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Effectiveness of a peracetic acid-based disinfectant against spores of *Bacillus cereus* under different environmental conditions

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ABSTRACT

A peracetic acid based disinfectant was tested for its efficacy against spores of different *Bacillus cereus* -strains (DSM 318, 4312, 4313 and 4384). To determine the influence of different factors like exposure-time, temperature and presence of protein quantitative and qualitative suspension tests were performed. Spore suspensions of *B. cereus* were treated with various concentrations of a representative peracetic acid based disinfectant at three temperatures (10, 15 and 20 °C), with protein load and with different exposure times (5, 30 and 60 min). Temperature, level of concentration and exposure-time had a significant influence on reduction of spores of *B. cereus* ($p < 0.05$). The susceptibility of spores of different strains greatly differed. A treatment of spores of DSM 4384 with 2.0% for 30 min even at 10 °C inactivated all present spores (initial number 6.18–6.71 log CFU/ml). Spores of *B. cereus* strain DSM 4313 had only reductions of 0.16–0.97 log CFU/ml at same treatment conditions. The presence of inactivated bovine serum as interfering substance had no significant influence on reduction ($p > 0.05$).

1. Introduction

Bacillus (*B.*) *cereus* can lead to two types of food-borne gastrointestinal illnesses, namely the emetic and diarrheal syndrome. It is also known as a causative agent of spoilage and as a spore former (Agata, Ohta, & Yokoyama, 2002; Andersson, Ronner, & Granum, 1995; Wijnands, Pielaat, Dufrenne, Zwietering, & van Leusden, 2009). Contamination of surfaces with spores of *B. cereus* is a severe problem in food processing plants, dairy industry, restaurants and even in private households (Gaulin, Viger, & Fillion, 2002; Svensson, Eneroth, Brendehaug, & Christiansson, 1999; Todd, 1992). Possible vehicles for *B. cereus* to enter the food chain are dust and soil (Kotiranta, Lounatmaa, & Haapasalo, 2000; Salustiano et al., 2009; Schoeni & Wong, 2005; Shaheen, Svensson, Andersson, Christiansson, & Salkinoja-Salonen, 2010). Once *B. cereus* adheres to surfaces in the kitchen, all kind of food treated in the kitchen can be cross-contaminated (Kramer & Gilbert, 1989; Kreuzberger, Moravek, Dietrich, Scheurer, & Märtlbauer, 2008). Particularly food facilities with a high number of customers, like large-scale catering establishments, refectories and canteen kitchens have to ensure a successful disinfection (Ernst et al., 2006; Kleer et al., 2001).

Spores of bacteria like *B. cereus*, unlike non-spore forming Gram-positives (Klein, Bonaparte, & Reuter, 1992) are a challenge for cleaning and disinfection. Routinely performed physical or chemical treatments are insufficient in inactivating spores. In addition spore germination can be initiated by heating procedures and *B. cereus* is able to proliferate at temperatures up to 60 °C (BfR, 2008; Martinez, Armesto, Franco, & Carballo, 2012; Russell, 1999; Sagripanti & Bonifacino, 1999).

Good hygienic practices include an adequate disinfection against bacterial spores. They are very important to improve quality and shelf life of food. Only few of frequently applied chemical disinfectants, that are suitable for the use in food areas, are sporicidal. Peracetic acid based disinfectants are known to be sporicidal and applicable in food areas, due to their chemical composition (Andre, Hedin, Remize, & Zuber, 2011; Blakistone, Chuyate, Kautter, Charbonneau, & Suit, 1999; Khadre & Yousef, 2001; Langsrud, Baardsen, & Sundheim, 2000; Sagripanti & Bonifacino, 1999).

Among spore formers *B. cereus*-strains are relatively highly resistant against chemical disinfectants and even among *B. cereus*-strains differences in susceptibility have been shown (Blakistone et al., 1999; Sudhaus, Pina-Perez, Martinez, & Klein, 2012). Hence,

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Table 1
Reduction of spores of *B. cereus* strain **DSM 318** (presented as $-\log_{10}(N_t/N_0)$) treated by different concentrations of PES 15/23 at three temperatures, with or without protein load and up to 60 min exposure time.

