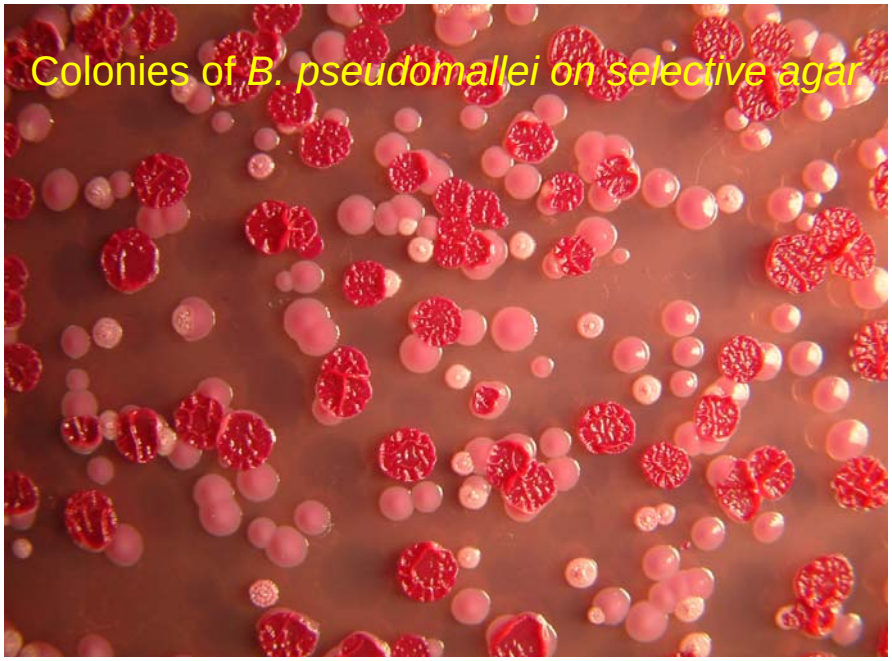
# Genetic diversity of *Burkholderia pseudomallei* isolated from agricultural land in northeast Thailand




***Vanaporn Wuthiekanun, Direk Limmathurotsakul, Narisara Chantratita, Aunchalee Thanwisai, Edward J. Feil, Nicholas P. Day and Sharon J. Peacock***

Mahidol-Oxford Tropical Medicine Research Unit

Faculty of Tropical Medicine

# Introduction

- The soil dwelling Gram-negative bacterium *Burkholderia pseudomallei* is the cause of melioidosis, a serious human infection with a mortality rate of 50% in Thailand and 20% in Australia

