ELECTROPHORESIS OF MOTILE BACTERIA[1]

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For over 30 years the technique of microelectrophoresis has been used to study the surface properties of bacteria and other particles of microscopic size. Since flagella undoubtedly act as cellular surface components, they would be expected to contribute to the electrophoretic behavior of the cells. Likewise, vital motion might be expected to complicate electrophoretic measurements of flagellated cells.

In preparing bacteria for the usual electrophoretic measurements, the bacterial cells are washed at least 3 times by centrifuging and resuspending in the desired buffer solution. This washing is necessary to remove the culture medium, waste products, and other constituents of the growth environment which might affect the cellular surfaces. Measurements are usually carried out within a few minutes after washing. In previous investigations (Harden and Harris, 1953; and Harris, 1953) of bacteria capable of vital motion, no motility due to flagella was observed. The objectives of this investigation were to prepare washed suspensions of motile bacteria for electrophoretic studies in such a way as to preserve their motility and to determine, if possible, the magnitude of the electrical potential necessary to overcome this vital motion.

EXPERIMENTAL METHODS

Young actively motile suspensions of the various bacteria were obtained by frequent transfers in broth and incubation at 30 C. For the electrophoretic measurements the organisms were grown 24 hr on a shaking machine in 300-ml Erlenmeyer flasks containing 100 ml broth. The composition of the medium used for growing and maintaining all cultures was: peptone (Difco), 5 g; yeast extract (Difco), 2 g; glucose, 10 g; K_2HPO_4, 1.8 g; KH_2PO_4, 0.7 g; and 1,000 ml distilled water. After incubation the cells were thrown down in an angle head centrifuge at 3,000 rpm. They were then suspended in a suitable buffer solution and recentrifuged. This was repeated 3 times. A mixture of M/100 K_2HPO_4 and M/100 KH_2PO_4 (pH 7.0) served as the buffer suspending solution for these studies. Microscopic observation immediately following washing revealed no vital motion. If the suspensions were allowed to stand at room temperature, motile cells could be seen within a few hours. The cells in most suspensions regained vigorous motility within 8 hr after washing. Washed cells refrigerated at 5 C did not regain motility during an observation period of 48 hr. For the electrophoretic measurements reported, the washed suspensions of cells were held at 30 C for 12 hr before study.

Flagella stains were made of the organisms following each step in the washing process and after electrophoresis using the procedure of Leifson (1951).

The electrophoretic assembly consisted of the usual flat-type all-glass cell, plaster of Paris–$CuSO_4$–copper electrodes and a direct current source with a variable (0–300 v dc) voltage supply (Moyer, 1936). The suspension of motile bacteria was placed in the cell and observed with the aid of a 24× objective and 10× ocular. When the current was applied the non-motile bacteria moved toward the positive pole while, with low electrical currents, the direction of movement of motile cells did not seem to be affected.

To measure the ability of motile cells to overcome the pull of an electrical potential, an individual, actively motile bacterium, moving in the opposite direction to the flow of the current and movement of the non-motile cells, was selected for observation; the current then was increased rapidly until the cell stopped and began to move in the opposite direction under the force of the electrical potential. The current necessary to reverse the direction of movement of the motile bacterium was measured with a milliammeter. After measuring the specific resistance of the suspending medium with a Wheatstone bridge

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circuit, application of Ohm's law made possible a calculation of the value of the electrical potential needed to overcome the vital motion of the bacterium.

RESULTS AND DISCUSSION

The first phases of the study dealt with measurements of the electrical potential required to stop motile organisms. As would be expected, considerable variation was found in the electrical potential necessary to counteract movement of cells in a given suspension. Marked differences in bacterial motility usually can be observed by ordinary microscopic means in any single preparation. The mean values for 20 observations with the standard error of the mean for 20 species from 11 genera are shown in table 1. It should be emphasized that these values are not considered absolute but represent an average which might be expected to vary with different experimental conditions. The potential necessary to overcome the vital motion of the cells in M/100 buffer varied from 0.66 v per cm for *Escherichia coli* to 2.14 v per cm for *Pseudomonas chlororaphis*.

TABLE 1

Electrical potential (v per cm) required to stop the movement of certain motile bacteria in M/100 phosphate buffer, pH 7.0

Aerobacter aerogenes	0.75 ± .06
Alcaligenes bookeri	1.50 ± .03
Bacillus cereus	1.31 ± 0.15
Bacillus megaterium	1.64 ± 0.32
Bacillus polymyxa	1.63 ± 0.43
Bacillus subtilis	1.89 ± 0.08
Cellulomonas rossica	1.84 ± 0.21
Erwinia carotovora	1.46 ± 0.12
Escherichia coli	0.66 ± 0.05
Proteus mirabilis	1.95 ± 0.46
Pseudomonas aeruginosa	1.88 ± 0.45
Pseudomonas chlororaphis	2.14 ± 0.65
Pseudomonas fluorescens	2.02 ± 0.43
Pseudomonas fragi	1.75 ± 0.40
Pseudomonas graveolens	1.57 ± 0.21
Pseudomonas indoloxidans	2.01 ± 0.54
Rhodospirillum rubrum	2.10 ± 0.76
Salmonella cholerasius	1.23 ± 0.37
Salmonella typhimurium	1.92 ± 0.48
Spirillum serpens	0.76 ± 0.21

Under the conditions of these experiments the application of the maximum voltages (5.0 v per cm) did not seem to injure the cells or flagella. After application of sufficiently high potential to cause all cells (both motile and non-motile) to move toward the positive electrode, the voltage could be reduced and the bacteria appeared as actively motile as before the test.

