

STRAIN DIVERSITY ALONG THE LIFE CYCLE OF *BACILLUS CEREUS*

SUGAR METABOLISM, SPORULATION, AND GERMINATION CAPACITY OF SPORES DERIVED FROM REFERENCE STRAIN AND UNDOMESTICATED ISOLATES

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Introduction

Undomesticated spore formers isolated from foods and food processing facilities may show altered characteristics compared to widely used domesticated reference strains that may have lost relevant traits. Analysis of sporulation and germination capacity, spore resistance, metabolism and outgrowth efficiency of undomesticated *Bacillus cereus* isolates therefore provides valuable insights in factors that determine survival and fitness in different environmental niches encountered during pathogen transmission from soil to host.

Methods

Carbohydrate utilization profiles, sporulation and germination characteristics were assessed for *B. cereus* ATCC14579 and 20 strains isolated from different food products. Germination of individual untreated and heated spores was analyzed in an assay using Flow cytometry (FCM).

Results

B. cereus isolates showed high diversity in carbohydrate metabolism (Table 1), in agreement with predicted performance based on known gene cassettes responsible for sugar utilization (not shown). All strains could metabolize glucose, maltose and trehalose, while only few metabolized galactose, mannose and lactose.

Sporulation efficiency was assessed for rice water and BHI (Fig. 1) and was highest in rice water for all strains.

The germination efficiency was determined after assessing the percentage of spores that remained dormant (unstained) in the population after exposure to selected germinants (Fig. 2A). A combination of alanine/inosine resulted in most efficient germination for all strains (Fig. 2B-F). Furthermore, heat activation stimulated germination for all strains, and was essential for isolate 13. Heat activated *B. cereus* ATCC14579 spores germinated even in the absence of added germinants. Spores of isolate 14 were most potent to germinate without heat activation.

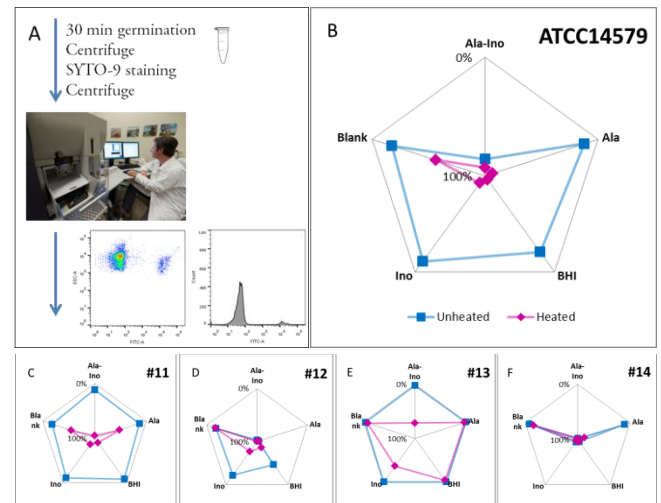


FIGURE 2. EVALUATION OF GERMINATION EFFICIENCY (a) SCHEMATIC OVERVIEW OF THE METHOD. (b-f) PERCENTAGE OF GERMINATED SPORES OF DIFFERENT *B. CEREUS* ISOLATES AFTER 30 MIN IN ALANINE (ALA), INOSINE (INO), MIX OF ALANINE AND INOSINE (ALA-INO) OR BHI WITH HEAT ACTIVATION (PINK) AND WITHOUT (BLUE)

TABLE 1. CARBOHYDRATE UTILISATION BY *B. CEREUS* ISOLATES

Isolate	Origin	Glycerol	D-Fructose	D-Xylose	D-Galactose	D-Glucose	D-Fructose	D-Mannose	N-Acetylglucosamine	Arabin	Esculin ferric citrate	Sialin	D-Cellobiose	D-Maltose	D-Lactose (bovine origin)	D-Sucrose (sucrose)	D-Trehalose	Glycogen	L-Fucose	Inositol	Glucosyl-Glucoside	Glucose-6-phosphate	
1	Chilled dessert	-(-)	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Unknown	(2/3)	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	can defect - chocolate beverage	-(1/3)	2/3	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Dried onion	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Provolone sauce	+	+	-	1/3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Asparagus ham sauce	(2/3)	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Torteloni con fughì	(2/3)	17/3	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Indian rice dish	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	Homemade asparagus soup	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	Boiled rice	+	?	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	Pea soup	+	?	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	Dressing	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	White sauce	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	Commercial pasteurised milk	+	?	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	(Ambiant) ice cream	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	Water continuous spread	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	Cereal products	+	?	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	(fermented) dairy products	+	?	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	Mayonnaise, dressings	-(1/3)	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	(fermented) vegetable products	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATCC14579	ATCC reference strain	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATCC10987	ATCC reference strain	-(-)	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

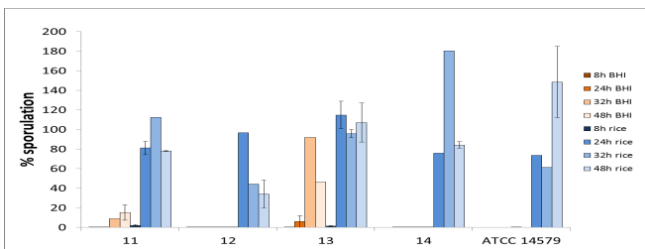


FIGURE 1. SPORULATION EFFICIENCY IN BHI (ORANGE) AND RICE WATER (BLUE) FOR A SELECTION OF *B. CEREUS* ISOLATES AT 30°C AFTER 8, 24, 32 AND 48 H

Conclusions

- B. cereus* strains displayed a highly diverse behavior with respect to carbohydrate metabolism, sporulation, and spore germination capacity
- This study provides insight in a range of performance parameters of *B. cereus* isolates that contribute to environmental transmission capacity of this food-born human pathogen