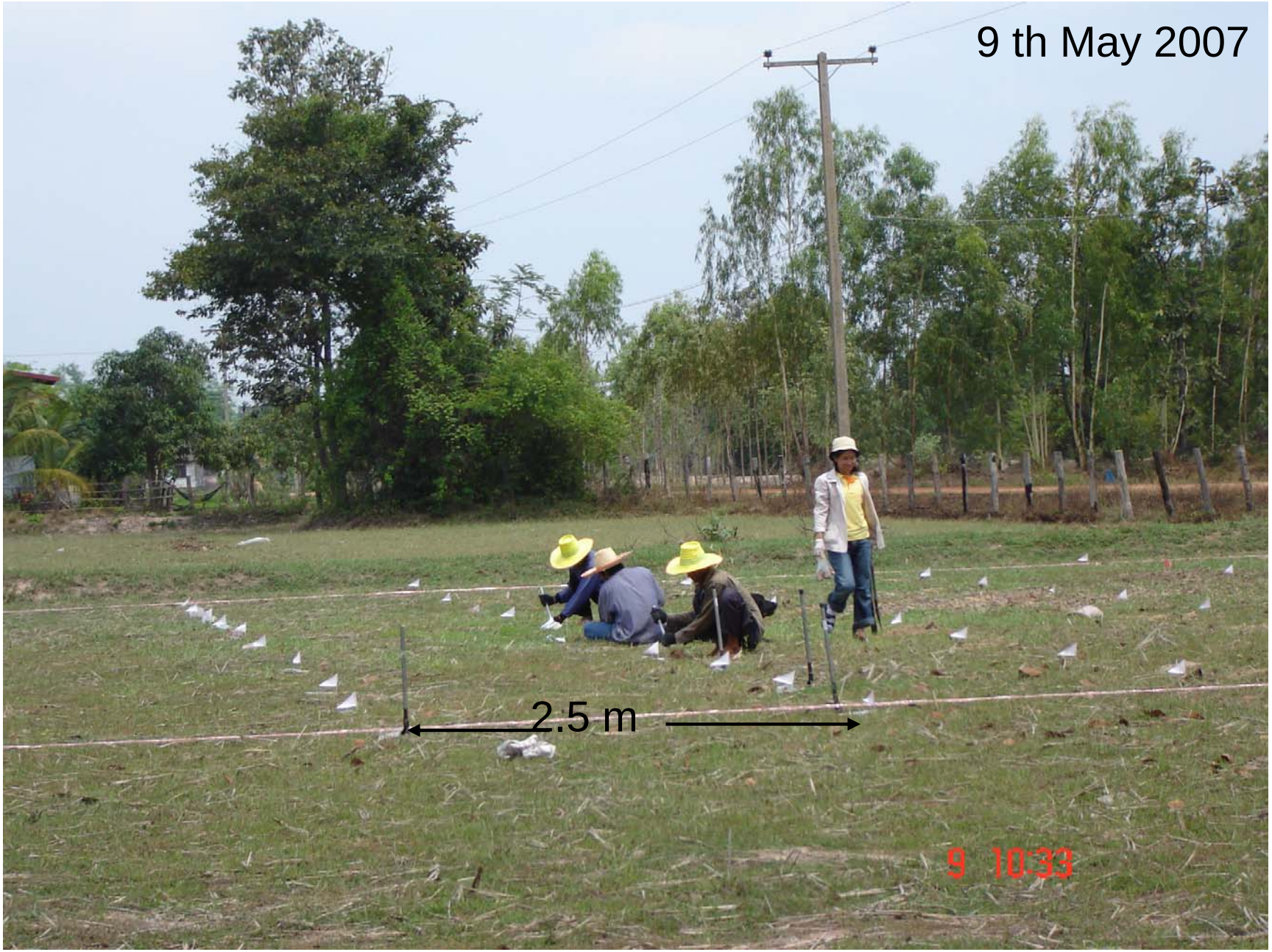
- Infection is acquired following bacterial inoculation and contamination of wounds, or more rarely via inhalation or ingestion

- The genetic diversity of this organism in the environment is poorly defined

# Objectives

1. Undertake soil sampling in a rice field, where melioidosis is usually acquired
2. Determine the proportion of soil samples positive for *B. pseudomallei* (out of 100 samples)
3. Determine the *B. pseudomallei* load (cfu/gm soil) at each positive site
4. Characterise the colonial appearance (type) on Ashdowns agar
5. Undertake genotyping to determine the genetic diversity of *B. pseudomallei* in soil

**Study site:** Amphoe Lao Sua Kok, Ubon Ratchathani, northeast Thailand



# Overview of methodology

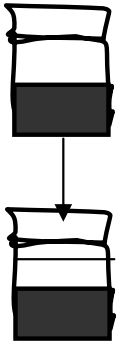
Soil culture for the presence of *B. pseudomallei*

Characterisation of colony morphotype using an existing algorithm

Genotyping using a combination of PFGE and MLST

# Soil culture

100 g of soil mixed with 100 ml sterile water and left overnight at room temperature

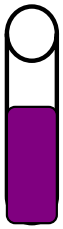


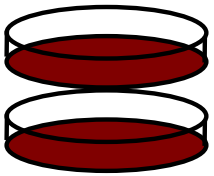
Separate supernatant



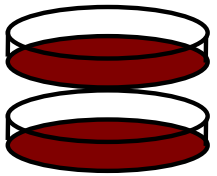
Spread onto 5 plates of Ashdown's selective agar (ASH)

