Identification of *Bacillus anthracis* by a specific chromosomal marker

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INTRODUCTION

Bacillus (B.) anthracis, the causative agent of anthrax, is an aerobe gram-positive ubiquitous soil bacterium.
This bacterium may infect livestock and humans by gastrointestinal, cutaneous, or respiratory route.

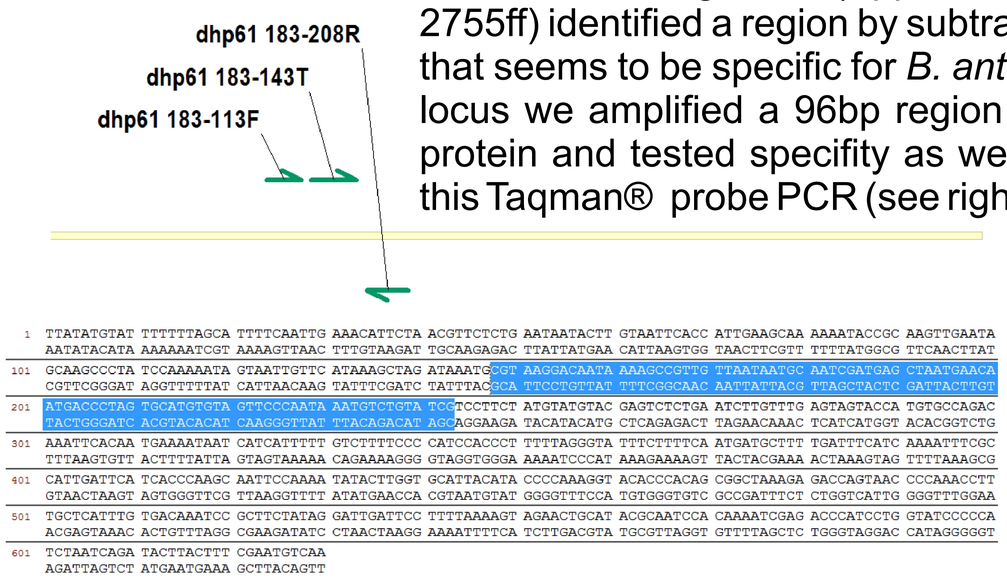
Spores of this bacterium can persist heat, UV- or γ-radiation or chemical desinfectants and maybe infectious for decades. Due to this properties and its high pathogenicity *B. anthracis* is considered as a potential biological weapon. To broad public awareness this pathogen was brought in 2001, when a set of anthrax-contaminated letters infected 22 persons and 5 of them died.

Correct identification of *B. anthracis* and closely related *B. cereus* and *B. thuringiensis* is still a big challenge in diagnostics. Since the appearance of more borderline organisms, classical microbiological methods do not play an important role in species identification anymore.

The genetically close relationship of these organisms hinders a correct identification. Commercially available assays detect virulence genes of “anthrax-specific” plasmids. Plasmidless strains or non-*B. anthracis* species harbouring anthrax-like plasmids will be misidentified. Only the use of a reliable chromosomal marker for a specific identification of *B. anthracis* and differentation from *B. cereus* and *B. thuringiensis* can solve the problem of a taxonomically correct species identification.

THE STUDY

In 2003 Radnedge *et al* (Appl Environ Microbiol, 69, 2755ff) identified a region by subtractive hybidization that seems to be specific for *B. anthracis*. Within this locus we amplified a 96bp region of a hypothetical protein and tested specifity as well as sensitivity of this Taqman® probe PCR (see right).



dhp61_183-113F	CGTAAGGACAATAAAAGCCGTTGT
dhp61_183-143T	TGCAATCGATGAGCTAATGAACAATGACCCT-FAM
dhp61_183-208R	CGATACAGACATTTATTGGGAACTACAC

Specificity

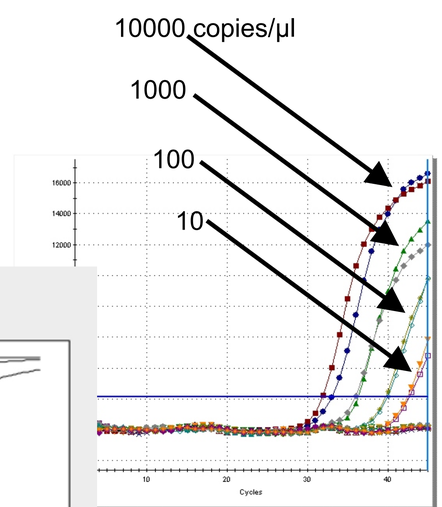
A total of 328 *Bacillus* strains belonging to 20 different *Bacillus* species were investigated.