T	P*	min.	Concentration of PES 15/23 [Vol. %]									
			0.25		0.5		1.0		1.5		2.0	
10 °C	+	5	0.35	-0.04	0.31	0.15	0.46	0.12	0.99	0.13	1.05	0.57
		30	0.47	0.03	0.81	0.46	1.27	0.72	2.50	1.19	3.59	2.14
		60	0.58	0.12	1.20	1.11	2.02	1.05	3.41	2.61	> d.l.	> d.l.
	-	5	0.09	0.39	0.16	0.14	0.07	0.22	0.43	0.45	0.31	0.52
		30	0.20	0.80	0.33	0.46	0.71	0.80	1.50	1.43	2.38°	2.11°
		60	0.31	0.76	0.84	0.72	1.10	1.15	2.48°	2.04°	> d.l.°	> d.l.°
15 °C	+	5	0.30	0.21	0.26	0.27	0.76	0.48	1.03	0.74	1.50	0.87
		30	0.40	0.41	0.67	0.65	1.84	1.89	2.97	> d.l.	> d.l.°	> d.l.°
		60	0.67	0.48	1.21	1.49	3.14	3.27	> d.l.°	> d.l.°	> d.l.°	> d.l.°
	-	5	0.38	0.09	0.33	0.17	0.57	0.53	0.69	0.49	1.01	0.78
		30	0.69	0.32	0.82	0.92	1.76	2.18	2.75	3.18	> d.l.°	> d.l.°
		60	0.80	1.22	1.46	1.67	3.42	2.58	> d.l.°	> d.l.°	> d.l.°	> d.l.°
20 °C	+	5	0.12	-0.03	0.36	0.11	0.81	1.13	1.70	1.40	1.80	2.34
		30	0.60	0.38	1.24	1.07	2.95	3.21	> d.l.	> d.l.	> d.l.°	> d.l.°
		60	0.95	0.78	2.44	2.56	> d.l.	> d.l.	> d.l.°	> d.l.°	> d.l.°	> d.l.°
	-	5	0.07	0.39	0.19	0.68	0.84	0.81	2.53	1.91	> d.l.	2.99
		30	0.42	0.75	1.27	1.55	> d.l.	> d.l.	> d.l.°	> d.l.°	> d.l.°	> d.l.°
		60	0.78	1.22	2.25	2.39	> d.l.	> d.l.	> d.l.°	> d.l.°	> d.l.°	> d.l.°

T = Temperature

*) P = Protein, - = without protein load, + = with protein load

> d.l. = reduction higher than detection limit

°) in qualitative suspension test always negative

min. = exposure time to disinfectant in minutes

N_t = CFU/ml after treatment, N_0 = initial CFU/ml

= effective test conditions according to the definition in our study (>3.0 log reduction and negative results in the qualitative suspension tests with respective concentration steps)

for testing efficacy of disinfectants it is important to take into consideration species and strains of spore formers. This study was performed in order to gain more information about the susceptibility of spores of different strains of *B. cereus* taking environmental conditions into account.

2. Material and methods

2.1. Material

Spore-suspensions of four different strains of *B. cereus* were prepared. The disinfectant PES 15/23 (Bactria GmbH & Co. KG, Kaiserslautern, Germany) contained peracetic acid (approx. 15%), hydrogen peroxide (approx. 25%), acetic acid and water. Dilutions of the disinfectant were done in accordance with guidelines for testing disinfectants of the German Association of Veterinarians (DVG, 2000) with water of standardized hardness (17.5 ml [10% $CaCl_2 \times 6H_20$] plus 5.0 ml [10.0% $MgSO_4 \times 7\ H_2O$] plus 3300 ml of distilled water). Different neutralization agents were tested. The most effective neutralization agent was composed of 3.0% Tween® 80 (8.22187, Merck, Darmstadt, Germany), 0.3% Lecithin (27608, Serva, Heidelberg, Germany), 0.1% L-histidin (3738, AppliChem, Darmstadt, Germany) and 0.5% sodium thiosulfate (6516, Merck). The protein agent was inactivated bovine serum (elocin lab GmbH, Mühlheim an der Ruhr, Germany) resulting in a final concentration of 10% protein agent in the disinfectant test solution.

2.2. Spore-suspensions

Spore-suspensions of three toxin-producing strains isolated from foodborne outbreaks (DSM No. 4312, 4313 and 4384) and one strain isolated from soil (DSM No. 318) were used in tests. Spore suspension was done as previously described by Ernst, Schulenburg, Jakob, Dahms, Martinez Lopez, Nychas, Werber, and Klein (2006). *B. cereus*-strains were spread on tryptone soy agar (1.05458, Merck) and incubated aerobically at 37 °C ± 1 °C. After ten days of incubation sodium chloride (0.9% NaCl) was used to rinse off the colonies. Three centrifugations of the gained suspension were performed (30 min, 20 °C, 4500 rpm) each followed by a washing step with sodium chloride. A heat shock at 70 °C for 30 min was performed to ensure the death of vegetative cells. The spore suspension was mixed with glycerine (end concentration of 7.5%) and stored at −4 °C. After production and before testing the spore suspension was examined under the microscope to ensure the absence of germinated spores or vegetative microorganisms.

2.3. Suspension tests

Quantitative and qualitative suspension tests were performed in accordance with guidelines for testing disinfectants of the German Association of Veterinarians (DVG, 2000). Five commonly used levels for disinfectants containing peracetic acid (0.25%, 0.5%, 1.0%, 1.5% and 2.0% (w/v)) (DVG, 2012) were tested at 10 °C ± 1 °C, 15 °C ± 1 °C and 20 °C ± 1 °C. The trials were made in a temperature controlled water bath (Julabo Labortechnik GmbH, Seelbach, Germany). Exposure times of 5 min, 30 min and 60 min were tested. Each trial was tested with and without the addition of inactivated bovine serum as interfering substance. Every combination of test conditions was at least done in duplicate.

In the **quantitative suspension test** with protein load 0.1 ml of the spore suspension (approximately 5.0×10^7 CFU/ml), 1.0 ml of the protein agent and 9.0 ml of the disinfectant test solution were