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Molecular Typing of *Bacillus anthracis* using Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA-8)

C.W. Stratilo and D.E. Bader

Defence R&D Canada

Technical Memorandum

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Abstract

Bacillus anthracis, the etiological agent of anthrax, lacks character homoplasy and is genetically very monomorphic. Variable number tandem repeats (VNTR) are rapidly evolving stretches of DNA that can serve as markers for strain differentiation (molecular typing). A collection of *B. anthracis* strains were characterized using multiple-locus VNTR analysis (MLVA). MLVA allowed these strains to be placed within a phylogenetic framework. Several strains were discriminated from each other; however, some isolates were indistinguishable including a group of nine Canadian isolates. This work allowed verification of strain designation within the DRDC Suffield collection and allowed for the development of a high throughput genetic typing capability at DRDC Suffield.

Résumé

Bacillus anthracis, l'agent étiologique du charbon bactéridien ne possède pas le caractère de l'homoplasmie et est génétiquement très monomorphique. Un nombre variable de répétitions en tandem (VNTR) évoluent rapidement en étendues d'ADN qui peuvent servir de marqueurs pour la différentiation des souches (typage moléculaire). Une collection de souches de *B. anthracis* a été caractérisée en utilisant des analyses VNTR de locus multiples (AVVM). Les AVVM ont permis à ces souches d'être placées dans un cadre phylogénique. Plusieurs souches ont été discriminées les unes des autres ; quelques isolats sont cependant demeurés non distinguables, y compris le groupe des neuf isolats canadiens. Ces travaux ont permis la vérification de la désignation des souches parmi la collection de RDDC Suffield et ont permis le développement d'une capacité de typage génétique de haut débit.

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Executive summary

Molecular Typing of *Bacillus anthracis* using Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA-8)

C.W. Stratilo; D.E. Bader; DRDC Suffield TM 2007-282; Defence R&D Canada Suffield; December 2007.

Introduction: Infectious biological agents comprise a potential threat to human life and health whether as a consequence of deliberate use (biowarfare or bioterrorism), accidental release, or natural outbreaks. Initial mitigation steps required to minimize the impact of an attack or outbreak involve identifying the agent, treating affected individuals, and containing the outbreak. After the anthrax letter attacks in 2001, molecular typing methods were employed in the United States to characterize the strain used in the attack. DRDC Suffield, in partnership with the Public Health Agency of Canada National Microbiology Laboratory, recognized the need to establish a Canadian capability to characterize potential biothreat agents at the genetic level using molecular typing methods. A joint CRTI project proposal to establish and apply molecular typing methods for the characterization of three biothreat agents was approved 22 April 2003 and progressed through to 31 Mar 2007 (CRTI 02-0069RD). This report describes the molecular typing method, MLVA-8, used for the genetic characterization of *B. anthracis* and the MLVA-8 pattern obtained for 29 strains of *B. anthracis*.

Results: MLVA-8 analysis of DNA from 29 *B. anthracis* strains revealed genetic relationships between strains in which many strains were distinguished from each other, but nine Canadian *B. anthracis* isolates were not distinguished from each other.

Significance: Knowledge and characterization of potential infectious agents, which the Canadian Forces as well as Canadian civilians might face in the event of a bioattack, bio-release, or outbreak is fundamental to Canadian biodefence and biosecurity. DRDC Suffield has established molecular strain typing capability for *B. anthracis*, characterized *B. anthracis* strains in the DRDC Suffield collection using this method, and established a phylogenetic framework that could be used to type and characterize genetic relationships of additional *B. anthracis* strains in the future. While MLVA subtyping could be used for forensic investigations where intentional use is suspected, a greater understanding of the genetic diversity of *B. anthracis* in natural populations within Canada and worldwide may be required to definitively distinguish a natural outbreak from an intentional use situation. This would require a large scale typing effort comprising a larger number of medical and environmental *B. anthracis* isolates than is currently present in the DRDC Suffield collection.

Future plans: MLVA databases could be expanded as additional *B. anthracis* strains are acquired in order to increase the resolving power of this technique.

