Genotyping *Bacillus anthracis*:
from global diversity to epidemiological and bioforensic investigations

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Fasanella et al., 2005

www.unsolved.com/0215-Anthrax.html

www.nature.com/news
Key tools and requirements for bioforensics and molecular trace-back

• Genetic signatures/molecular assays
  – subtyping on different scales

• Global strain and genetic fingerprint databases
  – assists epidemiological-bioforensic investigations
  – design and validate additional genetic signatures

• Real world application and validation

• Appreciation basis of diversity (what is probability of match, mutation rates of markers)
Finding genetic signatures in *B. anthracis*

- Few genotypic differences between *B. anthracis* and *B. cereus, B. thuringiensis* and among *B. anthracis* isolates.

- Dormant spore phase contributes to genetic monomorphism, complicating subtyping efforts.

- ‘VNTR’ based genetic typing discriminates among *B. anthracis* isolates (Anderson, 1996; Keim et al., 2000; Lista et al., 2006).

- Following 2001 attacks, funding to sequence *B. anthracis* strains to find rare differences, such as SNPs (Read et al., 2002).
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**SNPs and VNTRs**
Genetic signatures: Variable Number of Tandem Repeats (VNTRs)

- Range in evolutionary stability
  - Mutation rates: $10^{-4}$ to $10^{-8}$ mut/gen
- Can be highly mutable and discriminating
- Multiple-locus VNTR analysis, MLVA:
  - 8-marker VNTR system (Keim et al, 2000)
  - 15 marker (Van Ert, 2005)

Deletion

Insertion
Genetic signatures: Single Nucleotide Polymorphisms (SNPs)

- Sequencing identified SNPs among diverse strains (Read et al., 2002; Ravel et al., unpublished data)

- Pearson et al. 2004, mapped 1,000 SNPs across 26 diverse isolates
  - Evolutionarily stable, low mutation rates ($10^{-9}, 10^{-10}$ mut/gen)

- Multiple SNPs along a given phylogenetic branch

- 14 representative SNPs that define major clonal lineages
  - Reduce redundancy
  - Maximize subtyping power
  - Canonical SNPs or canSNPs

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TCTAGTACTCATGATGCTACTG
```

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TCTAGTACTCAOGATGCTACTG
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\[
\text{TCTAGTACTCA\textcolor{red}{T}GATGCTACTG} \\
\text{TCTAGTACTCA\textcolor{red}{C}GATGCTACTG}
\]
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Genetic Signatures: Subtyping using both canSNPs and VNTRs

- SNPs - defines major genetic groups
- VNTRs - provides resolution within the genetic groups
- Both: diversity on several evolutionary-spatial scales
  - global > regional > local
- 14 SNPs, 15 VNTRs across over 1,000 isolates from 42 countries

Keim et al., 2004
Global diversity and genetic database of *B. anthracis*: SNPs and VNTRs

- SNPs identify 12 major genetic groups, 3 major lineages
- With VNTRs, a total of 231 genotypes
- Geographic regions have distinctive genotype composition
- “A” geographically widespread, “B” geographically confined
- Establishes geographic-genetic reference database

Van Ert et al., in review
Application of molecular assays and global genetic databases for epidemiological and forensic investigations

Fingerprint unknown B. anthracis isolate

SNPs

A

G

T

C

G

T

C

A

VNTRs

Query global fingerprint database

Where is it found?

What does it match?

G

T

A

G

T

C

C

G

G

T

A

G

C

C

C

G
Global Diversity of *B. anthracis*: investigation of fatal anthrax case in Hong Kong
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Use of global genetic databases to design, validate molecular assays for epidemiological and forensic investigations
Design of strain-specific SNPs: distinguish particular strains from all others!

CanSNPs can be strain specific

Allows rapid, specific inclusion/exclusion of samples from forensic, epidemiological investigations
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- CanSNPs can be strain specific
- Allows rapid, specific inclusion/exclusion of samples from forensic, epidemiological investigations
Strain-specific SNPs coupled with real-time PCR: rapid, sensitive inclusion/exclusion of samples from an investigation

- High-throughput: SNP type hundreds of samples a day
- Sensitive: trace level analysis
- Technically simple

Van Ert et al, 2006
Validating *B. anthracis* species-specific SNPs

- *plcR* nonsense mutation suggested as definitive trait of *B. anthracis*
- tested across diverse *B. anthracis* strains
- not present in genetic near-neighbors, *B. cereus*, *B. thuringiensis*¹,²
- Rapidly allows powders, cultures, environmental samples to be screened as *B. anthracis*³

¹Slamti et al., 2004; ²Van Ert and Easterday, 2005a, ³Van Ert and Easterday, 2005b
Conclusions and what’s needed

- SNPs and VNTRs represent a powerful combination of markers for genotyping *B. anthracis*

- Understanding the global population genetic structure of *B. anthracis* is critical for epidemiological and bioforensic investigations

- Large strain/genetic databases are important for the design and validation of genetic signatures
  - Strain specific SNPs: with real-time PCR, results in sensitive, rapid assays
  - Species specific SNPs

- What’s needed
  - Expanded strain collections, databases
  - In vitro and in vivo mutation rates for modeling and inclusion/exclusion statistics/probabilities
Acknowledgements

- Midwest Research Institute
  - Ted Hadfield

- Northern Arizona University
  - PAUL KEIM
  - Lynn Huynh
  - Ryan Easterday
  - Shaylan Zanecki,
  - Tatum Simonson
  - SNP team: Tal Pearson,
    Jana U’reen et al.,
  - YP Team: Dave Wagner, Amy Vogler

- Collaborators:
  - Martin Hugh-Jones, LSU
  - Paul Jackson, Karen Hill, Rich Okinaka, LANL
  - Tim Read, Jacques Ravel, TIGR
  - Antonio Fasanella ARL Italy
  - Vincent Perreten, Univ. Bern
  - Alex Hoffmaster, CDC
  - A. Maho, LRVZ, Chad
  - Alim Aikembayev, KSCQZD, Kazakhsan
Different platforms-approaches to SNP typing in *B. anthracis*:

Electrospray Ionization Time of Flight Mass Spectrometry for Multiplex SNP Analysis

*Van Ert et al, 2004; IBIS and NAU unpublished*
NASBA Isothermal Amplification & Dipstick Microarray Detection

- R. Cary, Los Alamos National Laboratory, Patent Pending
- Isothermal amplification
- Hybridization sandwich assay: lateral flow and detection
- Based on plcR TaqMAMA primers (Van Ert and Easterday et al, 2005)
Evidence of Human impacts on the global transmission, diversity of *B. anthracis*

- ‘A radiation’ coincides with animal domesticate population expansions (mid-holocene (3,064-6,127 ybp)

- Colonial-era importation of *B. anthracis* from the Old World into the New World
  - New world sublineages exhibit low genetic diversity
  - Rarely observed outside of regions

- Trade/commerce: the repeated importation of diverse genotypes in developed countries
Introduction of anthrax into Australia

- Geering, W. A. (1997) hypothesized that anthrax was initially introduced into Australia in 1847, via contaminated bone-meal fertilizer from India
Fig. 01: Basic phylogeny of B. anthracis using 992 SNPs across 26 B. anthracis isolates and 3 genetic near-neighbors (adapted from Pearson et al, 2004). The position of the B. anthracis isolates are indicated in circles on the dendrogram and a description of the isolates is found in Table 1. The position of the canonical SNPs used in this study are indicated.