FORM 4587

OCT 31 1960

Lederberg

Henry Ford Hospital

DETROIT 2, MICHIGAN

Dr. J. Lederberg
Department of Genetics
Stanford University
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Palo Alto, California

October 20 1960

Dear Doctor Lederberg:

While working with recombination between "L forms" I obtained some odd results. I am enclosing some of my results so that you may look them over and criticize if you so desire.

I used two strains of E. coli K-12 which were previously marked as follows:
W-1177 (F-) Meth+, Threo -, Leuc -, B-1$^-$, Lact$^-$, Malt$^-$, Man$^-$, Xyl$^-$, Arab$^-$

Sent W1845 11/7/60 →

W-1922 (H fr) Meth$^-$, Threo$^+$, Leuc$^+$, B-1$^+$, Lact$^+$, Malt$^+$, Man$^+$, Xyl$^+$, Arab$^+$

Recombination was produced by spotting each strain in a different position within a petri dish so as not to be in contact with each other. Penicillin was added, then molten sucrose agar, and the dish agitated to insure thorough mixing. A mixture of "L forms" of both strains was produced by this method. As the "L forms" increased in size they met to produce conjugation. After 24 hours of incubation penicillinase was added and the "L forms" reverted to normal colonies. The mixture was then homogenized, diluted and streaked on minimal agar. Next, replica plates were made on synthetic EMB minimal agar for each non-selective fermentation marker. As a control, normal colonies were produced in exactly the same manner as the "L forms", with the exception that penicillin was omitted.

The results of this work are summarized in enclosed table V. Since the maltose marker appeared positive more frequently than any of the other non-selective markers, I assumed that it was the first gene to be transferred during conjugation. Do you feel that this assumption is valid? Recombinant colonies produced by methods other than "L form" recombination show very similar results, as seen on table XI.

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As further proof that recombination occurs between "L forms", I produced a mixture of the two different strains of "L forms" but did not treat them with penicillinase. The mixture was homogenized, 1.0 ml. of the mixture placed in a petri dish, penicillin added, and molten sucrose minimal agar added. After two to three days viable single "L forms" were cut out of the agar and placed in individual minimal sucrose broth tubes. The "L forms" were allowed to revert to normal colonies and tested on replica plates of synthetic EMB. Only eight such "L forms" were tested. Of the eight, seven produced cloudy growth in the sucrose minimal broth after about a week and two of these produced recombinant colonies on EMB minimal agar plates.

I would be very interested to know if you feel that this work indicates that "L form" recombination does occur and if the evidence indicates that the maltose gene enters first during conjugation.

I also wish to thank you for your letter telling me the origin and characteristics of strain W-1922. Unfortunately, I did not use the same markers and cannot apply this as proof that my work is correct.

Thank you again for your aid, and I hope to hear any criticism which you may have to offer on this work.

Very respectfully yours,

Robert M. Wilson

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