to assimilate arabinose. *B. pseudomallei* strains that utilize arabinose (Ara^+) constitute a population that is genetically distinct from other Ara^- environmental and clinical strains (Trakulsomboon *et al*, 1997). Moreover, Ara^- *B. pseudomallei* has recently been demonstrated to be a virulent biotype (Smith *et al*, 1997; Brett *et al*, 1997). Therefore, these data clearly demonstrate the presence of two phylogenetical different populations among isolates in the species leading to a proposal to name Ara^+ *B. pseudomallei* as a new species (Brett *et al*, 1997).

Melioidosis in Thailand is observed to be prevalent in the northeastern region and this endemicity of the disease is associated with the presence of *B. pseudomallei* in soil (V uddhakul *et al*, 1999). It is un-

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certain whether a high prevalence of the organism in soil is the only important factor contributing to a high prevalence of melioidosis. Since there is a clear evidence that there are two biotypes among this organism which one biotype (Ara^-) is more virulent than the other (Ara^+), this study aims to determine an association between the distribution of the arabinose assimilation biotypes of *B. pseudomallei* and the geographic distribution where the isolates are collected.

MATERIALS AND METHODS

Bacterial isolates

A total of 830 isolates of *B. pseudomallei* collected from patients and soil throughout Thailand was included. The details of collection sites and collection methods are described elsewhere (Vuddhakul *et al*, 1999; Suputtamongkol *et al*, 1999). All isolates were identified as *B. pseudomallei* by conventional biochemical tests. They included 412 clinical isolates and 418 soil isolates. Distribution of the isolates collected from different regions of Thailand is shown in Table 1.

Arabinose assimilattion test

Isolates were grown for 48 hours on nutrient agar and 5 colonies were suspended in sterile distilled water and adjusted the turbidity to 0.5 MacFarland. A ten-fold dilution of this suspension 0.3 µl was applied onto the surface of a minimal agar medium (MAM) (Clowes and Hayes, 1968) supplemented with 0.2% (w/v) L-arabinose (DIFCO, Detroit, USA). The same procedure was performed in MAM supplemented with glucose was carried out as a growth control. Results were recorded as growth after incubation at 37°C for 48 hours. Isolates that grow on both agar media are considered to utilize arabinose (Ara^+) whereas negative isolates (Ara^-) are identified if they grow only on the glucose plate as shown in Fig 1. The arabinose utilization of all isolates were tested twice.

Table 1
Geographical distribution of 830 isolates of *B. pseudomallei.*

Region	Total isolates	Sources of isolation	
		Human	Soil
Northeastern	638	350	288
Southern	88	11	77
Northern	32	15	17
Central	72	36	36
Overall	830	412	418

Data analysis

Data were analyzed by descriptive statistics. Comparing the isolation rates of Ara^- biotype and the ratio of Ara^- to Ara^+ among different collection regions was computed by chi-square statistics. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Human isolates

All 412 clinical isolates except one could not utilize arabinose (Ara^-). The infection caused by this only one Ara^+ isolate has been recently reported (Lertpatanasuwan *et al*, 1999).

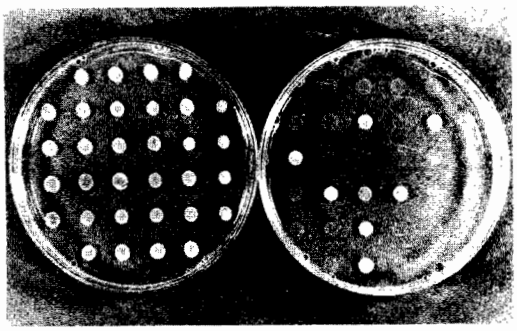
Soil isolates

Out of 418 soil isolates, 232 (55.5%) were identified as Ara^- biotype. The prevalences of Ara^- isolates were unevenly distributed among different regions of Thailand as shown in Table 2. Ara^- isolates were identified in 62.5%, 46.8%, 35.3% and 27.8% of the isolates collected from the northeastern, southern, northern and central regions respectively. Ara^-/Ara^+ ratio of the isolates from the northeastern region was 1.7. In the southern region, the numbers of Ara^- and Ara^+ isolates were very close giving an Ara^- to Ara^+ ratio of 0.9. On the other hand, the ratios of Ara^- to Ara^+ in the

Table 2
Arabinose assimilation of *B. pseudomallei* soil isolates from different regions of Thailand.