Rhodospirillum rubrum and *Spirillum serpens* were observed to exhibit a unique type of behavior. Definite evidence of polarity of the cellular surface was seen in both acetate and phosphate buffers. The cells moved toward the cathode in the usual manner, indicating the negative nature of the cellular surface charge. However, apparently one end of the spiral shaped organism possessed an electrical charge of a different magnitude than the other end. When the current was reversed the cells rotated so that when they moved in the opposite direction the same end of

TABLE 2

*Comparison of electrophoresis of flagellated bacteria rendered non-motile by washing and by formalin treatment**

Organism	Non-Motile Immediately After Final Washing	Formalinized	t-value (†)
Aerobacter aerogenes	23.8 ± 0.5	23.6 ± 0.6	0.66
Alcaligenes bookeri	18.7 ± 0.4	19.1 ± 0.4	0.80
Bacillus megaterium	21.3 ± 0.4	20.7 ± 0.6	0.71
Bacillus subtilis	8.9 ± 0.1	9.4 ± 0.3	2.56
Erwinia carotovora	23.5 ± 0.4	23.5 ± 0.3	0.00
Escherichia coli	19.1 ± 0.5	20.2 ± 0.6	1.52
Pseudomonas chlororaphis	15.2 ± 0.7	17.1 ± 0.5	2.18
Pseudomonas graveolens	8.8 ± 0.2	8.9 ± 0.3	0.43
Spirillum serpens	21.7 ± 0.6	21.2 ± 0.4	0.67

* The values given are time in seconds for cells to move 120 μ in the electrophoretic cell.

† Only values greater than 2.71 are significant at 1% level.

the bacterium was oriented toward the positive pole. This phenomenon was not observed with the other species studied, nor has any reference to such polarity been found in the literature on bacterial electrophoresis.

Flagella stains made at each step in the study revealed that washing and centrifugation did not appear to have any marked effect on retention of the flagella. The flagella on washed cells were similar in appearance to those on the cells prior to washing both as to length and number. Although the cells were non-motile, flagella were still present and presumably could contribute to the electrical properties of the cell surface. To investigate this relationship, electrophoretic measurements were made on the non-motile (but flagellated) cells immediately following centrifuging. The organisms were then allowed to remain at 30 C for several hours until they regained motility. Neutral formaldehyde was added to 0.004 per cent concentration. This stopped all vital movement of the bacteria but did not affect the flagella staining or the electrophoretic properties of the cells.

In table 2 comparisons of the time required for the bacteria to be moved 120 μ by electrophoresis are given for flagellated cells rendered non-motile by the preparatory washing treatment and by formaldehyde. Statistical analysis by the t-test showed no significant difference in the 2 treatments with 9 species from 6 genera of bacteria. These data indicate that with the usual procedure for preparing bacterial cells for electrophoresis, cells probably retain their flagella and that the electrophoretic rates determined are those of flagellated organisms.

There may be some question as to whether vital motion of bacteria has caused errors in past studies of electrophoresis. It is unlikely that such errors have arisen. Measurements are usually made soon after washing when cells would be immotile. Results in table 1 indicate the magnitude of the potential required to stop motile bacteria. Expressed in terms of current the range is from 1.0 to 4.0 ma. This is 3 to 5 times the current ordinarily used in electrophoresis. Furthermore, in the usual procedure the zero mobility of bacteria within the electrophoretic apparatus with a zero electrical potential must be ascertained for each separate experiment as a control against valve leakage, stray currents, and other factors which might introduce errors. Vital motion of bacteria would be seen in such instances.

The contribution that flagella make to the general electrical properties of the bacteria has not been determined. Certainly the magnitude of this effect would be expected to vary greatly from cells bearing a single flagellum to peritrichous cells which appear to be completely surrounded by flagella. While it is relatively easy to prepare flagella free from the cells, it is much more difficult to free the cells of flagella by treatments which would not alter the surfaces of the cell. Heating, chilling, distilled water washing, vigorous shaking with paint mixers and blendors, and tryptic digestion (used singly and in combination) failed to give satisfactory removal as indicated by flagella stains. No treatments were found to give complete removal of flagella without some accompanying cellular disintegration. Hence electrophoretic comparisons of flagellated and non-flagellated cells from the same suspension has not been possible.

SUMMARY

The electrical potential necessary to overcome the vital motion of twenty bacterial species was determined. Flagella stains and electrophoretic measurements of formalin-inactivated cells indicated that the flagella remained on bacterial cells during electrophoresis. Motile cells of *Rhodospirillum rubrum* and *Spirillum serpens* were observed to orient themselves in the electrical field, indicating differentially charged portions of the cell surface.

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