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Comparison of the Stomacher with other Systems for Breaking Clumps and Chains in the Enumeration of Bacteria

KURT E. DEIBEL and GEORGE J. BANWART*

Department of Microbiology, The Ohio State University, 484 West 12th Avenue, Columbus, Ohio 43210

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ABSTRACT

Five systems were compared for their ability to break up chains and clumps of organisms for enumeration. The highest aerobic plate counts of *Bacillus cereus* were obtained by mixing the organism in the Waring blendor or the Osterizer. Significantly lower counts were obtained by stomaching, shaking or shaking with beads. Results similar to those of *B. cereus* were obtained when *Staphylococcus aureus* and *Streptococcus faecalis* were prepared for enumeration using these five systems. There was no significant difference in aerobic plate counts obtained by using the five systems with *Yersinia enterocolitica* as the test organism.

Sharpe and Jackson (*8*) introduced the Stomacher for preparing food samples for microbial analysis. The Stomacher has been compared to a multitude of other mixers, including those that are commonly used in the United States (*5,7*).

Bacterial counts of foods blended in the Stomacher were similar to or higher than those of foods diluted in the Waring blendor (*5,6*). The microbial counts of certain foods and pure cultures were similar when mixed in either the Stomacher or Osterizer (*3,7*). Lower numbers of organisms were recovered from certain foods, especially those containing fat, when the Stomacher was compared to other mixing methods (*1,2,7,8*). Researchers attributed this to fat globules adhering to the Stomacher bag which could not be dislodged.

When enumerating microorganisms in a food, two things must be accomplished: (*a*) removal of organisms from food particles and (*b*) separation from each other. Thomas and McMeekin (*9*) showed with a scanning electron microscope that the Stomacher removed organisms from food particles. The main problem with the Stomacher may be with separating the organisms that are in chains or clumps.

One assumption of a plate count is that each colony arises from a single cell unit. Therefore, dispersal of clumps and chains of bacteria into unattached individual cells is imperative. The system used to mix a food or culture is a critical step in the dispersal of organisms. Goel and Marth (*4*) compared shaking and shaking with beads for their ability to break up chains of a chain forming bacterium. Their results showed no difference in chain breakage with or without glass beads being present.

This report compares the Stomacher to other mixing methods for the ability to separate bacteria that form chains or clumps.

MATERIALS AND METHODS

Cultures

Streptococcus faecalis 48 and *Staphylococcus aureus* 303 were obtained from The Ohio State University culture collection. *Bacillus cereus* 836/76 was obtained from the Central Public Health Laboratory, London, England. *Yersinia enterocolitica* 975 was obtained from the Food and Drug Administration, Cincinnati, Ohio. All cultures were maintained on Plate Count Agar (Difco) slants at 4°C.

Systems

Five systems were used to mix the cultures with sterile diluent. These systems were the Colworth Stomacher 400, Waring blendor model number PB-5A, Osterizer (John Oster Mfg. Co.) model 947, Pyrex wide mouth jar number 1373 and Pyrex wide mouth jar number 1373 with small glass beads.

Analysis

To obtain a working culture, the organisms were inoculated into nutrient broth and incubated for 24 h at 32°C. Then, 0.1 ml of each of the cultures of *B. cereus, S. aureus* and *Y. enterocolitica* was inoculated into separate bottles of sterile skim milk and incubated at 32°C for 24 h. The skim milk was prepared by weighing 95.9 g of instant nonfat dry milk (Carnation) into a liter of distilled water. This was mixed and then dispersed in 110-ml portions into Pyrex bottles (number 1372). The skim milk was heated at 118°C for 12 min. *S. faecalis* was inoculated (0.1 ml) into 110 ml of nutrient broth (Difco). When *S. faecalis* was inoculated into sterile skim milk, it clotted the milk during incubation in a preliminary test. The bottles were incubated at 32°C for 24 h.