Sommaire

Molecular Typing of *Bacillus anthracis* using Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA-8)

C.W. Stratilo; D.E. Bader; DRDC Suffield TM 2007-282; R & D pour la défense Canada – Suffield; Décembre 2007.

Introduction: Certains agents biologiques infectieux représentent une menace potentielle contre la vie et la santé en cas d'usage délibéré (guerre biologique ou bioterrorisme), d'émission accidentelle ou encore d'épidémie naturelle. Les étapes d'atténuation initiales requises pour minimiser l'impact de l'attaque ou de l'épidémie comprennent l'identification de l'agent, le traitement des individus affectés et le besoin de contenir l'épidémie. Après les attaques de lettres contenant du charbon bactéridien, en 2001, les méthodes de typage moléculaire ont été employées aux États-Unis pour caractériser la souche utilisée durant l'attaque. DRDC Suffield, en partenariat avec le service de la santé publique du laboratoire microbiologique national du Canada, a reconnu le besoin d'établir une capacité canadienne visant à caractériser les agents potentiels de menace biologique au niveau génétique au moyen des méthodes de typage moléculaire. Une proposition de projet conjoint de l'IRTC visant à établir et appliquer les méthodes de typage moléculaire pour la caractérisation de trois agents représentant une menace biologique a été approuvée le 22 avril 2003 et a progressé jusqu'au 31 mars 2007 (IRTC 02-0069RD). Ce rapport décrit la méthode de typage moléculaire, AVVM-8, utilisée pour la caractérisation génétique du *B. anthracis* ainsi que le modèle AVVM-8, obtenu pour 29 souches de *B. anthracis.*

Résultats : Les analyses AVVM-8 d'ADN provenant de souches de *B. anthracis* ont révélé des relations génétiques entre les souches parmi lesquelles beaucoup de souches ont été distinguées les unes des autres mais neuf isolats *B. anthracis* canadiens n'ont pu être distingués les uns des autres.

Portée des résultats: La connaissance et la caractérisation d'agents infectieux potentiels, auxquels les Forces canadiennes et les civils canadiens risquent de faire face en cas d'attaque biologique, d'émissions biologiques ou d'épidémie, est fondamentale à la biodéfense et biosécurité canadienne. RDDC Suffield a établi une capacité de typage moléculaire de souches pour *B. anthracis*, a caractérisé les souches de *B. anthracis* dans la collection de RDDC Suffield en utilisant cette méthode et a établi un cadre phylogénétique qui pourrait être utilisé, à l'avenir, pour typer et caractériser les relations génétiques de souches *B. anthracis* supplémentaires. Les analyses AVVM de sous typage pourraient être utilisées durant les investigations légistes, quand on suspecte une utilisation intentionnelle; cependant, une meilleure compréhension de la diversité génétique du *B. anthracis* chez les populations naturelles au Canada et dans le monde entier pourrait exiger un effort de typage à grande échelle, comprenant un plus grand nombre d'isolats de *B. anthracis* médicaux et environnementaux que ceux qui sont actuellement présents dans la collection de RDDC Suffield.

Perspectives d'avenir : On pourrait étendre les bases de données AVVM au fur et à mesure qu'on acquiert des souches de *B. anthracis* additionnelles pour être en mesure d'augmenter le pouvoir discriminateur de cette technique.

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Introduction

The causative agent of anthrax**,** *Bacillus anthracis,* is a spore forming bacterium endemic in soils throughout much of the world. It is a zoonotic disease affecting both animals and humans. Virulent strains of *B. anthracis* contain two virulence plasmids, pXO1 and pXO2. These plasmids contain genes that confer toxin producing ability and capsule synthesis activity, respectively, although there are chromosomally encoded factors that are crucial for the full virulence of *B. anthracis* [1]. Due to recent bioterrorism events involving anthrax, there has been an increase in interest in *B. anthracis*, especially with respect to identification, detection, and molecular subtyping of this pathogen.

B. anthracis belongs to the *B. cereus* group, whose members include *B. cereus* and *B. thuringiensis*. Amplified fragment length polymorphism (AFLP) analysis of the *B. cereus* group has revealed that *B. cereus* and *B. thuringiensis* are broadly interspersed across all branches of the AFLP phylogenetic tree, while *B. anthracis* shows very low genetic diversity and clusters to a sub-branch of the phylogenetic tree which is distinct from other members of the *B. cereus* group [2].