1 ml of supernatant add in to 9 ml of selective enrichment broth

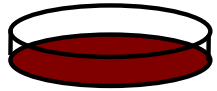




**10 µl**



**100 µl**

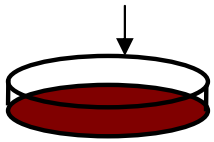


**500 µl**

Incubated at 40 °C, 48 h

Incubated at 40 °C, 4 days

10 ul of surface liquid plated onto ASH

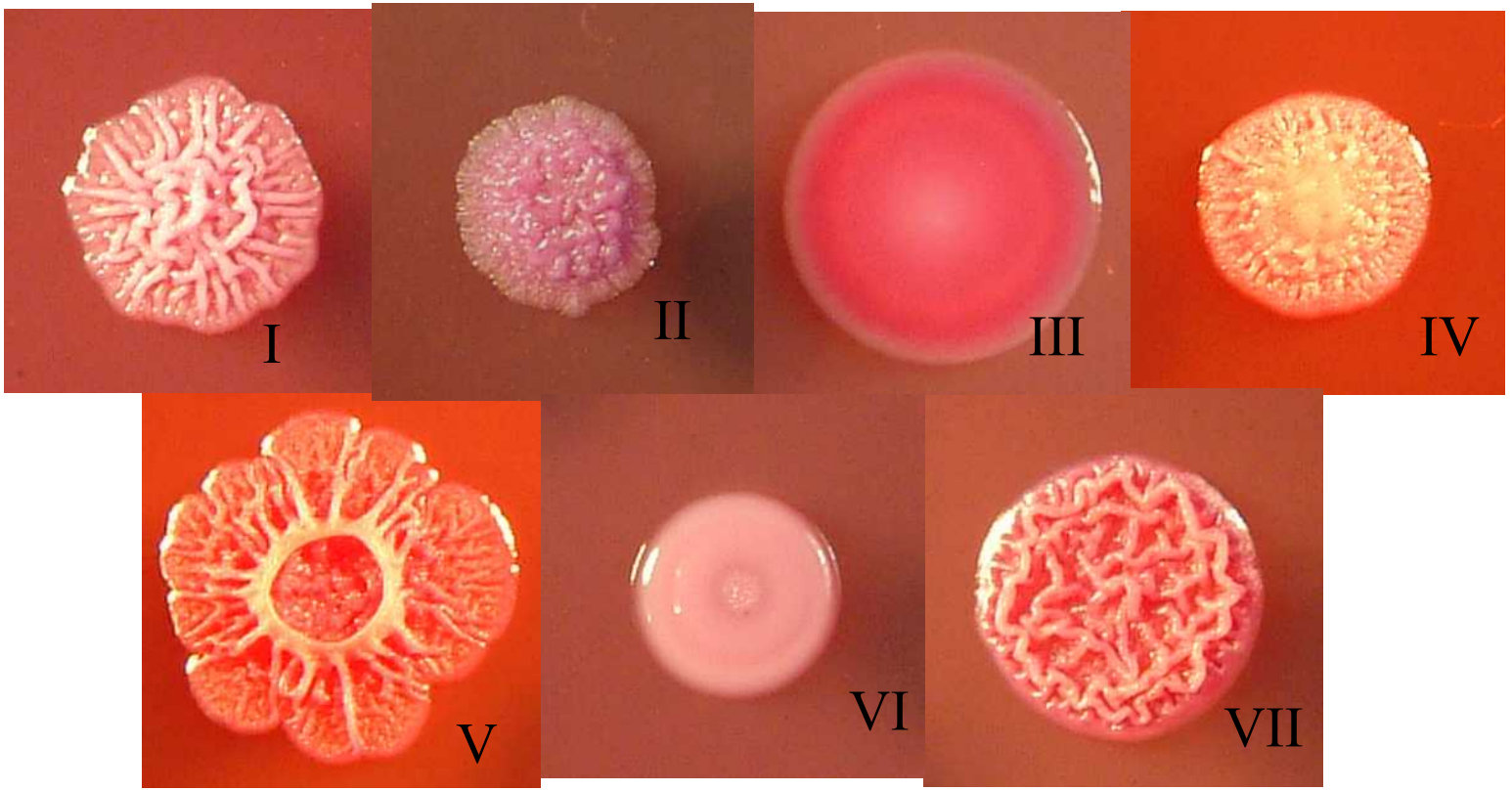


Incubated at 40 °C, 4 days

Identify and characterise colony morphotype

# Characterisation of colony morphotype

Seven morphotypes characterised to date




Chantratita et.al 2007 *J.Bacteriol. 189:807-817*

# Genotyping of *B. pseudomallei*

~600 primary colonies were picked from ASH for genotyping by:

