Distribution of Thermophilic Aerobic Sporeforming Bacteria in Food Ingredients

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ABSTRACT

RICHMOND, B. (University of Missouri, Columbia), AND M. L. FIELDS. Distribution of thermophilic aerobic sporeforming bacteria in food ingredients. Appl. Microbiol. 14:623–626. 1966.—Samples of sugar, starch, spices, and miscellaneous products were tested for thermophilic sporeformers of Bacillus to determine the dominant species present. Surface colonies selected at random were identified. Six species of Bacillus were isolated: B. stearothermophilus, B. coagulans, B. licheniformis, B. subtilis, B. circulans, and B. pumilus. Samples of starch and pepper were tested for thermophilic sporeformers of Bacillus to determine the distribution of rough and smooth variants. Colonies were classified as rough or smooth variants by colonial characteristics. The distribution of variant forms in these two products was significantly different. Starch samples showed predominantly rough variants; pepper samples showed predominantly smooth variants.

The distribution of thermophilic aerobic sporeforming bacteria in food ingredients is of interest to the food microbiologist because of their potential importance as spoilage organisms in canned foods. Cameron and Esty (3) selected 55 and 37 C for temperatures to determine the kind of thermophile. With their classification, cultures which grew at 55 C but not at 37 C were classified as obligate thermophiles, and those cultures which grew at both 55 and 37 C were considered as facultative thermophiles. Gordon and Smith (5), however, found that temperature alone was not a good taxonomic tool to differentiate species of the genus Bacillus, with the exception of distinguishing B. coagulans from B. stearothermophilus. If one selects 55 C as the temperature to make the primary isolation, fewer strains of B. coagulans would grow as well as strains of B. subtilis, B. brevis, and B. circulans (5). According to Smith, Gordon, and Clark (9), the following species grow at 50 C or higher: B. licheniformis, B. subtilis, B. stearothermophilus, B. coagulans, B. brevis, B. pumilus, and B. macerans.

Species which have caused spoilage in canned goods are: B. stearothermophilus, B. coagulans, and B. licheniformis. B. stearothermophilus is the most heat-resistant of the aerobic sporeforming bacteria. B. stearothermophilus causes the typical flat-sour spoilage in low-acid foods such as cream-style corn, peas, and beans. B. coagulans causes flat-sour spoilage in tomato juice and firm coagulation in evaporated milk. B. licheniformis was isolated from spoiled banana purée (7).

The presence of spores of thermophilic bacteria in sugar and starch has been recognized as a potential source of spoilage organisms for some time (1, 2, 8). There are no references in the literature on the distribution of species of the genus Bacillus or in regard to the distribution of rough and smooth variants of members of the genus Bacillus in various types of food ingredients.

The distribution of the thermophilic species as well as the variant type is of interest because of the difference in the heat resistance between species such as B. stearothermophilus and B. subtilis, and between the rough and smooth variant forms of species such as B. stearothermophilus, where the smooth variant is more resistant than the rough (4). Since a process time (time and temperature to cook and "sterilize" a canned food) is based upon a definite bacterial spore load, ingredients containing high levels of the flat-sour type may raise the spore load of the product above the level for the process so that spoilage results. The objectives of this research were to determine the distribution of thermophilic species (growth at 52 C) in various food ingredi-
ents and to determine the ratio of smooth to rough in starch and black pepper (at 55 C).

**Materials and Methods**

**Distribution of species.** The objective in this part of the study was to examine many different ingredients rather than many samples of a few ingredients to determine the distribution of species. The following food products were used as test samples: sugar (brown, eight samples; granular, five samples; and raw, six samples), starch (corn starch, 13 samples; cake flour, 1 sample; oat flour, 1 sample; potato starch, 1 sample), spices (11 samples and 9 kinds), milk powder (whole and skim, 1 sample each), green split peas (1 sample), strain G yeast (1 sample), cocoa (1 sample), gelatin (1 sample), and dried soup (1 sample each of tomato and vegetable). The food ingredients samples were obtained from canning companies and local supermarkets. The procedures used in this study were based on methods recommended by the National Canners Association as given by Hersom and Hulland (6).

The isolation procedure for sugars, spices, and other samples, excluding the starches, consisted of placing a 10-g sample in a sterile 300-ml flask marked to indicate a volume of 100 ml. Sterile water was added to make 100 ml. The contents were thoroughly mixed, brought to a boil, and held at boiling for 5 min. A 1-ml amount of each sample was placed in each of three sterile petri plates, covered with dextrose-tryptone-agar (Fisher Scientific, Fair Lawn, N.J.), and thoroughly mixed. The plates were incubated at 52 C for 48 hr so that a maximal number of species that grow at 50 C and above could be isolated but still be within 3 C from the recommended temperature.

Due to the thickening property of starch, certain modifications were necessary for handling the starch and flour products. A 10-g amount of starch was placed in a sterile 300-ml flask, 100 ml of sterile water was added, and the mixture was thoroughly stirred. A 20-ml amount of this prepared suspension was added to 100 ml of melted dextrose-tryptone-agar; the mixture was stirred as the starch began to flow and then was held for 30 min with occasional stirring in a simmering hot-water bath. At the end of this time, the mixture was poured into five petri plates and incubated at 52 C for 48 hr.

**Identification of isolates.** Randomly selected surface colonies of rough and smooth (same number of each) variants were transferred to nutrient agar slants for taxonomic studies. Each slant was coded with a number according to the food product from which it was isolated, and the letter “R” or “S” according to whether it was a rough variant or a smooth variant judged by colonial characteristics.