B. anthracis is considered one of the most genetically monomorphic pathogenic bacteria described, lacking character homoplasy and containing few single nucleotide polymorphisms (SNPs), and is therefore considered to be evolutionarily □young□[3]. This lack of homoplasy may be due to its life history, which includes spending long periods of time as dormant endospores. Most molecular techniques used to differentiate between strains in other species, such as AFLP [2, 4], multiple-locus sequence typing (MLST) [5], and pulsed field gel electrophoresis (PFGE) [6], fail to discriminate *B. anthracis* strains.

Several molecular typing methods, including SNP analysis and multiple□locus variable number tandem repeat analysis (MLVA), have been more successful in discriminating *B. anthracis* strains and have allowed the exploration of its phylogenetics [7].

A MLVA method exploiting the copy number differences of nucleotide repeat sequences at 6 chromosomal loci and one locus for each of the two plasmids has been developed [8]. MLVA analysis is useful since there is an increase in mutation rates compared to SNPs, as well as a greatly increased number of allelic states compared to biallelic SNPS [7]. The MLVA technique was used to analyze DNA from *B. anthracis* strains in the DRDC Suffield strain collection as well as DNA from *B. anthracis* isolates in the Publice Health Agency of Canada National Microbiology Laboratory (PHAC-NML) collection. This report describes the results obtained from this analysis.

Materials and Methods

Bacterial Strains and DNA isolation

Bacterial strains used as a source of DNA for this study comprised those in the DRDC Suffield and PHAC-NML collections (Table 1).

Table 1. B. anthracis strains used

DRDC Suffield Strains	PHAC-NML Strains
Vollum	9807
Vollum 1B	9937
SK61	NML 03-0139
ATCC-4229	NML 03-0191
ACB	9609
Ames	9911
NH	Sterne Colorado
VH	ATCC-6602
9604	2000-77
9610	9946
9614	
94188C	
93212C	
9619	
93-189C	
Buffalo	
SK31	
SK162	
17T5	

DNA from DRDC Suffield *B. anthracis* strains was prepared in the DRDC Suffield BSL3 bacterial biocontainment suite as follows. *B. anthracis* strains were grown on sheep blood agar Petri dishes at 37 ˚C overnight. Strain DNA was isolated using MasterPure DNA & RNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA), Phase lock (Eppendorf, Westbury, NY, USA) or GNOME DNA Isolation Kit (Q.BIOgene, Irvine, CA, USA). DNA preparations were checked for sterility by broth and plate culture prior to removal for MLVA analysis. MLVA-8 analysis was performed outside BSL3 biocontainment. DNA from strains in the PHAC-NML collection was provided by PHAC-NML.

MLVA

MLVA of the *B. anthracis* strain DNA was performed for loci as described by Keim *et al*. [8]. PCR reactions contained 1□AmpliTaq gold PCR buffer and 0.5 U of AmpliTaq gold DNA polymerase (Applied Biosystems Inc., Foster City, CA, USA); 2mM $MgCl_2$ (for amplification of *vrrA*, *vrrB*$_1$, or *HCvrrC,1 HCvrrC2* loci) or 4mM $MgCl_2$ (for amplification of *vrrB*$_2$, *CG*$_3$, *pXO1-att*, *pXO2-at* loci); dNTPs (0.2 mM each); and 0.2 µM of forward and reverse primers (Table 2).