- Pulsed field gel electrophoresis (PFGE)

(Analysed using the BioNumerics software version 2.5 (Applied Maths, Belgium))

- Multi Locus Sequence Typing (MLST)

http://bpseudomallei.mlst.net/

# Pulsed Field Gel Electrophoresis

PFGE is a technique used to separate especially long strands of DNA by length in order to tell differences among samples. It operates by alternating electric fields to run DNA

# Multilocus Sequence Typing (MLST)

- MLST is a nucleotide sequence based approach for the unambiguous characterization of isolates of bacteria and other organisms via the internet.

- Developed by Godoy et al., 2003 for typing of *B. pseudomallei* and closely related species

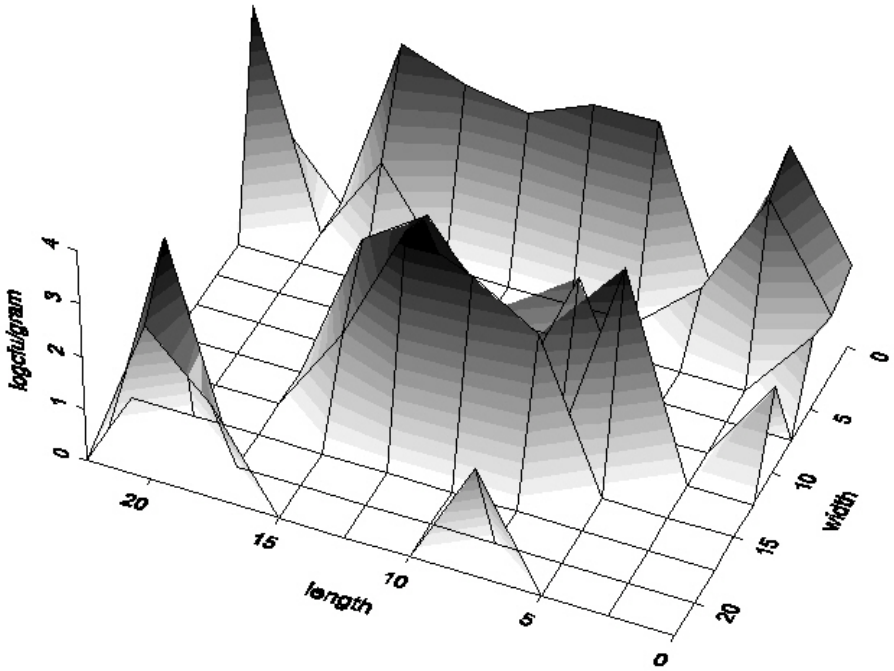
- 7 housekeeping genes (*ace*, *lepA*, *lipA*, *nark*, *ndh*, *gltB* and *gmhD*) were designed base on gene sequence of *B. pseudomallei* K96243

