

Investigation on the Influence of Aerial-Earth Circuit on the Biological Activities.

III. Mechanism of the influence on *Azotobacter* *chroococcum* as to its electrophoresis.*

By

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Two preceding reports¹⁾ dealt with the influence of the aerial-earth circuit on *Azotobacter chroococcum* as to its growth and fixation of nitrogen, and the static potential respectively. Further enquiry was made on the mechanism of the influence regarding the electrophoresis or electrokinetic potential of *Azotobacter chroococcum* and the results are presented in this paper.

Experimental procedure :— The same strain of *Azotobacter chroococcum* as used in the preceding experiments was taken and grown for three days in ASHBY'S liquid medium, with and without an addition of calcium carbonate, and in both closed and open circuit. At the end of period, a clear portion of culture was pipetted out and the electrophoresis was determined in the following manner :

NORTHRUP-KUNITZ cell²⁾, as shown photographically (Fig. I), was used in combination with Leitz microscope (Plate VIII) (M) having 10X Huygenian ocular ; ocular micrometer ; achromatic objective 20X, 9 mm. focus, 0.45 N. A., and "Stella" lamp (L) of 100 Watts. The ocular micrometer was standardized against the stage micrometer, and the time of migration of the organism over a known divisions equal to 100μ on the ocular micrometer was taken by means of a stopwatch. A source of direct current of 100 volts was used thru a proper resistance (R) so that any desired voltage can be drawn and indicated by a voltmeter (V) which is connected up with a mercury commutator (K) by which the poles can be changed. The cell (C) of which description is given in detail in Fig. I, is placed on the microscopic stage and connected to the electrode tubes. The electrode tubes (T₁ & T₂) are filled with saturated zinc sulfate and the zinc electrodes are connected to a source of potential. The stopcocks (S₁ & S₂) are

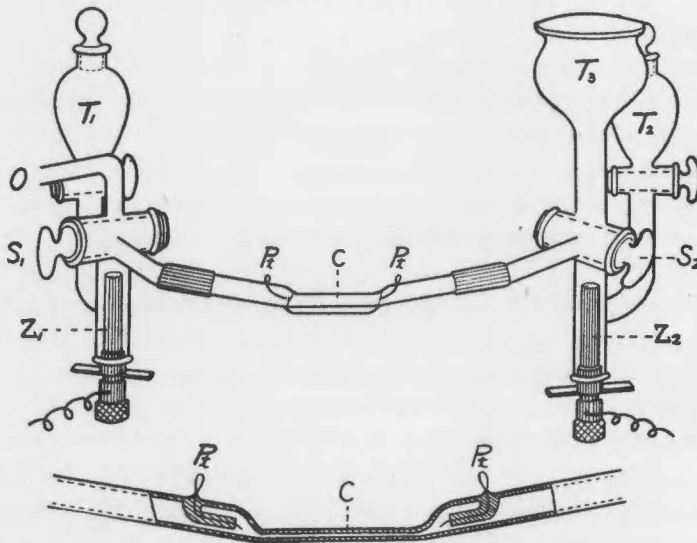
* Reported in : The Proceeding of the Imperial Academy, IX, No. 10 : 592—595, 1933.

1.) A. ITANO, Proc. Imp. Academy (Japan), IX, 47—50 ; 309—312, 133.

2.) J. H. NORTHRUP and M. KUNITZ, J. Gen. Physiol., 7 : 729, 1925.

turned so that the cell is connected to the overflow (O), and wash out any excess of zinc sulfate with distilled water, applying suction at the overflow tube. After the cell is rinsed out with a small amount of sample, it is filled thru tube (T₃) carefully avoiding any air bubble. The stopcocks (S₁ & S₂) are turned so that the cell is connected with zinc sulfate; the circuit is now closed, and the velocity of migration of organism is measured at the different depth of the cell; reverse the current and the same observation is made to ascertain the charge of organism, by means of a commutator (K). The platinum wires (Pt) placed in the cell are used to determine the potential gradient of the cell itself, which was found to be 4.3 volts per cm. in this case.

Fig. I.
Northrup-Kunitz Cell.



The results are given in the following table :

(See Table I on next page.)

Table I indicates that *Azotobacter chroococcum* carries the negative charge and migrates toward the anode. The velocity of migration varies somewhat by the medium, with or without CaCO₃. That is slightly faster in the medium to which no CaCO₃ added. In both of these media, the closed circuit retards the rate of migration.

Discussion :— *Azotobacter chroococcum* carries the negative charge and the velocity of migration is depressed by closing the circuit in both media used, but how this depression effect the physiological activity of the organism remains to be interpreted.

Table I.
Rate of Migration of *Azotobacter chroococcum*.

Treatments.	Depth* (mm.)	Time to go 100 μ	Average time (seconds.)	Average μ per (seconds.)	
-CaCO ₃ Control.	0.10	-6.5; -7.0	-6.63	-15.00	
		-8.0; -5.0			-6.11
	0.20	-5.7; -5.5	-5.58	-17.94	
		-5.8; -5.3			
	Circuited.	0.10	-7.3; -8.2	-8.88	-11.27
			-11.0; -9.0		
0.20		-6.2; -6.8	-6.88	-14.55	
		-8.0; -6.5			
+CaCO ₃ Control.	0.10	-8.2; -7.8	-8.05	-12.41	
		-7.2; -9.0			-6.72
	0.20	-5.2; 5.3	-5.38	-18.60	
		-5.5; 5.5			
	Circuited.	0.10	-9.3; -10.5	-10.75	-9.30
			-11.5; -9.0		
0.20		-5.5; -5.8	-5.83	-17.17	
		-6.2; -5.8			

Notes: (-) sign indicates the apparent sign of charge of *Azotobacter* and means to migrate toward the anode; * distance from cover-glass at which determination was made.

Summary:— The mechanism of the influence of aerial-earth circuit on *Azotobacter chroococcum* as to its electrophoresis was investigated and the following summary may be made:

1. *Azotobacter chroococcum* carries the negative charge and migrate toward the anode in the electric field.
2. The presence of excess calcium salt in the medium depresses the velocity of migration slightly.
3. In the closed circuit, the velocity of migration is depressed in both media, with or without CaCO₃.

PLATE VIII

Entire Apparatus.