Table 2. MLVA primers

Marker	Primers	Primer Sequences (5' to 3')	Allele Size Range
vrrA	vrrA-F1-HEX	CAC AAC TAC CAC CGA TGG CAC A	289-314
	vrrA-R1	GCG CGT TTC GTT TGA TTC ATA C	
vrrB1	vrrB1-F1-HEX	ATA GGT GGT TTT CCG CAA GTT ATT C	184-256
	vrrB1-R1	GAT GAG TTT GAT AAA GAA TAG CCT GTG	
vrrB2	vrrB2-F1-FAM	CAC AGG CTA TTC TTT ATC AAA CTC ATC	153-171
	vrrB2-R1	CCC AAG GTG AAG ATT GTT GTT GA	
HCvrrC1	HCvrrC1F-FAM	CAG AAG AAT CAG TGA TTG TC	405-522
	HCvrrC1R	CAC CAT TTC TTG TTG CAT TTC CTC	
HCvrrC2	HCvrrC2F-HEX	TGG CTG ATG AAC AAA CGA A	444-516
	HCvrrC2R	GCA TGT CTT TCC ATT AAT CGC GCT	
CG3	CG3-F1-NED	TGT CGT TTT ACT TCT CTC TCC AAT AC	153-158
	GC3-R1	AGT CAT TGT TCT GTA TAA AGG GCA T	
pXO1-aat	pXO1-AAT-F3-NED	CAA TTT ATT AAC GAT CAG ATT AAG TTC A	117-141
	pXO1-AAT-R3	TCT AGA ATT AGT TGC TTC ATA ATG GC	
pXO2-at	pXO2-AT-F1-HEX	TCA TCC TCT TTT AAG TCT TGG GT	135-155
	pXO2-AT-R1	GTG TGA TGA ACT CCG ACG ACA	
vrrC1	vrrC1-F	GAAGCAAGAAAGTGATGTAGTGGAC	362-690
	vrrC1-R	CATTTCCTCAAGTGCTACAGGTTC	
vrrC2	vrrC2-F	CCAGAAGAAGTGGAACCTGTAGCAC	528-603
	vrrC2-R	GTCTTTCCATTAATCGCGCTCTATC	

Approximately 2–20 ng of template DNA was used per 50 µL reaction. Phosphoramidite fluorescent dye (Fam, Hex, Tamra) covalently linked to the forward primer was used to allow direct analysis of the amplicons. Amplicon sequencing required the use of unlabeled forward and reverse primers. Thermocycling conditions were 95 °C for 5 min and 35 cycles of 94 °C for 30 sec, 60 °C for 30 sec, 65 °C for 30 sec and 65 °C for 7 min. HiDi formamide (8 µL) and 1 µL of the diluted PCR products were combined with 1 µL of size standard Rhodamine-X Mapmaker 70 to 400 bp and CST ROX 420-800 (BioVentures Inc., Murfreesboro, TN, USA). These products were analyzed on an ABI 3100 genetic analyzer and sized using GeneMapper (Applied Biosystems).

Some of the MLVA amplicons were sequenced to establish the size of the amplicon and the VNTR. These MLVA PCR products were purified using Montage PCR_{96} plates (Millipore, Nepean, ON, CA) and sequenced using the MLVA primers as sequencing primers. All sequencing reactions were carried out in 20 µL reactions with Big Dye 3.1 Terminator chemistry (Applied Biosystems) and analyzed on an ABI 3100 automated sequencer (Applied Biosystems). Contig assembly of the *B. anthracis* MLVA loci sequences was performed with Sequencher (Gene Codes Corp., AnnArbor, MI, USA). Clustering analysis of the MLVA was done using a Categorical coefficient and UPGMA options using Bionumerics software (Applied Maths, Inc., Austin, TX, USA).

Results

The nature and amount of known variability of the various VNTR loci are described in Table 3.

Table 3. Characterization of B. anthracis VNTR used for MLVA

Marker Locus	Consensus Repeat Motif	Repeat Size	Array Size
vrrA1	CAATATCAACAA	12	2-6
vrrB1	CA(T/C)CA(T/C/A)(G/C)(G/A)(C/A/T/G)	9	11-23
vrrB2	CATCAAGGT **or** CATCACCAC	9	2-4
vrrC1	AA(C/T)TGC(A/G)GAA(A/G)CAGAAGAGT(T/C) AGAAGAAGT(G/A)GAAGT	36	4-12
vrrC2	G(C/T)AGA(A/G)GAA(G/C)(A/C)A(C/T)C(G/A)(G /A)(T/C)(T/C)	18	16-19
CG3	ATATT	5	1-2
pXO1-aat	TAA	3	4-13
pXO2-at	AT	2	6-15

Direct sequencing of the eight marker loci allowed for exact sizing of the alleles present at those loci. Allele names were based on sizing data established by direct sequencing (Table 4). The size of the amplicons obtained during fragment analysis are also listed in Table 4 (location plus or minus the offset).