# Results of soil culture for *B. pseudomallei*

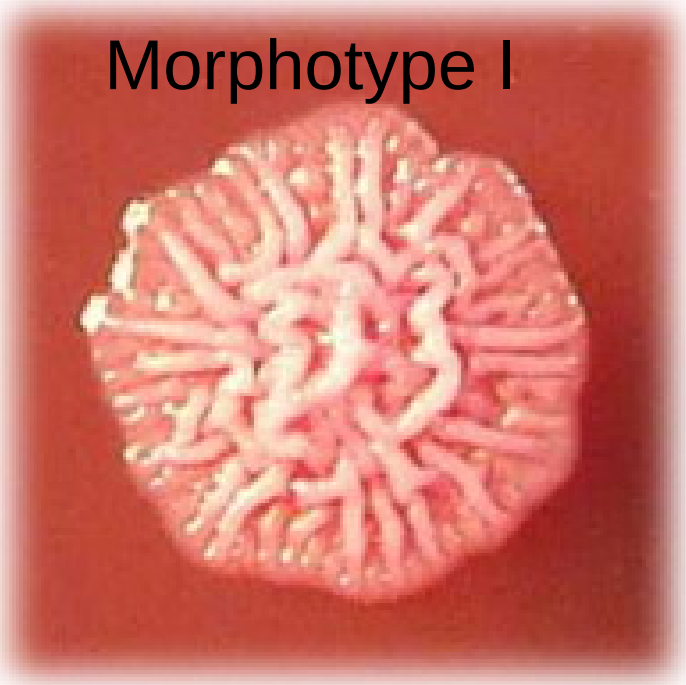
**28/100 soil samples were culture positive**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | A1 | | A3 | A4 | A5 | A6 | A7 | | A9 | A 10 |
| B | B1 | | B3 | | | | | B8 | B9 | B 10 |
| C | C1 | | | | | | | | | |
| D | | | | | | | | | | |
| E | | | | | | | E7 | | | E 10 |
| F | | | | F4 | F5 | F6 | | F8 | | |
| G | | | | G4 | G5 | G6 | G7 | | | |
| H | | | | | | | | | | |
| I | | I2 | | | | | | | | |
| J | | J2 | J3 | | | | J7 | | | |

*Presence & count (log cfu/gm) B. pseudomallei*
*median 700 range 3 to >10,000*
*IQR 50 to 2810*



