Quarterly Progress Report for the Period 4/1/00 to 6/30/00

Project Title: Multiple-Locus VNTR Analysis (MLVA) for Bacterial Strain Identification

DOE Project Number: DE-FG03-00NN20102   Northern Arizona University

B&R CODE: **Date:** November 7, 2000

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Progress During the Quarter:

Section 3.6.7 Year 1 Goals and Deliverables

Goal 1. A 25 Marker MLVA System for *Y. pestis*

The genomic sequence has been analyzed for repeat structure towards finding VNTR loci.

Goal 2. MLVA genotype data for 40 *Y. pestis* samples from USAMRIID

Analysis of 24 core samples from USAMRIID has been completed with the full 42 VNTR markers.

Goal 3. MLVA analysis of California, New Mexico and Arizona *Y. pestis* collections.

NM samples have been transferred from NM State Health to BYU for DNA extraction.

Goal 4. A 25 marker MLVA system for *B. anthracis*

Primers for 28 potential VNTR loci have been developed.
These are in addition to the original 8 VNTR loci.

Goal 5. MLVA genotypic data for ~97 unique *B. anthracis* strains

DISCLAIMER

We have identified a set of 89 unique B. anthracis strains by analysis with the 8 marker MLVA system. These are paritially analyzed using new primers from goal 4.

Goal 6. Identification of 25 potential VNTR regions for *F. tularensis*

We are screening ~ 20 additional repeated regions for VNTR potential.

Comments

Section 3.6.7 Year 1 Goals and Deliverables

Goal 1. A 25 Marker MLVA System for *Y. pestis*

We will be developing primers, optimizing reactions and testing for primer compatibility in multiplex reactions in the next few months. A 25 marker MLVA system will be developed by the end of the contract period.

Goal 2. MLVA genotype data for 40 *Y. pestis* samples from USAMRIID

The core set of *Y. pestis* samples from USAMRIID represent a very diverse collection and its analysis is intended as a broad assay of diversity. A full analysis of 40 samples may not be necessary in order to accomplish this goal and we have selected a representative core set of 12, instead. These will be used in combination with additional samples as they are obtained

Goal 3. MLVA analysis of California, New Mexico and Arizona *Y. pestis* collections

MLVA analysis of the NM samples will begin as soon as the DNAs are prepared by BYU. They preparing the samples for analysis at both LANL and NAU. This will slow our analysis due to the greater requirements of the LANL analysis. We expect receive these in the next quarter.

Goal 4. A 25 marker MLVA system for *B. anthracis*

The additional potential VNTRs are being screened for variation in our 89 strain collection.

Goal 5. MLVA genotypic data for ~97 unique *B. anthracis* strains

See above.

Goal 6. Identification of 25 potential VNTR regions for *F. tularensis*

The sequence data from Franscella tularensis is very limited and of poor quality. We are continuing to screen potential regions but our failure rate is rather high due to poor quality of data which results in primers sets that don't work or repeated sequences that are artifacts.

Funding Status	Operations	Capital
Uncosted from previous FY.	0	0
Current FY	$ 270,000	$ 30,000
Total Funding Available	$ 270,000	$ 30,000
$ Spent this quarter (est.):	$ -	$ -
$ Spent year-to-date (est.):	$ 100,000	$ 0
$ Remaining for this FY	$ 170,000	$ 30,000
Anticipated uncosted current FY funds	0	0

Technical Reports/Presentation

Talk: June 30, Ba MLVA Epi, Alan Bosomworth, Nevada Department of Agriculture, Reno, Nevada.

Journal Article: Keim, P., L.B. Price, A.M. Klevytska, K.L. Smith, J.M. Schupp, R. Okinaka, P. Jackson, and M.E. Hugh-Jones. 2000. Multiple-Locus VNTR analysis (MLVA) reveals genetic relationships within *Bacillus anthracis*. *J. Bacteriology*. 182:2928-2936.

Journal Article: Turnbull, P., P. Jackson, K. Hill, A-B, Koltso, Keim, P. 2000. Systematic Relationships within *Bacillus anthracis*. "In: Applications and Systematics for Bacillus and Relatives. 2001. (Berkeley,RCW, Heyndrickx M, Logan NA, de Vos P, eds). Blackwell Science, Oxford" In press.

Journal Article: Keim, P, and KL Smith. 2000. Genome analysis for strain discrimination. In: "Anthrax Toxins." ed. T. Koehler. Current Topics in Microbiology and Immunology. Springer Verlag. In review.