Table 4. Allele calls for B. anthracis MLVA analysis

Marker Name	Bin Parameters	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7
vrrA1	Sequence (bp)	290	302	314	326			
	Location	285.8	297.4	309.7	321.8			
	Offset(left/right)	0.54/0.5	0.95/0.5	0.94/0.5	0.61/0.5			
vrrB1	Sequence (bp)	229	256					
	Location	225.3	252.44					
	Offset(left/right)	0.5/0.5	0.73/0.5					
vrrB2	Sequence (bp)	135	153	162	171			
	Location	128.7	147.15	156.38	165.38			
	Offset(left/right)	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5			
HCvrrC1	Sequence (bp)	405	450	486				
	Location	398.2	442.46	480.5				
	Offset(left/right)	0.5/0.5	0.5/0.7	0.5/0.5				
HCvrrC2	Sequence (bp)	444	516					
	Location	436.82	510.1					
	Offset(left/right)	0.5/0.5	0.5/0.5					
CG3	Sequence (bp)	153	158					
	Location	149.12	154.23					
	Offset(left/right)	0.5/0.5	0.5/0.5					
pX01-AAT	Sequence (bp)	123	126	129	132	135	138	144
	Location	117.96	121.0	124.1	127.0	130.0	133.0	139.0
	Offset(left/right)	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5
pX02-AT	Sequence (bp)	135	137	139	141	143	145	
	Location	131.5	133.56	135.4	137.5	139.71	141.4	
	Offset(left/right)	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5	0.5/0.5	

The difference between amplicon length established by fluorescent primer fragment length analysis, and direct sequencing, can be ascertained by comparison of the allele name established by direct sequencing and the location of the allele established by fragment length analysis (Table 4). This difference was found to be between +/- 0 to 8 bp. This discrepancy may be attributed to the type of phosphoramidite fluorescent dye used in 5' labelling reactions for fragment analysis. The designated size of the allele for a given locus is based on the size of the VNTR amplicon determined by sequence analysis as opposed to fragment size analysis. The results of the MLVA analysis are listed in Table 5. The genetic relationship of the strains analyzed by MLVA are shown in Figure 1.

Table 5. Amplicon size (bp) for MLVA analysis of B. anthracis

Strain	vrrA	vrrB1	vrrB2	HCvrrC1	HCvrrC2	CG3	pXO1-aat	pXO2-at
ATTC-4229	314	229	153	486	516	153	NEG	137
ATTC-6602	326	229	135	486	516	158	NEG	NEG
9604	314	229	162	486	516	153	129	137
9807	314	229	162	486	516	153	129	137
9911	314	229	162	486	516	153	129	137
NML 03-0139	314	229	162	486	516	153	129	137
NML 03-0191	314	229	162	486	516	153	129	137
17T5	302	256	171	450	444	158	123	143
2000-77	314	229	162	486	516	153	129	137
93-189C	314	229	162	486	516	153	129	135
93212C	314	229	162	486	516	158	135	135
94188C	314	229	162	486	516	153	129	137
9614	314	229	162	486	516	153	129	135
9619	314	229	162	486	516	153	126	135
9609	314	229	162	486	516	153	129	135
9610	314	229	162	450	444	158	129	141
9937	314	229	162	486	516	153	129	137
9946	314	229	162	486	516	153	129	137
ACB	302	229	153	486	444	158	129	141
Ames	314	229	153	450	444	158	126	141
Buffalo	314	229	162	486	516	153	129	139
NH	314	229	162	405	516	158	138	139
SK162	314	229	162	486	516	153	129	139
SK31	302	256	171	450	444	158	126	143
SK61	314	229	162	486	444	158	135	145
Sterne	314	229	162	450	444	158	132	NEG
VH	326	229	162	405	516	158	144	139
Vollum	290	229	153	405	516	158	135	139
Vollum 1B	290	229	153	405	516	158	135	139