# Colony morphology

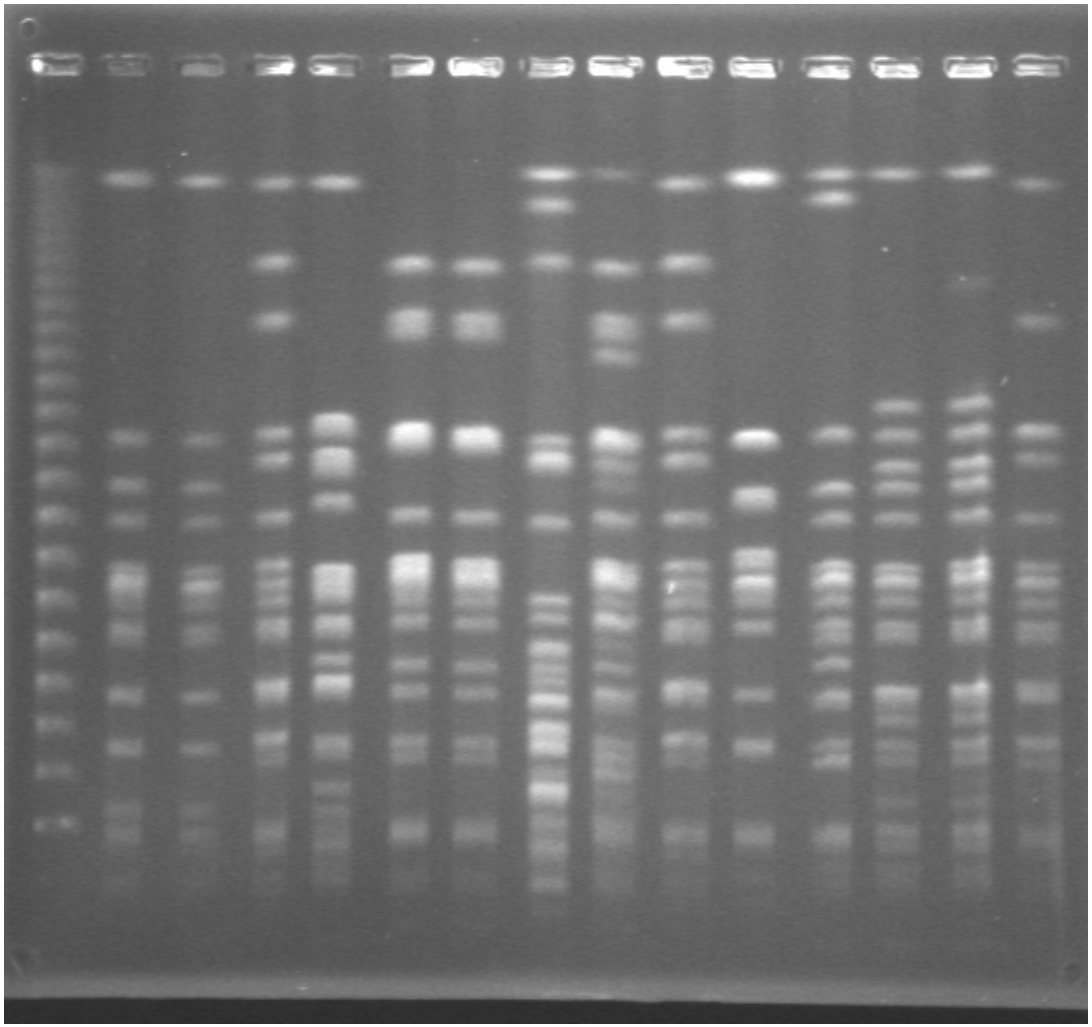



Only type I was identified

It is the preferential morphotype in the environment

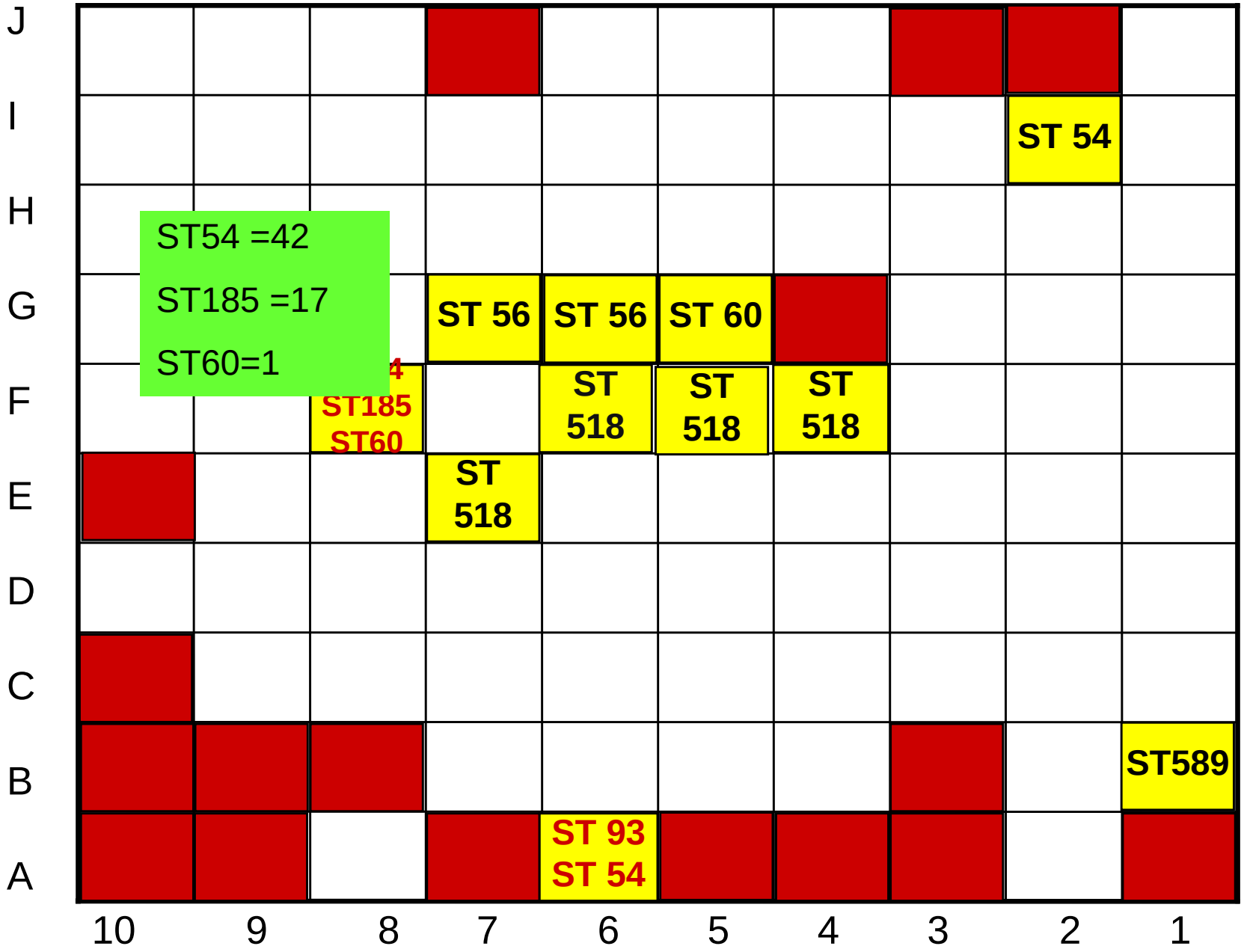
# Genotyping using PFGE and MLST

A total of 630 colonies were typing using PFGE. This gave 11 PFGE banding pattern types. MLST of a representative of each type resolved 7 sequence types



| PFGE Type | ST |
|---|---|
| A | ST 93 |
| B,F,K | ST 54 |
| C | ST 589 |
| D, E | ST 518 |
| G | ST 185 |
| H | ST 60 |
| I, J | ST 56 |

# Measures of genetic diversity in 11 positive sites



# Summary

- *B. pseudomallei* was present in 28 / 100 sampling points
- Only colony morphotype I was identified in this rice field
- Eleven PFGE types and 7 multilocus sequence type were identified from a total of 11 independent sampling points
- Multiple *B. pseudomallei* genotypes were present within a single soil sample
- Different *B. pseudomallei* genotypes were present at independent but nearby sampling points

# Acknowledgements

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- During continuous field electrophoresis, DNA above 30-50 kb migrates with the same mobility regardless of size. This is seen in a gel as a single large diffuse band. If, however, the DNA is forced to change direction during electrophoresis, different sized fragments within this diffuse band begin to separate from each other.
- With each reorientation of the electric field relative to the gel, smaller sized DNA will begin moving in the new direction more quickly than the larger DNA. Thus, the larger DNA lags behind, providing a separation from the smaller DNA.

