International Journal of Food Microbiology 145 (2011) S129–S136

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Evaluation of *Bacillus* strains as model systems for the work on *Bacillus anthracis* spores

Martina Fricker [a,1], Joakim Ågren [b,1], Bo Segerman [b], Rickard Knutsson [b], Monika Ehling-Schulz [a,*]

[a] Food Microbiology Unit, Clinic for Ruminants, Dept. for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria
[b] National Veterinary Institute (SVA), Department of Bacteriology, SE-751 89 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 26 March 2010
Received in revised form 30 July 2010
Accepted 31 July 2010

Keywords:
Bacillus anthracis
Surrogate
SpoIIIAB typing
Heat inactivation
Germination
SEM

ABSTRACT

Available strain collections of *Bacillus anthracis* and *Bacillus cereus* were screened for *B. cereus* strains sharing major genotypic characteristics with *B. anthracis*. Based on the comparison of partial spoIIIAB sequences, whole genome sequences and MLST, a strain set representing different lineages including candidate model strains for *B. anthracis* was compiled. Spores from the selected strain set and two *B. anthracis* strains were prepared according to a newly optimized protocol transferable to biosafety level-3 (BSL3) conditions and phenotypic characteristics including scanning electron microscopy (SEM), heat inactivation, and germination were evaluated. Two *B. cereus* isolates were identified that were genetically related to *B. anthracis* and showed high similarity to *B. anthracis* spores in their heat inactivation profile and their response to the germinants L-alanine and inosine. In addition, these isolates were also mimicking *B. anthracis* on modified PLET, a selective plating medium for *B. anthracis*, and shared various other biochemical characteristics with *B. anthracis*. Therefore these two strains are not only appropriate models for *B. anthracis* in experiments based on spore characteristics but also in trials working with plating media. These two strains are now used within the BIOTRACER consortium as validated models for *B. anthracis* and will facilitate the development and optimization of tracing and detection systems for *B. anthracis* in the food and feed chain.
© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Deliberate contamination of food and feed is of increasing concern, not only in the USA but also in the EU, especially as the two recent melamine-scandals highlighted the deficits in public and industrial preparedness for intentional contaminations of food and feed. One of the research areas of the European project BIOTRACER (www.biotracer.org) addresses intentional and accidental contamination scenarios with potential bioterror (BT-) agents in feed and food chains. The model scenarios studied in frame of Biotracer are deliberate contamination by spore-forming bacteria, such as *Bacillus anthracis* and *Clostridium botulinum*.

Since the well known anthrax letters from 2001 in the United States (Jernigan et al., 2001), numerous new methods and technologies for the detection and identification of *B. anthracis* have been developed and are still optimized. The increased interest in biosafety has also stressed the issue of a bioterrorist act involving the food production, processing and distribution chain (Danzig, 2003; Erickson and Kornacki, 2003). Food and feed are both composed of a complex matrix, and detection and isolation of potential threat agents is often difficult. Therefore new methods for the tracing and tracking of pathogens in different food or feed chains have to be carefully evaluated concerning their applicability. According to the United States Centers for Disease Control and Prevention (CDC) Select agent program, *B. anthracis* is classified as a critical biological agent of category A (Khan et al., 2000). Work with *B. anthracis* is performed in biosafety level 3 (BSL-3) containment environments, normally requiring dedicated laboratories, safety equipment, trained staff and external approval of the methods applied according to national regulations. This makes the development of new detection methods, their optimization and implementation in BSL-3 laboratories very laborious and time consuming compared to similar work in BSL-2 laboratories. It is not possible to work with *B. anthracis* outside BSL-3 even if the isolate is pXO1- and/or pXO2-cured in several European countries, as for instance Germany and Sweden. Therefore respective models are very important for the development and evaluation of new methods involving *B. anthracis* spores. The utmost importance of such simulants was recently addressed in the action plan of the Council conclusions on strengthening chemical, biological, radiological and nuclear (CBRN) security in the European Union (Anonymous, 2009).

In the past, different spore-forming bacteria have been used as surrogate for *B. anthracis* to minimize the risk arising from the work with this pathogen during the development and first validation steps of new methods. Mainly members of the closely related *Bacillus cereus* group, but also more distant *Bacillus* species as *B. atrophaeus*, *B. subtilis*, or *B. licheniformis* have been selected for this purpose (Nicholson and Galeano, 2003; Montville et al., 2005; Novak et al., 2005; de Siano et al., 2006; Majcher et al., 2008). These studies have shown that the selected strains not always behave in the same way as *B. anthracis*. Since *B. anthracis* belongs to the *B. cereus* group, which

* Corresponding author.
E-mail address: monika.ehling-schulz@vetmeduni.ac.at (M. Ehling-Schulz).
[1] Contributed equally.

0168-1605/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2010.07.036

comprises the genetically highly related species *B. anthracis*, *B. cereus*, *B. thuringiensis*, *B. weihenstephanensis*, and *B. mycoides*, it is most likely that members of this group share major phenotypic characteristics. However, due to the great diversity and strain variability within the *B. cereus* group of organisms (Ehling-Schulz et al., 2005; Fricker et al., 2008) the selection of appropriate strains is very important.

To our knowledge, this is the first study that combines information on genotypic characteristics with the phenotypic characterization of both *B. anthracis* and *B. cereus* group strains in order to evaluate surrogates for *B. anthracis*.

2. Materials and methods

2.1. Bacterial strains and cultivation

Details on bacteria used for the phenotypic spore characterization are given in Table 1. The work with *B. anthracis* was conducted under Biosafety Level 3 (BSL-3) conditions according to the regulations mandatory at SVA (National Veterinary Institute, Sweden). All strains were stored on Luria-Bertani (LB) agar (10 g tryptone, 5 g yeast extract, 5 g NaCl, and 15 g agar per liter).