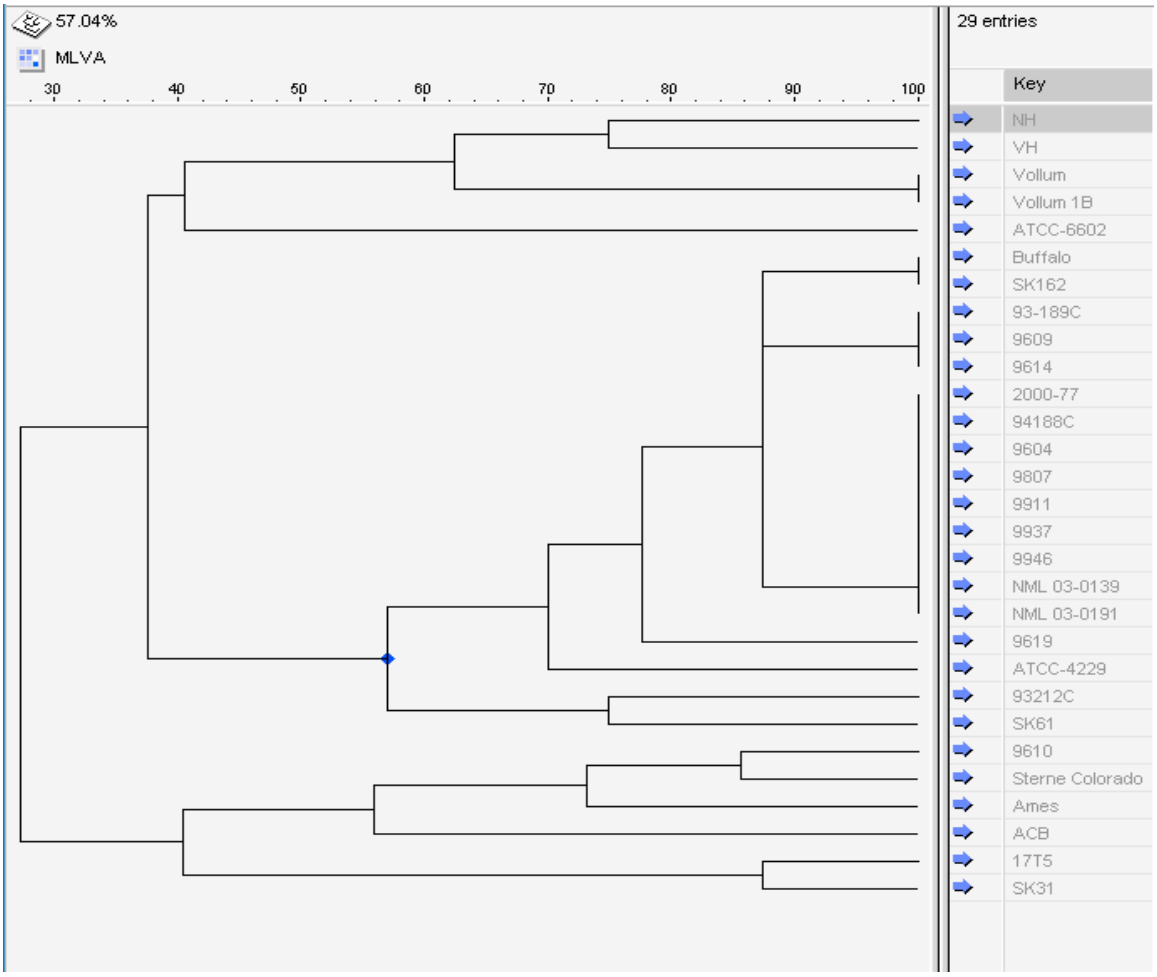

Figure 1. MLVA based dendogram of B. anthracis isolates. The eight MLVA marker loci were used to calculate clustering analysis using a Categorical coefficient and UPGMA options. The genetic distance is presented as percent similarity.

The vaccine strain (Sterne) is missing locus pXO2 since it lacks the plasmid. Strain 4229 lacks the pXO1 plasmid hence it lacks an allele for the pXO1 locus. Strain 6602 lacks both plasmids and does not have alleles for the pXO1 or pXO2 loci.