After incubation, the inoculated broth and milks were analyzed for the total number of colony-forming units using the pour plate technique with Plate Count Agar (Difco). All dilutions were made with

0.1% peptone water. Each organism was analyzed by the five systems. The broth or milk bottle was shaken gently by rotating the bottle over the palm of the hand and inverting the bottle several times (shake method). Any residue on the inner bottom of the bottle was shaken loose and suspended into the medium. This bottle was shaken just enough to disperse the organisms evenly throughout the broth or milk. This sample was further diluted and plated. After the shaking system was performed, the other four systems were done randomly to not influence the results. The sample was blended for 2 min in the Waring blendor, Osterizer or the Stomacher. Shaking with beads was done by shaking the sample 30 times in a 1-ft arc for 7 s.

After preparation, plates were incubated at 32°C for 48 h and the colony-forming units were enumerated with the aid of a Quebec colony counter. Ten replicates of each organism using the above procedures were done.

Approximately 0.05 ml of the 1/10 dilution from each system was pipeted onto separate microscope slides. The slides were air-dried, heat-fixed, and simple-stained with crystal violet. The number of cells per chain was microscopically determined using the 1.30 mm objective lens, resulting in 100× magnification. The percent of organism per chain length was determined.

RESULTS AND DISCUSSION

Some organisms tend to form chains or clumps and others normally are found as single cells. Usually cells of *B. cereus* and *S. faecalis* are observed in chains. Cells of *S. aureus* are found in clumps or clusters and *Y. enterocolitica* occurs as individual cells. When determining the total number of organisms in a food, all chains and clumps of cells should be broken apart to yield single cells.

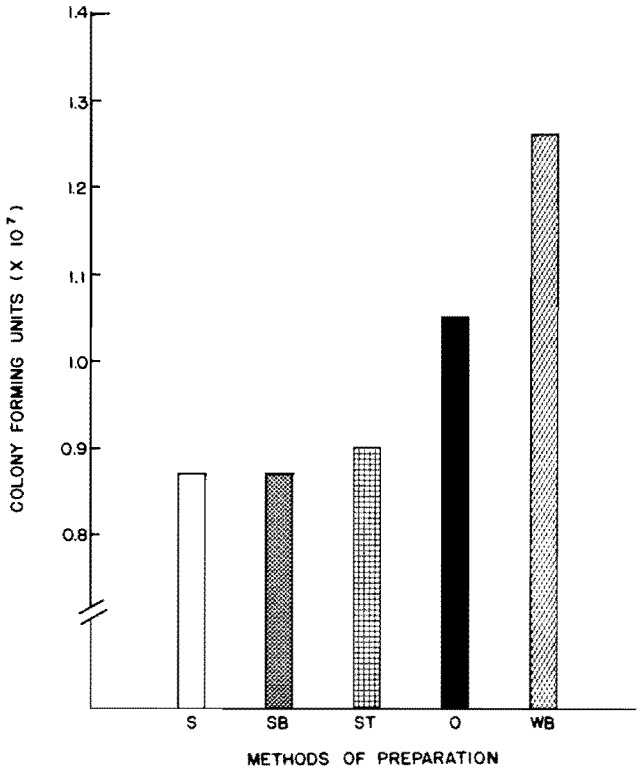

Figure 1. *Comparison of colony forming units of* Bacillus cereus *836/76 using five methods of preparation (shake (S), shake with beads (SB), Stomacher (ST), Osterizer (O), Waring blendor (WB)).*

TABLE 1. *Number of* Bacillus cereus *836/76 per chain using different methods of mixing.*

Method of mixing		Number of organisms per chain				
		1	2	3	4	5 or more
Shake	Ave. number observed[a]	11	19	8	2	2
	Percent	26	45	19	5	5
Shake with beads	Ave. number observed[a]	17	19	2	0	0
	Percent	45	50	5	0	0
Osterizer	Ave. number observed[a]	26	16	0	0	0
	Percent	62	38	0	0	0
Waring blendor	Ave. number observed[a]	33	7	0	0	0
	Percent	82.5	17.5	0	0	0
Stomacher	Ave. number observed[a]	15	17	3	0	0
	Percent	43	49	8	0	0

[a]Values are the averages of 10 tests.