Table 5. Relationship between 11 PFGE banding pattern and 7 MLST analysis of soil isolates

| **PFGE Type** | **ST** | **MLST profile** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ***ace*** | ***gltB*** | ***gmhD*** | ***lepA*** | ***lipA*** | ***narK*** | ***ndh*** |
| A | ST 93 | 1 | 1 | 2 | 1 | 1 | 4 | 1 |
| B,F,K | ST 54 | 3 | 1 | 3 | 3 | 1 | 2 | 1 |
| C | ST 589 | 3 | 1 | 11 | 1 | 1 | 1 | 1 |
| D, E | ST 518 | 1 | 1 | 13 | 2 | 1 | 1 | 1 |
| G | ST 185 | 1 | 4 | 2 | 2 | 1 | 4 | 1 |
| H | ST 60 | 3 | 1 | 12 | 1 | 1 | 3 | 1 |
| I, J | ST 56 | 3 | 1 | 4 | 1 | 1 | 4 | 1 |

Table 6. Genotyping results for 630 colonies of *B. pseudomallei* from each of independent sampling

| **Sequence type** | **Number of colonies per point** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **A6** | **B1** | **E7** | **F4** | **F5** | **F6** | **F8** | **G5** | **G6** | **G7** | **I2** |
| **ST 93** | 56 | | | | | | | | | | |
| **ST 54** | 1 | | | | | | 42 | | | | 60 |
| **ST 589** | | 60 | | | | | | | | | |
| **ST 518** | | | 45 | 60 | 60 | 45 | | | | | |
| **ST 185** | | | | | | | 17 | | | | |
| **ST 60** | | | | | | | 1 | 60 | | | |
| **ST 56** | | | | | | | | | 60 | 60 | |

# Result

## 1. Soil culture

| | Number of positive culture points | Number of negative culture points | Total |
|---|---|---|---|
| Disused land | 77 | 23 | 100 |
| Rice paddy | 28 | 72 | 100 |

A possible explanation for the lower number of positive samples in the rice paddy is the detrimental

- Effect of chemical pesticides and fertilisers.
- Difference in positivity between different sampling sites represents natural variation within the region.