Region	Number (%)		Ratio of Ara^- to Ara^+	Total
	Ara^-	Ara^+		
Northeastern	180 (62.5) *	108 (37.5)	1.7 *	288
Southern	36 (46.8)	41 (53.2)	0.9	77
Northern	6 (35.3)	11 (64.7)	0.5	17
Central	10 (27.8)	26 (71.2)	0.4	36
Total	232 (55.5)	186 (44.5)	1.3	418

* $p < 0.0001$



MAM supplemented with glucose MAM supplemented with arabinose

Fig 1–Growth characteristics of *B. pseudomallei* isolates on Minimal Agar Medium (MAM). Isolates that grow on both agar media are considered to utilize arabinose (Ara^+) whereas negative isolates (Ara^-) grow only on the glucose media.

northern and central regions where clinical melioidosis is very uncommon were much less than 1 (Table 2). Ara^- isolates were significantly more common in the soil collected from the northeastern region when compared with the other regions ($p < 0.0001$).

DISCUSSION

Our finding of Ara^- biotype in a large proportion of clinical isolates confirmed the results of a prior study of 2,500 clinical strains which were all Ara^- isolates (Wuthiekanun *et al*, 1996). These consistent observations indicate that Ara^- *B. pseudomallei* is a major virulent biotype. However, all Ara^+ strains are not always non-pathogenic since there was one clinical isolate of Ara^+ biotype in this study. The mode of acquisition of Ara^+ *B. pseudomallei* infection in this previously healthy patient was by infection after having a traumatic wound heavily contaminated with Ara^+ *B. pseudomallei* in the soil (Lertpatanasuwan *et al*, 1999). This first case report demonstrates that Ara^+ *B. pseudomallei* can be pathogenic even in immunocompetent subjects if the inoculum size is heavy. The pathogenicity of this strain needs to be studied in order to understand more regarding the role of Ara^+ *B. pseudomallei*.

In contrast to the results seen in clinical isolates, only 55% of overall soil isolates were Ara^- biotype. When the data on arabinose assimilation of soil isolates were further analysed according to the geographic distribution where the isolates were recovered, we found that the prevalence of Ara^- biotype in soils collected from the northeastern region (62.5%) was significantly more than those collected from the southern (46.8%), northern (35.3%) and central (27.8%) regions of Thailand as shown in Table 2. This observation corresponds to the results of our previous study (Vuddhakul *et al*, 1999) showing that 1) *B. pseudomallei* infection rate per 100,000 in-patients from the northeastern region (137.9) was significantly more prevalent than those from southern (14.4), northern (18) and central (13.4) regions respectively and 2) the isolation rate of *B. pseudomallei* in soil collected from northeastern region (20.4%) was significantly more prevalent than those collected from southern (5.8%), northern (4.4%) and central (6.1%) regions respectively. This finding suggests that in addition to the presence of *B. pseudomallei* in soil which is an important factor contributing to the uneven distribution of melioidosis in different regions of Thailand, the distribution of the more virulent biotype (Ara^-) soil isolates is a factor contributing to a high prevalence of melioidosis in northeast Thailand as well. However these two well defined factors may not be completely sufficient to explain the burden of melioidosis in northeast Thailand since Ara^- *B. pseudomallei* could also be recovered from the soil collected from other regions. Further studies of discrepancies among *B. pseudomallei* isolates collected from different regions of Thailand in addition to arabinose assimilation property are being carried out. The mechanisms or factors contributing to high prevalence of *B. pseudomallei* and high prevalence of Ara^- biotype in the soil collected from northeastern Thailand, and the role of Ara^+/Ara^- biotype interaction or switching should be explored in order to develop environmental control instruments for melioidosis in the future.

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