



Molecular Characterization and Proposed Taxonomic Placement of the Biosimulant 'BG'

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Report Documentation Page

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INTRODUCTION

- A brief history of the biosimulant “BG”
 - Migula describes *Bacillus globigii*, 1900
 - Smith reclassifies, *B. subtilis* var. *niger*, 1952
 - Gordon eliminates variation designation, 1973
 - Nakamura proposes *B. atrophaeus* sp. nov., 1989
 - Fritze and Pukall reclassify ATCC 9372, 2001



INTRODUCTION

- ATCC 9372 used as a “surrogate” of *B. anthracis* by the Dugway Proving Ground
- Ambiguous status of other pigmented *Bacillus* strains



INTRODUCTION

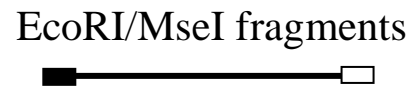
- Amplified Fragment Length Polymorphism (AFLP)
 - high resolution genotyping technique
 - use in bacterial taxonomic studies



INTRODUCTION



Restriction digest, EcoRI, MseI
Adaptor ligation



Selective amplification, EcoRI-primer*,
MseI-primer



Capillary Electrophoresis





METHODS

- 16S rRNA gene sequencing
 - MicroSeq® 500 (Applied Biosystems)
- AFLP
 - 16 selective primer sets
 - Internal size standard
 - Data analysis w/ GelCompar II (Applied Maths)