The tests and descriptions outlined for *Bacillus* species by Smith et al. (9) were followed in identifying the species of the isolates. All tests requiring incubation were carried out at 52 C with time intervals of 24 hr to 7 days.

**Distribution of variants.** Because the number of spores was so low in some of the samples tested, and since one cannot determine the distribution of variant types adequately in samples containing only a few colonies, samples of starch and black pepper were selected for the study of the distribution of variants, since they gave the highest consistent spore load. These were prepared as previously stated, except that these plates were incubated at 55 C. The temperature of 55 C was selected in this case because it is the usual temperature used for thermophiles and, in this phase of the study, the emphasis was on the ratio of rough to smooth rather than on distribution of species. The plates were then viewed at a magnification of 45 times under a dissecting microscope, classified as rough or smooth, and the number of rough and smooth colonies was counted. The colonies were classified as rough and smooth according to colonial characteristics (4). The average per cent rough and smooth was determined and recorded.

Since each colony was classified as rough or smooth, enumeration data were collected in this phase of the study, and the data were pooled so that each colony became a separate determination. Enumeration data so collected may be analyzed by the chi-square method (10), and were so analyzed in this study to determine whether the null hypothesis (the ratio of S-R variants in pepper was the same as the ratio of S-R variants in starch) would be rejected or accepted.

**Results and Discussion**

**Distribution of species.** Figure 1 shows the distribution of thermophilic aerobic sporeforming bacteria which were isolated from 18 food ingredients. Of the 76 randomly selected colonies for the taxonomic studies, the largest number of strains was in the species *B. coagulans*. Not only were there more strains of *B. coagulans*, but *B. coagulans* was also found in 14 of the 18 samples tested. This is significant, since most of the food ingredients harbored *B. coagulans* which could establish itself in a food-processing plant if conditions were right and there was poor sanitation. According to Gordon and Smith (5), strains of this organism can grow from 28 to 60 C, increasing their potential occurrence. Sixteen strains of *B. coagulans* were found in starch and sugar, and 9 strains were found in spices. The remaining seven strains were isolated from mustard, dried milk, split dried peas, dried yeast, and dried tomato soup. Standards for the number of spores allowed in sugar and starch have been set by the National Canners Association (6). There are no standards for spices.

The number of samples of *B. stearothermophilus* was low, being only two strains. One strain was isolated from sugar and the other from turmeric. Two strains of *B. pumilus* were isolated from raw sugar and from oat flour. One strain of *B. circulans* was isolated from raw sugar, one from black pepper, and one from cinnamon.

*B. subtilis* was found in 8 of the 18 food ingredients (17 strains) and was next to *B. coagulans*
in having the greatest distribution among food ingredients tested. Six strains of B. subtilis were isolated from corn starch, one from raw sugar, one from turmeric, three from black pepper, one from onion powder, two from curry powder, two from nutmeg, and one from cocoa. B. licheniformis was isolated from 7 of 18 food ingredients. However, 13 of the 19 isolates were from starch, the largest number of strains of any species in this study. The remaining six strains were isolated from turmeric, garlic powder, mustard, dried milk, dried tomato soup, and black pepper.

**Distribution of variants.** The distribution of rough and smooth variants in starch and pepper is given in Table 1. Twenty-one samples of corn starch were examined. The aerobic spore count at 55°C varied from 12 to 849 spores per gram, with the mean being 70 spores per gram. The percentage of rough variant on the plate ranged from 64 to 99, with an average of 82%.

The distribution of variant forms in black pepper was different from that in starch. Twenty-eight samples were examined. Higher spore counts (600 to 570,000 per gram, with an average of 9,200 per gram) were observed. The percentage of rough colonies was considerably lower than in the starch samples (1 to 56%, with an average of 21%). When the total number of colonies examined (4,633 for pepper and 4,913 for starch) were analyzed by chi-square, the results were highly significant, indicating that the ratio of S–R is not the same in these two ingredients. The reason that the variant forms are present in such different ratios is not known, and is an area where further research is indicated.

**General discussion.** Gordon and Smith (5) listed five species of *Bacillus* which grow at 55°C, the usual temperature for the determination of "flat-sour spores." Only *B. coagulans* and *B. stearothermophilus* are true flat-sour types which are associated with flat-sour spoilage in canned foods. According to Gordon and Smith (5), *B. subtilis*, *B. brevis*, and *B. circulans* also grow at this temperature and can produce acid in dextrose-tryptone-agar, and so could be counted as "flat-sour type" spores. However, these spores would not be potential spoilage producers like *B. coagulans* and *B. stearothermophilus* because of the lack of heat resistance of spores of these species. If one were attempting to measure the "flat-sour type" exclusively, an incubation temperature of 60°C would allow only the strains of

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*Fig. 1. Distribution of 76 isolates of thermophilic bacteria taken at random from platings of 18 food ingredients*

**Table 1. Distribution of rough and smooth colonial types of thermophilic aerobic sporeforming bacteria in corn starch and black pepper**

<table>
<thead>
<tr>
<th>Food ingredient</th>
<th>Total samples tested</th>
<th>Avg. spore count per g&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Range of spore counts per g</th>
<th>Range of per cent R colonies</th>
<th>Avg. percentage of R colonies per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>21</td>
<td>70</td>
<td>12–849</td>
<td>64–99</td>
<td>82</td>
</tr>
<tr>
<td>Black pepper</td>
<td>28</td>
<td>9,200</td>
<td>600–570,000</td>
<td>1–56</td>
<td>21</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ingredients plated with dextrose-tryptone-agar. Incubated at 55°C for 48 hr.
B. coagulans and B. stearothermophilus to grow. This temperature would, however, decrease the strains of B. coagulans which would grow (5).

LITERATURE CITED