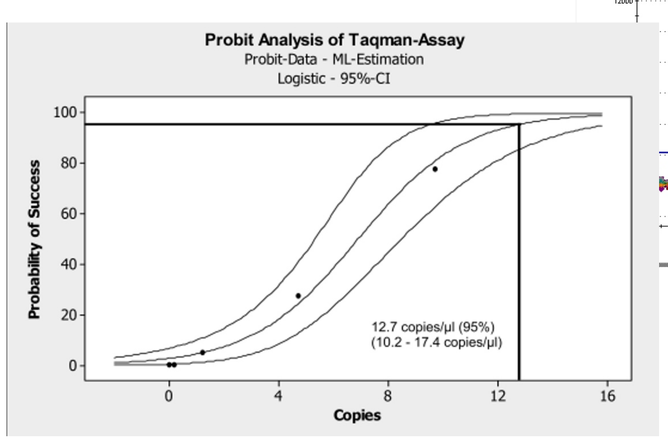
Bacillus species	Number of strains	PCR positive
Bacillus anthracis	**92**	**92**
Bacillus alvei	3	0
Bacillus brevis	4	0
Bacillus cereus	53	0
Bacillus circulans	13	0
Bacillus coagulans	6	0
Bacillus firmus	5	0
Bacillus laterosporum	3	0
Bacillus lentus	2	0
Bacillus licheniformis	11	0
Bacillus megaterium	7	0
Bacillus mycoides	20	0
Bacillus polymyxa	10	0
Bacillus pseudomycoides	2	0
Bacillus pumilus	9	0
Bacillus sphaericus	11	0
Bacillus stearothermophilus	1	0
Bacillus subtilis	14	0
Bacillus thuringiensis	33	0
Bacillus weihenstephanensis	29	0
total	**328**	**92**

All *B. anthracis* isolates (n=92) were specifically detected whereas 236 strains belonging to 19 *Bacillus* species other than *B. anthracis* showed negative results.

Sensitivity

The detection limit was calculated using Probit-analysis to be 12.7 copies per µl (95% confidence interval, 10.2 17.5 copies).





Several authors tried to developed an assay for a specific identification of *B. anthracis* based on a chromosomal marker. However, there are several draw-backs obtained with some *B. cereus* (see table) strains. In addition the primer and probe sequences targeting the *saspB* gene have not been stated in the publications.
The results of this study suggest the marker BA5345 is indeed *B. anthracis*-specific. These findings may be useful for future development of efficient diagnostic tools for the rapid identification of *B. anthracis* and differentiation from other members of the *B. cereus* group.

Marker	Number of strains testes versus amplified		Notes	Reference
	B. anthracis	B. cereus		
Ba831	47/47	4/60		Ramisse et al 1996, JAM, 87, 224ff
rpoB	14/14	5/36		Ellerbrock et al FEMS Microb Lett. 2002, 214, 51ff
BA5510	4/4	2/289		Olsen et al JMM 2007, 71, 265ff
gyrB	1/1	2/23		La Duc et al JMM 2004, 56, 383ff.
saspB	392/392	0/56	Primer not published	Hoffmaster et al EID 2002, 8, 1178ff
plcR	89/89	0/29	Probe based single Nucleotide differentiation needed	Easterday et al JCM 2005, 43, 1995ff
gyrA	43/43	0/49	Probe based single Nucleotide differentiation needed	Hurtle JCM 2004, 42, 179ff
BA5345	**142/142**	**0/236**		**Antwerpen et al, MCP 2008, 22, 313ff**

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