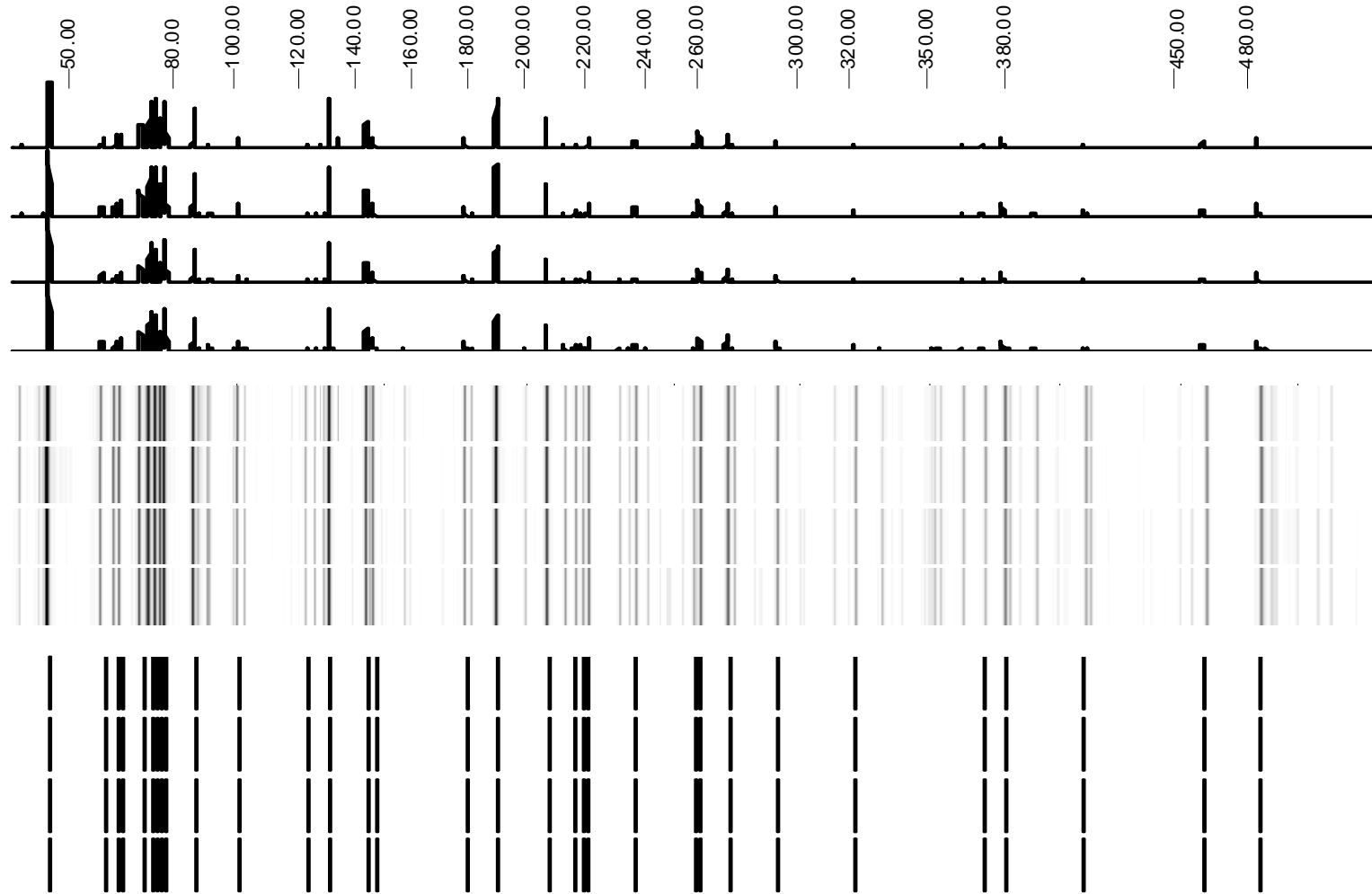


METHODS

- AFLP
 - EcoRI +C/MseI +CC, EcoRI +C/MseI +CA
 - Reproducibility and baseline variability
 - Removal of ambiguous band classes
 - Creation of a composite data set of Dice coefficients
 - UPGMA dendrogram constructed
 - Bootstrap analysis



METHODS





RESULTS

- 16S rRNA gene sequencing - ATCC 49337 vs 9372

	1	11	21	31	41	51	61	71
Bacillus 49337	TGGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCCTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTG							
Bacillus 9372	TGGAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCCTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTG							
Consensus	tggagagtttgatcctggctcaggacgaacgctggcggcctgcctaatacatgcaagtcgagcggacagatgggagcttg							
	81	91	101	111	121	131	141	151
Bacillus 49337	CTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGG							
Bacillus 9372	CTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGG							
Consensus	ctccctgatgtttagcggcggacgggtgagtaaacacgtgggtaacctgcctgtaagactgggataactccgggaaaccggg							
	161	171	181	191	201	211	221	231
Bacillus 49337	GCTAATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCG							
Bacillus 9372	GCTAATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCG							
Consensus	gctaataccggatgcttgtttgaaccgc atggttcaaacataaaaaggtggcttcggctaccacttacagatggaccgcg							
	241	251	261	271	281	291	301	311
Bacillus 49337	GCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTG							
Bacillus 9372	GCGCATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTG							
Consensus	gcgcattagctagttggtgaggtaa ggctcaccaaggcaacgatgcgtagccgacctgagagggatcgggccacactg							



