

BACILLUS ISOLATES FROM REFRIGERATED DOUGHS, WHEAT FLOUR, AND WHEAT¹

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ABSTRACT

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Bacillus species were isolated from refrigerated doughs, wheat flour, and wheat samples. Fifty-three isolates from refrigerated doughs were identified as *B. cereus*, *B. licheniformis*, *B. pumilus*, or *B. subtilis*. In addition to these four species, *B. brevis*, *B. coagulans*, and *B. megaterium* were among 31 isolates from wheat flour. Eleven isolates from wheat were identified as *B. brevis*, *B. cereus*, *B. circulans*, or *B. licheniformis*. None of the species was found in all of the samples.

Bacillus spp. have been implicated in food spoilage and food poisoning problems. As endospores, these mesophilic species resist a wide temperature range. Some strains survive baking temperatures between 350 and 450° F (1), and others tolerate cold temperatures (2-5). Therefore, if present in baked or chilled foods stored at room temperatures, the spores may germinate, and the bacteria may cause problems. *B. subtilis* and *B. licheniformis* are associated with a food spoilage known as ropey bread, and *B. cereus* and *B. subtilis* produce food poisoning endotoxin in starchy foods (1). This article reports a continuation of the examination of the microflora of wheat, wheat flour (6), and fresh and spoiled dough products (7,8). A taxonomic study was conducted on the *Bacillus* isolates.

MATERIALS AND METHODS

Samples of refrigerated doughs were purchased from supermarkets. Milled flour and unwashed wheat samples were obtained from mills in Kansas, Nebraska, and the Pacific Northwest.

Total bacterial counts were determined on all samples as described previously (6-8), and 25 colonies were randomly selected from dilution plates containing 30-300 colonies. After grouping bacteria by their morphologic and physiologic characteristics (9), the aerobic spore-forming bacteria were identified (10) by methods of Smith et al (11). Cultures were preserved by lyophilization.

RESULTS AND DISCUSSION

The distribution of *Bacillus* organisms varied in refrigerated doughs, wheat flour, and wheat (Table I). Among 53 isolates from refrigerated dough samples, three species were found. Approximately 50% were *B. subtilis*, 30% *B. cereus*, and 20% *B. pumilus*. Refrigerated doughs are one example of chilled convenience foods in which wheat flour is a main ingredient. Although these foods are stored at low temperatures, nutrients and moisture are readily available

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TABLE I
Distribution of Bacillus Organisms Isolated From Samples of Refrigerated Doughs, Wheat Flour, and Wheat

Samples	Number of Bacillus Isolates	B. brevis	B. cereus	B. circulans	B. coagulans	B. licheniformis	B. megaterium	B. pumilus	B. subtilis	Other Bacillus spp
Refrigerated doughs										
R-I	14		5					3	6	
R-II	14		1			2		1	10	
R-III	14		6					4	4	
R-IV	11		5					2	4	
Wheat flour										
F-1	4	1					1		1	1
F-2	0									
F-3	1								1	
F-4	10	1	2		1	1		1	4	
F-5	9							7	1	1
F-6	1							1		
F-7	4							1	3	
F-8	1							1		
F-9	1									1
Wheat										
W-2	1		1							
W-5	9	1	1	6		1				
W-8	1		1							

TABLE II
Summary of Bacillus Isolates From Refrigerated Doughs, Wheat Flour, and Wheat

Samples	Number of Bacillus Isolates	B. brevis	B. cereus	B. circulans	B. coagulans	B. licheniformis	B. megaterium	B. pumilis	B. subtilis	Other Bacillus spp
Refrigerated doughs	53		17			2		10	24	
Wheat flour	31	2	2		1	1	1	11	10	3
Wheat	<u>11</u>	<u>1</u>	<u>3</u>	<u>6</u>	<u>—</u>	<u>1</u>	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>
Total	95	3	22	6	1	4	1	21	34	3

for microbial growth. Contaminated ingredients such as wheat flours may be the source of *Bacillus* organisms in refrigerated doughs.

Several species were detected among the 31 wheat flour isolates, but no species was found in all of the samples. In five of eight samples, however, *B. pumilus* and *B. subtilis* were detected; these two species were common in dough samples. *B. cereus*, which also was common in doughs, was detected in only one flour sample. *Bacillus* spp. are occasionally found in dry foods, but flour containing 20 or more mesophilic spores per 100 g is considered objectionable (1).

The distribution of isolates from wheat flour shows little relationship to those in wheat. Wheat and flour samples were obtained the same day from each mill, but detection of *Bacillus* organisms was not consistent. Though *B. cereus* was found in three wheat samples, *Bacillus* organisms were not always detected in flour, ie, sample F-2 flour. Different *Bacillus* spp. were found in flour samples F-5 and F-8 than were found in the corresponding wheat samples W-5 and W-8.

Table II is a summary of *Bacillus* isolates. Only two species, *B. cereus* and *B. licheniformis*, were isolated from the three sample groups but were not detected in every sample in each group. *B. pumilus* and *B. subtilis* were common in dough and flour but were not detected in wheat. Although only four species of aerobic spore-forming bacteria were detected in wheat, seven were isolated from flour, and three species dominated in the refrigerated dough isolates.

A taxonomic study of isolates aids in evaluating potential spoilage and health problems. In this case, various *Bacillus* species were detected in three different types of products—chilled moist, dry, and natural.

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