The average microbial counts of ten replicates of *B. cereus* using the five methods of preparation are shown in Fig. 1. A statistical test using the two-way analysis of variance showed that the five methods were significantly different at a level of 1.0%. Using the paired T test, each individual method was tested against each of the other methods. There was no significant difference in counts obtained by either of the shaking methods or the Stomacher. Also no significant differences in counts were obtained when the mixing methods of the Waring blendor and the Osterizer were compared. There was a significant difference between counts of samples prepared with the mechanical blenders and those prepared by shaking, shaking with beads, or with the Stomacher. Results of the Waring blendor, Osterizer, and two shaking methods can be expected. The results obtained with the Stomacher are in contrast to those of Sharpe and Harshman (*4*). With the pure cultures they used, higher counts were reported when prepared in the Stomacher than when the Osterizer was used. Results of the Stomacher may support and explain the statement of Andrews and associates (*1,2*), that the Stomacher is a food-specific mixing method which provided poor counts in some foods. These results in this report show that significantly lower counts were obtained with the Stomacher when compared to the Waring blendor or Osterizer, which relates to the ability of the system to separate the cells of *B. cereus*. Some of the food samples that Andrews et al. (*1,2*) analyzed might have contained chain- or clump-forming organisms and the Stomacher was not as effective as the Osterizer in separating the organisms from each other.

The microscopic counts (Table 1) made to support the aerobic plate counts showed that there was a difference in the number of cells that were found singly and in chains. Even though the Waring blendor did not break up all of the chains to single cells, it did break up most (82%) of them. This was higher than for any of the other methods.

The aerobic counts of *S. faecalis* revealed significant differences in the methods of preparation (Fig. 2). Paired T tests showed the same results as were described for *B. cereus*. Microscopic analysis (Table 2) showed that the Osterizer and Waring blendor yielded the highest percent of cells as single cells and the shake, shake with beads, and the Stomacher methods gave the highest percent of organisms with two or more cells per chain.

The aerobic plate counts of *S. aureus* are presented in Fig. 3. Statistical analysis of these data revealed a significant difference in the five methods of preparation, similar to those reported for *B. cereus*. Microscopic counts (Table 3) also showed counts that were similar to those obtained for the other two organisms.

TABLE 2. *Number of* Streptococcus faecalis *48 per chain using different methods of mixing.*

Method of mixing		Number of organisms per chain				
		1	2	3	4	5 or more
Shake	Ave. number observed[a]	12	25	8	4	3
	Percent	23	48	15	8	6
Shake with beads	Ave. number observed[a]	17	28	9	3	6
	Percent	27	44	14	5	10
Osterizer	Ave. number observed[a]	29	16	1	0	0
	Percent	63	35	2	0	0
Waring blendor	Ave. number observed[a]	45	8	0	0	0
	Percent	85	15	0	0	0
Stomacher	Ave. number observed[a]	26	37	5	0	0
	Percent	38	54	8	0	0

[a]Values are the average of 10 tests.

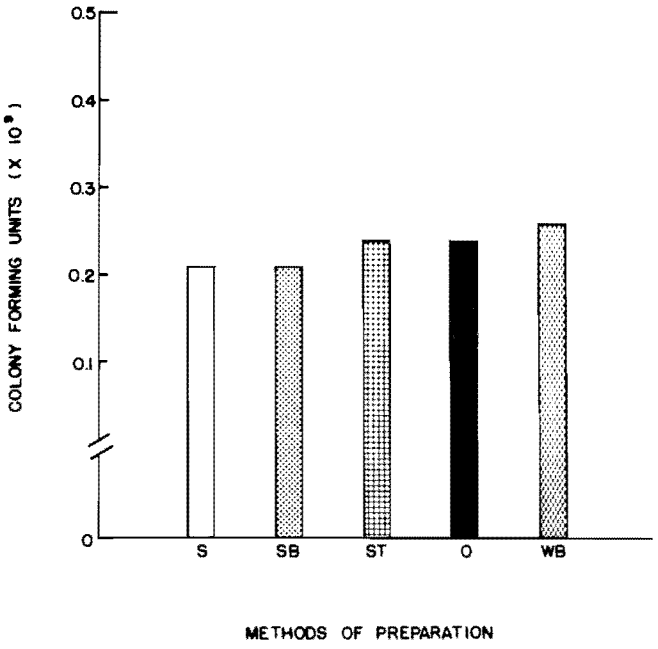

Figure 2. *Comparison of colony forming units of* Streptococcus faecalis *48 using five methods of preparation (shake (S), shake with beads (SB), Stomacher (ST), Osterizer (O), Waring blendor (WB)).*

