

Research Note

REFRIGERATION BEFORE INCUBATION AND ITS EFFECT ON RECOVERY OF TWO TYPES OF BACTERIA FROM FOOD¹

The plate count is by far the most widely used method for determining the number of viable cells or colony-forming units (cfu) in a food product.² By this method, portions of food samples are blended or homogenized, serially diluted in an appropriate sterile diluent, plated in or onto a suitable agar medium, incubated at an appropriate temperature for a given time, after which process all visible colonies are counted with a Quebec or electronic counter.

When this procedure is used, the plates should be counted promptly after the incubation period. Only if it is impossible to count at once, may the plates be stored after the required incubation, under refrigeration for a period of no more than 24 hours.³ Nowhere have we found published data as to the effect of refrigeration before incubation on the aerobic plate counts of food samples. Moreover, we found no published information as to whether a refrigerated storage period of more than 24 hours before counting plates was possible without significantly affecting the results obtained.

A small study was carried out to obtain information to these effects. This was part of a current research project in which numerous plate counts have to be made.

The nature of the work requires that approximately seven working days be dedicated to the preparation and completion of such plate counts.

Twenty tubes, each with 8 ml of sterile ripe plantain puree, were inoculated with 0.1 ml of a *Bacillus coagulans* spore suspension and 20 tubes with 0.1 ml of *Bacillus macerans* spore suspension. Ten tubes of each type of spore were incubated at 37° C (98° F), and 10 tubes at 50° C (122° F) for 3 days. After incubation, 1 ml of puree from each tube was diluted in sterile phosphate buffer⁴ and pour plated in quadruplicate with Plate Count Agar (DIFCO Laboratories, Detroit, Michigan).⁵ Duplicate plates of each tube were immediately incubated at 37° C (98° F) for 3 days. The other 2 plates from each tube were stored at 7° C (45° F) for 2 days and then incubated at 37° C (98° F) for 3 days. At the end of the corresponding incubation period, aerobic plate counts were made and the data submitted to statistical analysis.

Table 1 shows the results obtained. We observed no significant differences ($P=0.05$) in the plate counts obtained when plates were stored for 2 days under refrigeration before incubation from those ob-

¹Manuscript submitted to Editorial Board 19 February 1988.

²Jay James M., 1986. Modern Food Microbiology, 3rd ed, Van Nostrand Reinhold Co., New York.

³Speck, Marvin L., Ed, 1976. Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington, D. C.

⁴Fernández-Coll, F. and W. Rodríguez Toro, 1986. Thermal resistance of spores of two species of the genus *Bacillus*. *J. Agric. Univ. P. R.* 70 (3):189-96.

⁵Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

TABLE 1.—Effect of refrigerating¹ inoculated plates before incubation² on the Aerobic Plate Count³ on *Bacillus coagulans* and *Bacillus macerans* in ripe plantain puree incubated⁴ at two different temperatures

Bacteria	Not Refrigerated		Refrigerated
<i>B. coagulans</i>	37° C	3.9 × 10 ⁴ a ⁶	3.8 × 10 ⁴ a
	50° C	2.3 × 10 ⁴ b	2.1 × 10 ⁴ b
<i>B. macerans</i>	37° C	2.5 × 10 ⁴ c	2.4 × 10 ⁴ c
	50° C	2.1 × 10 ⁴ d	1.9 × 10 ⁴ d

¹At 7° C for 2 days.

²At 37° C for 3 days.

³Per ml of puree. These counts are the average of 20 plates (duplicate plates for each of 10 different tubes of inoculated puree).

⁴For 3 days.

⁶Counts in the same row followed by the same letter do not differ significantly at $P > 0.05$.

tained when the plates were incubated immediately. This held true for both *B. coagulans* and *B. macerans* and for both incubation temperatures (37° C and 50° C).

These results indicate that pour plates prepared under our particular circumstances can be stored under refrigeration before their incubation with no significant effect on the counts obtained. Also, refrigeration for more than 24 hours after pouring and before counting plates was possible without affecting results. According to our knowledge, none of these observations have been previously reported.

Because of the voluminous amount of follow-up work and lack of time inherent in microbiological analyses of foods, often the need arises to postpone work. Reasons to postpone work could be other professional commitments, avoiding paying technicians overtime for weekend follow-up work, or

avoiding overincubating plates if they cannot be counted immediately after the incubation period.

The results of this study indicate that plates could be stored in the refrigerator and incubated later so that the time at which they could be counted coincides with the availability of personnel. It is possible in one day to process the samples, make the corresponding dilutions, pour the plates and, after they solidify, store them in the refrigerator (for up to 2 days), and then incubate them on another day. We recommend that each laboratory that wishes to apply this procedure test it for the samples or microorganisms assayed to confirm that our findings apply to their particular circumstances.

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