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Growth characteristics of *Bacillus anthracis* compared to other *Bacillus* spp. on the selective nutrient media Anthrax Blood Agar® and Cereus Ident Agar®

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Abstract

Anthrax Blood Agar® (ABA) and Cereus Ident Agar® (CEI) were evaluated as selective growth media for the isolation of *Bacillus anthracis* using 92 *B. anthracis* and 132 other *Bacillus* strains from 30 species. The positive predictive values for the identification of *B. anthracis* on ABA, CEI, and the combination of both were 72%, 71%, and 90%, respectively. Thus, less than 10% of all species were misidentified using both nutrient media. Species which might be misidentified as *B. anthracis* were *B. cereus*, *B. mycoides*, and *B. thuringiensis*. Particularly, 30% of *B. weihenstephanensis* strains were misidentified as *B. anthracis*.

Keywords: *Bacillus anthracis*; *Bacillus cereus*; Selective nutrient media; Growth media

Anthrax is a serious disease in animals and humans caused by a Gram-positive, rod-shaped, non-motile, spore-forming bacterium. In humans, cutaneous anthrax accounts for the majority of clinical cases in naturally acquired infections. Cutaneous anthrax can be a self-limiting disease but up to 20% of patients develop septicemia. Gastrointestinal and inhalation anthrax commonly progress to fatal bacteremia and toxemia with a mortality rate >80% [3]. *Bacillus anthracis* was weaponized in biological warfare programmes in the 1950s and was used in bio-terrorist mail attacks in the United States in 2001. Identification of *B. anthracis* requires a combination of tests including direct fluorescent antibody staining of the capsule and the cell wall, biochemical identification, and PCR of plasmid-borne species-specific markers [1,2,4,9]. Phenotypic properties of *B. anthracis* such as amotility, sensitivity to penicillin and gamma phages can be assessed easily but may lack sensitivity [1]. On sheep blood agar *B. anthracis* forms large white to grey non-hemolytic colonies with irregularly tapered outgrowths (a "Medusa's head" appearance). Colonies have a dull appearance and are rather flat. The closely related species *B. cereus*, *B. mycoides* and *B. thuringiensis* of the "*B. cereus* group" can be misidentified as *B. anthracis* based on these morphological criteria [1,4]. For the isolation of *B. anthracis* from clinical or environmental samples, polymyxin lysozyme-EDTA thallous acetate (PLET) agar was traditionally

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used as selective growth medium [6]. However, PLET agar contains highly toxic thallium acetate at a high concentration, which excludes its use in many countries due to work-safety regulations.

The aim of this study was to assess the growth characteristics of *B. anthracis* in comparison with other *Bacillus* species on Cereus ident agar™ (CEI, Heipha, Eppelheim, Germany) and Anthrax blood agar™ (ABA, Heipha, Germany). ABA was developed on the basis of a recipe for an agar which is in use at the anthrax laboratory of the University Hohenheim since 1987 [7]. We demonstrate that *B. anthracis* isolates show characteristic morphological properties on both media useful for the differentiation from other *Bacillus* spp. Thus, the number of suspicious colonies requiring further confirmation can be reduced markedly.

We investigated 92 *B. anthracis* isolates of environmental and animal origin from a wide range of regions and 132 other *Bacillus* strains of 30 species (Table 1). Strains were obtained from the American Type Culture Collection (MD, USA), the German Collection of Microorganisms and Cultures (DSMZ, Braunschweig, Germany), B. Niederwoehrmeier (Wehrwissenschaftliches Institut für Schutztechnologien, Munster, Germany), and K. Noeckler (Bundesinstitut für Risikobewertung, Berlin, Germany). For inoculation, overnight cultures of bacteria grown on Columbia agar (Merck, Darmstadt, Germany) supplemented with 5% sheep blood (Oxoid, Wesel, Germany) were used. Single colonies were selected and cultured aerobically on ABA and CEI at 37 °C. Growth characteristics were determined after 24 h. ABA (Heipha, Prod. Nr. 1611e, Eppelheim, Germany) contains 14 g/l casein peptone,

Table 1. On Cereus Ident Agar® (CEI) and Anthrax Blood Agar® (ABA) 64 of 132 *Bacillus* isolates grew with a colony diameter >1 mm after 24 h of incubation

Species	Number of strains	CEI		ABA		CEI and ABA
		Growth	*B. anthracis* like	Growth	*B. anthracis* like	*B. anthracis* like
Bacillus alvei	2	0	0	0	0	0
Bacillus atrophaeus	1	0	0	0	0	0
Bacillus benzoevorans	1	0	0	0	0	0
Bacillus brevis	2	1	1	1	1	0
Bacillus cereus	15	13	4	13	6	1
Bacillus circulans	4	1	0	2	0	0
Bacillus coagulans	7	0	0	0	0	0
Bacillus firmus	3	0	0	0	0	0
Bacillus flexus	1	0	0	0	0	0
Bacillus fusiformis	1	0	0	0	0	0
Bacillus gibsonii	1	0	0	0	0	0
Bacillus laterosporus	2	0	0	0	0	0
Bacillus lentus	3	0	0	0	0	0
Bacillus licheniformis	6	0	0	0	0	0
Bacillus macerans	1	0	0	0	0	0
Bacillus megaterium	4	0	0	0	0	0
Bacillus mesenterius	1	0	0	0	0	0
Bacillus mojavensis	1	0	0	0	0	0
Bacillus mycoides	15	15	15	15	9	2
Bacillus olenorius	1	0	0	0	0	0
Bacillus polymyxa	5	1	1	0	0	0
Bacillus pseudomycoides	3	2	1	2	0	0
Bacillus pumilis	3	0	0	0	0	0
Bacillus simplex	1	0	0	0	0	0
Bacillus sphaericus	6	0	0	0	0	0
Bacillus stearothermophilus	1	0	0	0	0	0
Bacillus subtilis	9	0	0	0	0	0
Bacillus thuringiensis	14	14	1	14	11	1
Bacillus vallismortis	1	0	0	0	0	0
Bacillus weihenstephanensis	17	17	14	17	9	6
Sum	132	64	37	64	36	10

On CEI and ABA, 37 and 36 isolates were indistinguishable from *Bacillus anthracis*. The combined use of both media reduces the number of strains to 10 out of four species that require further tests.