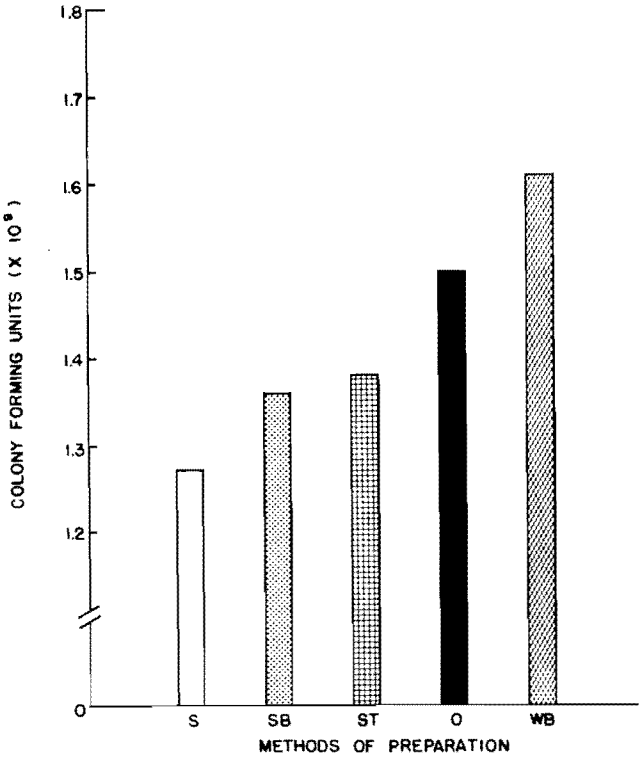

Figure 3. *Comparison of colony forming units of* Staphylococcus aureus *303 using five methods of preparation (shake (S), shake with beads (SB), Stomacher (ST), Osterizer (O), Waring blendor (WB)).*

TABLE 3. *Number of* Staphylococcus aureus *303 per clump using different methods of mixing.*

Method of mixing		Number of organisms per clump				
		1	2	3	4	5 or more
Shake	Ave. number observed[a]	17	32	11	6	5
	Percent	24	45	15.5	8.5	7
Shake with beads	Ave. number observed[a]	27	31	6	5	2
	Percent	38	43.5	8.5	7	3
Osterizer	Ave. number observed[a]	31	11	3	0	0
	Percent	69	24	7	0	0
Waring blendor	Ave. number observed[a]	38	10	0	0	0
	Percent	79	21	0	0	0
Stomacher	Ave. number observed[a]	25	44	5	0	1
	Percent	33.5	58.5	6.5	0	1.5

[a]Values are the average of 10 tests.

Y. enterocolitica was used as a control organism because it does not form chains or clumps. If all of the methods were equally adequate for preparing samples of individually grown pure cultures, then the only difference in the methods with chain formers is their ability to separate the cells. This assumption turned out to be valid. Statistical analysis showed that there were not significant differences between all methods. Figure 4 shows that all microbial counts of *Y. enterocolitica* were very similar. Microscopic values (Table 4) support this finding.

This study reveals that reliable counts were obtained using the Stomacher to prepare organisms for enumeration. However, it must be kept in mind that if chain- or clump-forming organisms are prevalent in a food sample the Stomacher might not be the system of choice when determining microbial counts.