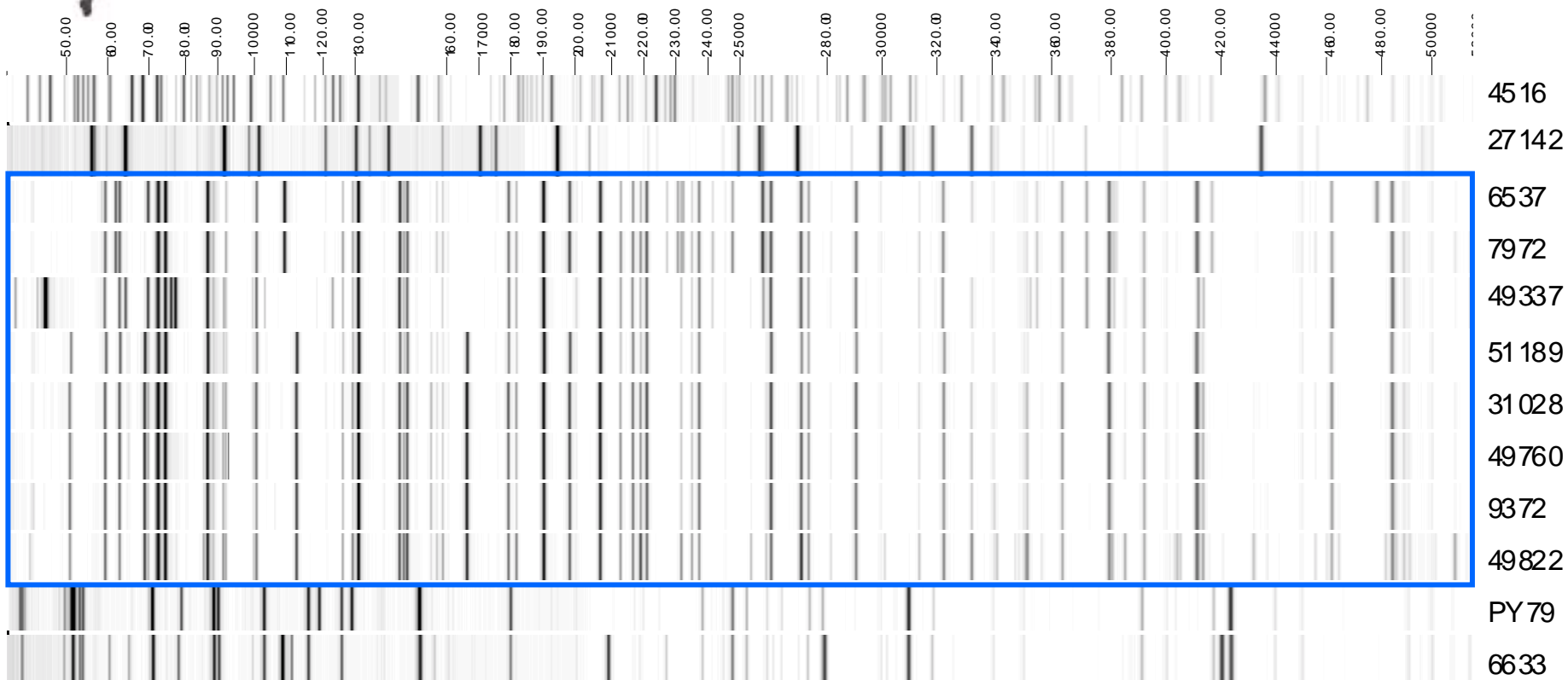


RESULTS

- 16S rRNA gene sequencing
 - *B. atrophaeus* vs *B. subtilis*
 - 99.3% identity (10 base substitutions in ~1500 bases)



