SHORT COMMUNICATIONS

Counting and Sizing of the Individual Cells of Chain-forming *Streptococcus thermophilus* with an Electric Particle Counter

By J. HEINONEN

Department of Biochemistry, University of Turku, Turku 2, Finland

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The number and volume distribution of microbes can be estimated with an electronic particle counter (Kubitschek, 1969). However, when chains of bacteria are present each chain is counted as one large particle. An attempt to overcome this difficulty was made using *Streptococcus thermophilus* KQ, a chain-forming bacterium, as a test organism. Chains of cells were fixed in formaldehyde and then subjected to ultrasonic treatment to break them down into single units.

METHODS

Streptococcus thermophilus strain KQ (Nurmikko & Kärhä, 1961) was grown and maintained as described by Heinonen (1968). Two ml. of culture was suspended in 4 ml. of formaldehyde solution (35 %, *pro analysi*, E. Merck AG, Darmstadt, Germany) and stood at room temperature for 2 to 16 h. and the chains of bacteria were broken by ultrasonic pulse (30 sec.; ultrasonic disintegrator, 20 kHz, 60 W; Medical & Scientific Equipments Ltd, London). The treated suspension was diluted with dust-free 0.14 M-NaCl solution (Natr. Chlorid Physiol. infudible, Oy Leiras Ab, Turku, Finland) to about 1×10^5 particles/ml. Particle numbers and volumes were measured by an electronic particle counter (Celloscope 302; Ab Lars Ljungberg & Co, Stockholm, Sweden) with a 30 μ m. aperture at maximal aperture current ($P = 10.0$). The limit particle volume was selected by changing the settings of pulse amplification (F) and discriminator (D). The volume counted was 0.1 ml. and this took 35 sec. Corrections were made for background counts of all solutions without culture.

RESULTS AND DISCUSSION

When chains of *Streptococcus thermophilus* KQ were broken by ultrasound without preincubation in formaldehyde solution, the number of large particles decreased during counting, although the total number of particles remained constant (Table 1). This is evidently due to shrinkage of the organisms damaged by the ultrasonic treatment; it was largely prevented by incubating the organisms in formaldehyde solution before the treatment (Table 1*a*). One h. in the formaldehyde solution was sufficient to fix the cell walls, but the incubation could be prolonged to 1 day without altering the count.

Table 1*b* illustrates the effect of short ultrasonic pulses. The total number of particles increased approximately sixfold during the first 15 sec. pulse but rose only about 20 % in the prolonged treatment, indicating that the chains were easily broken by ultrasound. If bacteria had remained in chains after the first ultrasonic pulse the total count of particles should have increased rapidly during the later pulses. Since this was not observed it appears that almost all the chains were broken down to single bacteria within 15 sec. This was

confirmed microscopically. The slight increase in the total number of particles occurring in the prolonged ultrasonic treatment may be due to the breakdown of bacterial pairs which had not formed a complete wall between the daughters after the division of the parent. Such pairs distort the count of dividing microbial populations, even if ultrasonic treatment is not used, and their numbers can only be assessed by microscopical examination (Kubitschek, 1969). The proportion of such pairs and the error caused by them is rather small in non-synchronized microbial populations.

Table 1. (a) *Prevention of the shrinkage of large organisms after ultrasonic treatment by preincubation in formaldehyde solution.* (b) *Number and volume distribution of particles in suspension of Streptococcus thermophilus KQ before and after ultrasonic pulses.*

(a) Samples from an exponential phase culture of *Streptococcus thermophilus* KQ were suspended in NaCl or formaldehyde solution for 2 h., treated by ultrasound, diluted and counted as described in Methods. Large particles are those which exceed 1.25 μm . diam.

Min. after dilution	Counts/0.1 ml. NaCl		Counts/0.1 ml. formaldehyde	
	Total	Large	Total	Large
3	10,379	2,332	10,621	2,815
10	10,829	1,337	10,490	2,768
20	10,537	1,016	10,527	2,625
40	10,745	1,020	10,720	2,578

(b) One sample, preincubated in formaldehyde solution, was subjected to ultrasonic pulses of 15 sec., diluted and counted as described in Methods. The particles with an apparent diameter larger than 1.7 μm . are recorded as multicelled particles. The volumes are expressed in arbitrary units, but one unit is approximately the average volume of a cell of *Streptococcus thermophilus* KQ.

Ultrasonic pulse (sec.)	Particles/0.1 ml.			Volumes		
	Single cell	Multi-celled	Total	Single cell	Multi-celled	Total
0	662	1,268	1,930	1,160	16,020	17,180
15	12,200	510	12,710	14,300	2,850	17,150
30	14,370	460	14,830	15,250	1,840	17,090
45	16,160	245	16,405	16,080	1,220	17,300

Table 1b shows also that the combined volume of all size fractions did not change after ultrasonic treatment, although the distribution of the particles in single-cell and multicelled fractions was altered drastically. This indicates that bacteria are not destroyed by the treatment. The particles remaining in the multicelled fraction after an ultrasonic pulse of 45 sec. were considered to be dust as their number slowly increased during prolonged treatment.

The precision of the method was tested by treating, diluting and counting 10 identical samples as described in Methods. The mean value of the results was 10,247 particles/0.1 ml. of the diluted suspension. The standard deviation (Bauer, 1960) was 256 particles, or about 2.5 % of the mean value, and the range of the values was 831 particles, or approximately 8 % of the mean value. Calibration with 1.86 μm . latex spheres indicated that most exponential phase *Streptococcus thermophilus* KQ cells had diameters between 0.9 and 1.3 μm .

This technique of sonic disruption in formaldehyde has been used in studying the correlation of enzyme formation with division in bacterial cultures (Heinonen, 1970). The applicability of the method to other microbes forming multicelled groups has not yet been tested.

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