The primers for vrrC1 and vrrC2 were redesigned in this study (HCvrrC1 and HCvrrC2) to produce smaller amplicons than those used by others [8]. Amplicons that are smaller than 600 bp in length allowed for a standard sizing algorithm to be used for analysis by the ABI Genemapper software. HCvrrC1 and HCvrrC2 were used for fragment analysis, but the traditional primers for those loci were used for sequencing.

While relationships between classical *B. anthracis* strains using cluster analysis were found to be as expected, based on previous reports [8], nine Canadian *B. anthracis* isolates displayed highly conserved MLVA types and were not resolved using this technique.

Discussion

The MLVA typing method presented in this report is a robust and well accepted approach to characterizing *B. anthracis* isolates. The use of fragment analysis allows for quick and reproducible results. Analysis of strains between laboratories worldwide is convenient and easily converted into simple numerical data, unlike AFLP or PFGE. VNTR analysis is required for *B. anthracis* typing due to its extremely low mutation rates, likely due to its life history. Other researchers have performed passage experiments for more than a 100,000 generations and observed only a single mutation at the vrrA locus [8]. This indicates that the eight loci used in MLVA (i.e. MLVA-8) are stable enough to be confidently used for typing this species.

The use of MLVA at eight loci did not allow most of the Canadian isolates to be distinguished from each other, as they appear to be quite monomorphic. It is possible that increasing the number of MLVA loci might be an option to increase the resolving power of this assay.

Discrepancies between the allele calls used in this paper and those found in other publications [8] are due to designations adopted based on fragment sizing analysis as opposed to sequence data. Our methods are based on direct sequencing of new alleles to determine the actual size of the amplicon.

B. anthracis has been used as a biological threat agent and could be used against the Canadian Forces by hostile forces or as bioterrorist threat against civilians. This project allowed DRDC Suffield to establish the capability to perform high resolution subtyping of *B. anthracis*. The methods and cluster database that was developed in this project can be used to type and characterize genetic relationships of additional *B. anthracis* strains in the future. The molecular typing data obtained for the *B. anthracis* strains in our collection could prove valuable should we encounter contamination of our reference stocks and need to trace the source of the contamination in order to maintain the integrity of our reference stocks for the R&D program. High resolution subtyping could be used for forensic investigations where intentional use is suspected; however understanding the genetic diversity of *B. anthracis* in natural populations within Canada and worldwide would help to distinguish a natural outbreak from an intentional use situation. This would require a large scale typing effort comprising a larger number of medical and environmental *B. anthracis* isolates than is currently present in the DRDC Suffield collection.

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List of symbols/abbreviations/acronyms/initialisms

AFLP	Amplified Fragment Length Polymorphism
bp	Base Pair
CRTI	Chemical, Biological, Radiological, Nuclear and Explosives Researc
DND	Department of National Defence
DRDC	Defence Research & Development Canada
DRDKIM	Director Research and Development Knowledge and Information Management
MLVA	Multi-Locus Variable-Number Tandem Repeat Analysis
MLST	Multi-Locus Sequence Typing
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PHAC-NML	Public Health Agency of Canada - National Microbiology Laboratory
R&D	Research & Development
SNP(s)	Single Nucleotide Polymorphism(S)
VNTR	Variable Number Tandem Repeats

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Bacillus anthracis, the etiological agent of anthrax, lacks character homoplasy and is genetically very monomorphic. Variable number tandem repeats (VNTR) are rapidly evolving stretches of DNA that can serve as markers for strain differentiation (molecular typing). A collection of *B. anthracis* strains were characterized using multiple-locus VNTR analysis (MLVA). MLVA allowed these strains to be placed within a phylogenetic framework. Several strains were discriminated from each other; however, some isolates were indistinguishable including a group of nine Canadian isolates. This work allowed verification of strain designation within the DRDC Suffield collection and allowed for the development of a high throughput genetic typing capability at DRDC Suffield.

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Bacillus anthracis; molecular typing; MLVA; VNTR

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