Voltage-Producing Capabilities of Escherichia coli Exposed to Sonic Radiation

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INTRODUCTION

The processes of life generated electrical fields in every organism examined with suitable and sufficiently sensitive measuring techniques (Scott, 1962). Studies of the effect of sonic energy upon bacteria were used to attack problems of classification of bacteria and to isolate specific components of these organisms by Lamanna and Mallette (1959).

The purpose of this research was to identify the voltage-producing capabilities, if any, of *Escherichia coli*, when exposed to various frequencies of sound to determine if there are relationships between growth and voltage production.

EXPERIMENTAL PROCEDURE AND MATERIALS

A pure agar plate culture of the rod-shaped bacterium, *E. coli*, was obtained from a local medical institution. A bacterial transfer was then made to a sterile nutrient agar test-tube slant and stored at 10 C. Additional transfers were made at two-week intervals from the most recently cultured agar slant to insure proper growth characteristics.

Commercially prepared, dehydrated nutrient broth (Difco), containing a beef extract and simple proteins, was mixed with the proper proportions of distilled water to serve as the medium supporting the growth of *E. coli*. This medium was sterilized as soon as possible after preparation in order to eliminate rapid multiplication of contaminating organisms which could alter the composition. Nutrient agar was prepared by adding dehydrated Bacto-agar to nutrient broth, treated as above, and poured aseptically into sterile petri dishes.

Sterile technique was maintained throughout experimentation. All bacterial transfers were conducted in a room free from drafts. The working surface was disinfected by washing it down with Zephiran chloride. Glassware containing media was plugged with nonabsorbent cotton, capped with two layers of brown wrapping paper, and secured by a rubber band to prevent contamination. Sterilization was accomplished by autoclaving at 121 C for 15 min.

A 50-ml medicine glass was chosen as the voltage test chamber to contain the medium in which *E. coli* would thrive during the electrical measurement. An electrode system, consisting of two nickel wire screens soldered to stiffened, twisted, copper wiring connected to terminals on top of an aluminum cover, was devised. This electrical circuit was insulated from the aluminum cover and periodically checked against a short that might result from moisture condensation produced during autoclaving.

The electrodes were immersed in the nutrient broth and connected to a Heath servo recorder, Model EUW-20A, which measured and recorded on graph paper the electrical potential of the medium in millivolts.

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E. coli was transferred from the agar slant to a 10-ml test-tube of sterilized nutrient broth by means of an inoculating loop. This culture was incubated at 37 C for 18 hr in order to allow the organisms to establish a highly reproductive phase. Approximately 1 to 2 ml of actively growing culture was poured into the sterile nutrient broth of the voltage test chamber. This new culture was then incubated at 37 C to begin data gathering of bacterial voltage.

A viable bacterial population count was conducted at intervals so that various voltage outputs could be compared to the number of living bacteria producing certain voltages. A 0.01 ml-inoculating loop was flamed and allowed to cool. Using this loop 0.01 ml of bacterial medium was transferred from the voltage test chamber to 10 ml of sterile nutrient broth. If the bacterial population was expected to be high, a second transfer was made. The purpose of this dilution was to reduce the number of actual bacteria to a number which could be mathematically calculated in direct proportion. A 0.01 ml-loop transfer was made from the dilution tube to the surface of sterile nutrient agar in a petri dish. Assuming that each bacterium in the sample would reproduce and develop into a colony, then a count of the colonies would represent the number of bacteria in the sample.[2] After the petri dish had been incubated for 24 hr at 37 C, a colony count was made to determine the number of living bacteria per ml in the medicine glass at the time of the population counting.

E. coli was introduced into the voltage test chamber as described above. Several hours after the inoculation, sonic radiation was applied to the immediate environment of the culture being tested. A variable frequency oscillator and a permanent magnet speaker served as the signal source. The intensity of sound was kept constant. The two frequencies used were 8,000 and 10,000 cps. Population counts provided a method of determining the number of viable bacteria producing voltage.

Nutrient broth sterilized, but not inoculated, was similarly subjected to incubation and voltage tests and served as a control.

The voltages from a series of sterile nutrient broth control tests indicated a slight electrical potential of generally 0.5 mv. Therefore, in Tables I and II, 0.5 mv was subtracted from the voltage measurements to correct for voltage caused by undetermined physical factors.

Results and Discussion

E. coli cultures indicated a definite voltage increase after several hours of growth. The voltage increased steadily to a maximum and a constant voltage usually persisted for about 2 hr before a slow decrease appeared. A viable bacterial population count at various stages revealed a definite increase in population coinciding with an increase in voltage as indicated in Table I. Voltage production increased very gradually in cultures of less than 10^6 living bacterial cells per ml. A rapid increase in voltage was then observed with relatively slow increase in population.

E. coli exposed to sonic energy showed a higher voltage production than those not exposed (Table II). Bacteria exposed to 8,000 cps of sonic radiation produced slightly more voltage than cultures not so exposed. Cultures exposed to 10,000 cps of sound produced considerably more voltage than those subjected to 8,000 cps.

Conclusion

E. coli cultures exhibit a voltage production increase coinciding with an increase in population, indicating that the number of living cells and

[2]Theoretically this may not always be true for variable factors; however, this method is reasonably practical for most purposes.

TABLE I. RELATION OF VOLTAGE TO BACTERIAL COUNT

Number of viable bacterial cells/ml	Voltage, mv.
9.70×10^{4}	0.500
7.20×10^{6}	3.750
2.43×10^{7}	1.000
6.83×10^{7}	1.000
1.00×10^{8}	2.750
5.00×10^{8}	5.000
7.00×10^{8}	8.000
9.00×10^{8}	6.250
3.66×10^{10}	11.500

TABLE II. RELATION OF BACTERIAL NUMBERS TO FREQUENCY OF SONIC RADIATION AND TO VOLTAGE.

Sonic radiation Frequency cps	Number of viable bacterial cells/ml	Voltage, mv
8,000	1.68×10^{8}	7.500
10,000	1.00×10^{8}	11.000
10,000	6.00×10^{8}	13.750
10,000	5.00×10^{9}	13.750

perhaps certain growth factors directly influence the amount of voltage produced. Cultures of this organism exposed to sonic energy are capable of producing more voltage. Those bacteria subjected to higher frequencies of sound produce more voltage, possibly suggesting a useful technique in the classification of bacteria according to their electrical response to different frequencies of sound stimuli. Further investigations of bacterial response to a wider frequency range would be desirable to determine if a peak voltage-production, response curve, caused by a maximum frequency endurance exists. The effect of a constant frequency accompanied by varied sound intensities might induce elaborative speculation. Similar evaluations of other species of bacteria could constitute interesting additional research.

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