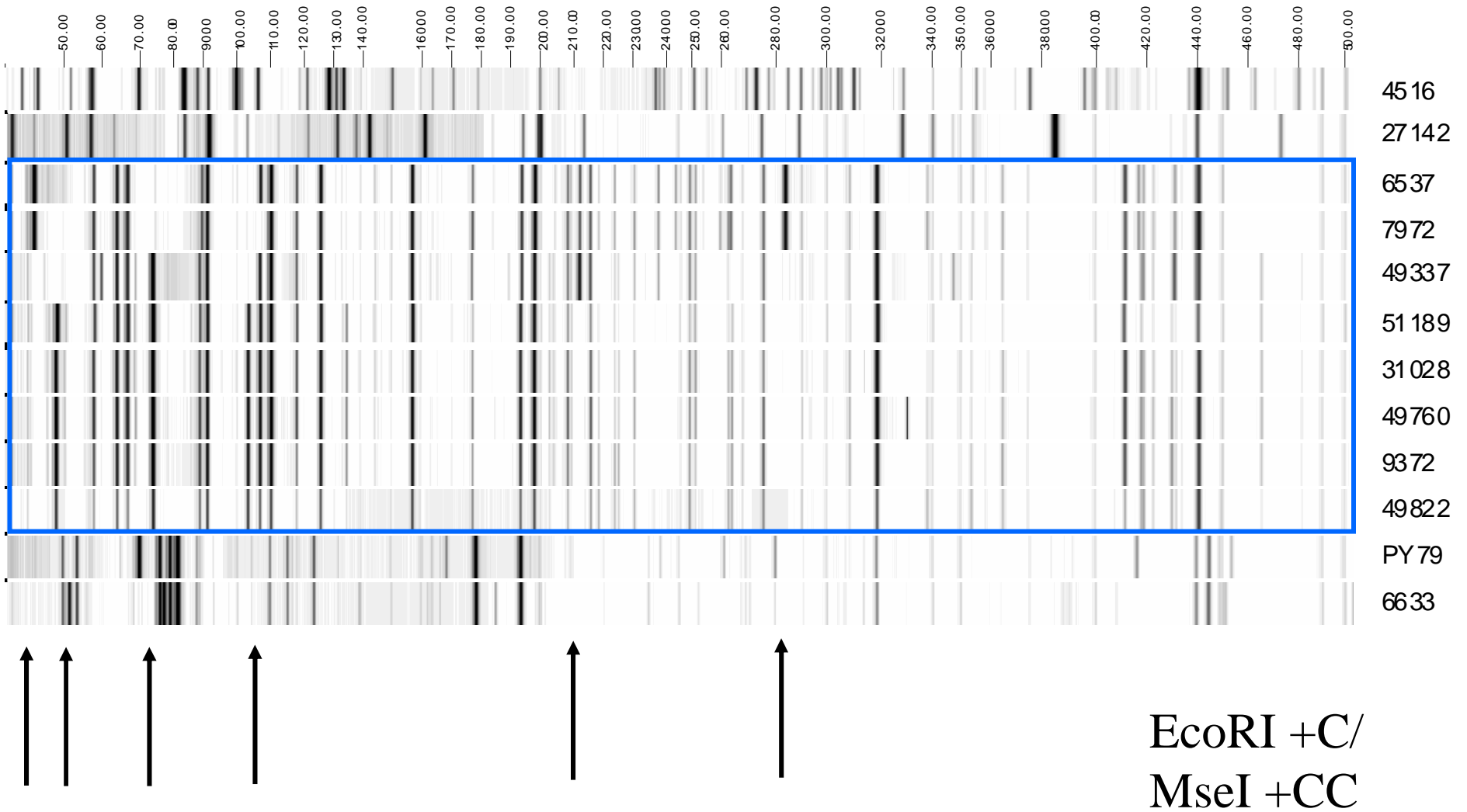
RESULTS



EcoRI +C/
MseI + CA



RESULTS



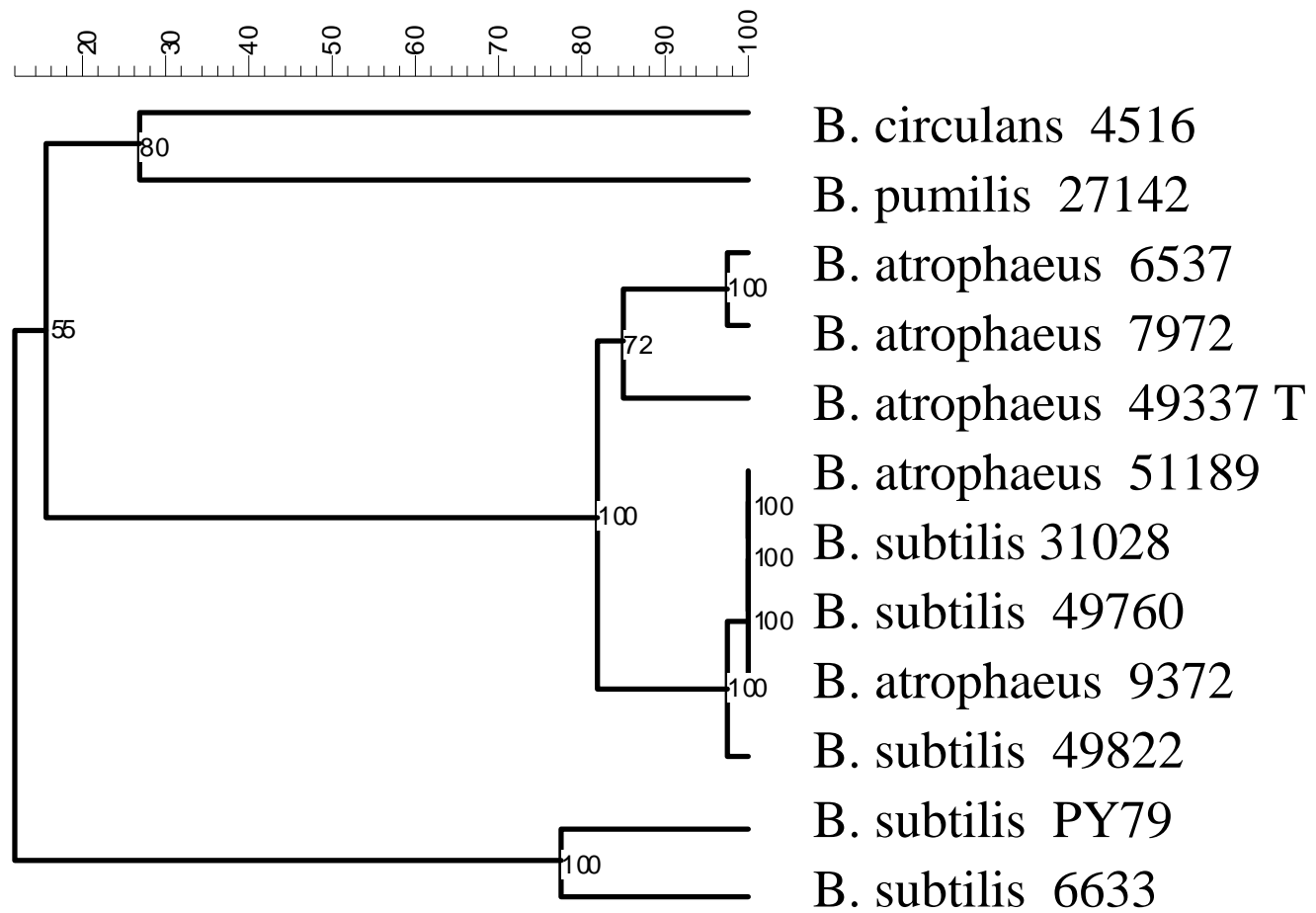


RESULTS

- AFLP
 - total number of fragments = 164
 - total among pigmented Bacilli = 72
 - 41 common fragments
 - 31 polymorphisms



RESULTS





SUMMARY

- AFLP data were useful in discriminating between closely related taxa of *Bacillus*
- Species level variation was easily detected with AFLP
- A distinct cluster of *B. atrophaeus* isolates was revealed
- AFLP data indicate a clear difference between *B. atrophaeus* type strain and Dugway “BG”
- A subspecies of *B. atrophaeus* is proposed; *B. atrophaeus* subsp. *globigii*
- Dugway “BG” is *B. atrophaeus* subsp. *globigii*