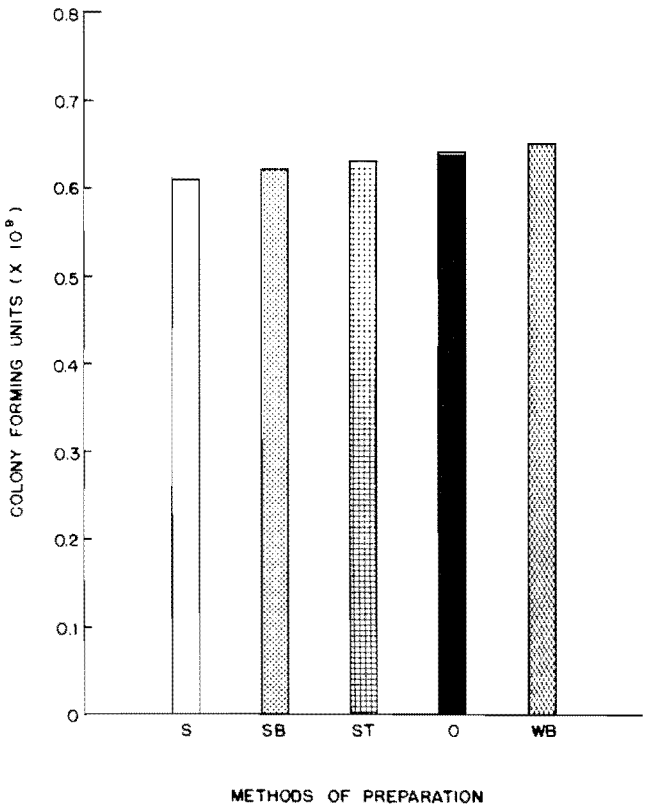

Figure 4. *Comparison of colony forming units of* Yersinia enterocolitica *975 using five methods of preparation (shake (S), shake with beads (SB), Osterizer (O), Waring blendor (WB)).*

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TABLE 4. *Number of* Yersinia enterocolitica *975 per chain using different method of mixing.*

Method of mixing		Number of organisms per chain				
		1	2	3	4	5 or more
Shake	Ave. number observed[a]	34	2	0	0	0
	Percent	94	6	0	0	0
Shake with beads	Ave. number observed[a]	29	1	0	0	0
	Percent	97	3	0	0	0
Osterizer	Ave. number observed[a]	35	0	0	0	0
	Percent	100	0	0	0	0
Waring blendor	Ave. number observed[a]	32	0	0	0	0
	Percent	100	0	0	0	0
Stomacher	Ave. number observed[a]	33	1	0	0	0
	Percent	97	3	0	0	0

[a]Values are the average of 10 tests.

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TABLE 1. *Yield (%), pH and moisture content of cottage cheese made from milk naturally contaminated with AFM_1*[a].

Trial	Yield (%)[b]	pH[c] at days					Moisture content[c] at days				
		0	3	7	10	14	0	3	7	10	14
1	14.6	4.6	4.6	4.7	4.5	4.5	81.0	81.2	80.9	79.4	80.3
2	15.8	4.6	4.4	4.5	4.5	4.6	79.9	82.3	80.1	80.6	78.0

[a]1.5 L of AFM_1-contaminated skim milk was used to prepare cheese.
[b]% yield based on a weight:weight basis.
[c]Values represent the mean of triplicate samples.

TABLE 2. *Concentration of AFM_1 in curd, whey, wash water, dressing, cheese and skim milk from which cottage cheese was made*[a].

Trial	Milk		Curd			Whey	Wash[b] water	Dressing	AFM_1 in cheese at days (μg/kg)				
	AFM_1 (μg/L)	Total AFM_1 (μg)	kg	AFM_1 (μg/kg)	Total AFM_1 (μg)	(μg/L)	(μg/L)	(μg/L)	0	3	7	10	14
1	2.33	3.50	.21	18.33	3.85	1.42	0.40	1.25	14.0	14.0	14.4	18.3	14.1
2	2.48	3.72	.23	20.48	4.71	0.92	0.47	3.73	23.1	13.1	17.5	16.6	32.1

[a]Values for AFM_1 concentrations represent the mean of duplicate samples.
[b]Average AFM_1 concentration of the combined wash waters from three washings of curd.

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