2.2. Phenotypic characterization of isolates

Selected isolates were grown on modified PLET as described recently (Luna et al., 2009) with 25 g Bacto heart infusion broth, 14 mg EDTA, 1.9 mg thallous acetate, 15,000 U polymyxin B, 150,000 U lysozyme, 38 mg sulfamethoxazole, and 2 mg trimethoprim per liter. Isolates were incubated at 30 °C for 24 to 96 h. In addition, carbohydrate utilization patterns of selected isolates were determined using the API 50 CH (bioMérieux, France). Isolates were grown on horse blood agar plates. Colonies were suspended in sodium chloride solution (0.85% NaCl) and used subsequently as inoculum for the API 50 CHB/E medium (bioMérieux, France). API strips were inoculated according to the manufacturer's instructions, incubated at 29 °C, and analyzed after 24 h and 48 h. Experiments were repeated three times for *B. anthracis* and twice for other *Bacillus* isolates.

2.3. Sequence typing (spoIIIAB and MLST)

The sporulation stage III AB gene (*spoIIIAB*) was used for sequence typing as described by Ehling-Schulz et al. (2005). Calculations were performed with the software package Clustal_X (Thompson et al., 1997), and trees were constructed using the TREECON software (Van de Peer and De Wachter, 1997). Distances were calculated based on Kimura's two parameter model (Kimura, 1980), and tree topology was interfered by neighbor joining. Newly sequenced data was compared to data published by Ehling-Schulz et al. (2005) and available data from databases (NCBI). Isolates selected for spore characterization were additionally typed with the optimized MLST scheme as described by Tourasse et al. (2006) and analyzed as described above for spoIIIAB.

2.4. Whole genome comparison

All available completed and draft genomes from the *B. cereus* group were downloaded and the average genomic distance to the *B. anthracis* strain ‘Ames’ based on pair wise BLAST comparisons of sequences divided into units of 200 base pairs were calculated using the ASC method (Segerman et al., 2011-this issue). In brief, the BLAST scores were normalized against the maximal possible score (against a 100% identical sequence) and averaged. Only sequence units scoring over a threshold separating the core genome from the accessory genes were included in the average. BLASTN were used with low complexity filtering turned off.

2.5. Spore preparation

One colony from solid media was used to inoculate 5 ml LB medium (see above but without agar). This pre-culture was incubated overnight at 37 °C inside a plastic cell culture flask with a sterile filter lid. The overnight culture was diluted 1:200 into 50 ml sporulation medium modified from de Vries et al. (2004). The inoculated medium was incubated at room temperature with shaking at 200 rpm in a plastic 500 ml Erlenmeyer flask fitted with a sterile filter lid until the cultures contained >95% free endospores (usually between 48–72 h), as determined by microscopy. The spores were harvested by centrifugation at 2500 *g* for 5 min, resuspended in 40 ml sterile pre-cooled washing buffer (2 mM KH_2PO_4, 8 mM K_2HPO_4) and vortexed vigorously. This washing step was performed 6 times and then once every week. Spore suspensions were stored at 4 °C as it has been recently shown that *B. anthracis* spores were stable at this storage temperature for at least 900 days (Almeida et al., 2008). Prior to experiments, the spore suspensions were washed with pre-cooled washing buffer.

2.6. Scanning electron microscopy

As *B. anthracis* spores in high concentrations were analysed outside the BSL-3-laboratory, the spores had to be inactivated before

Table 1
Bacterial strains used in this study. The length, diameter and aspect ratios are from spores measured with a scanning electron microscope.

Species	Name	Growth on modified PLET in 24 h	Spore length (μm)		Spore diameter (μm)		Aspect ratio	Number of spores measured
			Mean ± SD	Min–max	Mean ± SD	Min–max	Mean ± SD	
B. anthracis	7702 (Sterne)	+[a]	1.69 ± 0.10	1.50–1.85	0.82 ± 0.04	0.76–0.90	2.05 ± 0.14	19
B. anthracis	4229	n.d.[b]	1.16 ± 0.07	1.04–1.24	0.77 ± 0.04	0.70–0.82	1.52 ± 0.08	10
B. cereus	F2085/98	+	1.68 ± 0.12	1.43–1.92	0.84 ± 0.05	0.72–0.92	2.00 ± 0.13	45
B. cereus	NVH1518-99	+						
B. cereus	NVH0597-99	+	1.58 ± 0.07	1.48–1.70	0.80 ± 0.07	0.69–0.93	1.99 ± 0.16	15
B. cereus	F837/76	(+)[c]						
B. cereus	ATCC 10987	−	1.78 ± 0.07	1.66–1.92	0.86 ± 0.05	0.79–0.94	2.06 ± 0.13	9
B. cereus	F4810/72	−						
B. cereus	NVH1105-98	−						
B. cereus	IH41385	−						
B. cereus	ATCC 14579[T]	−[a]	1.40 ± 0.08	1.25–1.54	0.82 ± 0.04	0.75–0.93	1.71 ± 0.11	19
B. cereus	SDA VI273	−						
B. thuringiensis	var. *kurstaki* HD-1 (ATCC 33679)	−[a]	1.78 ± 0.19	1.49–2.11	0.86 ± 0.05	0.77–0.94	2.09 ± 0.20	16
B. atrophaeus	CCUG 11738 (NCTC 10073)	n.d.[b]	1.36 ± 0.07	1.21–1.52	0.76 ± 0.04	0.67–0.83	1.79 ± 0.09	22

[a] According to Luna et al. (2009).
[b] n.d.: not determined.
[c] Growth as pin point colony.

Download English Version:

https://daneshyari.com/en/article/4367888

Download Persian Version:

https://daneshyari.com/article/4367888

Daneshyari.com