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DEPARTMENT OF ZOÖLOGY

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Dr. Joshua Lederberg
Department of Genetics
University of Wisconsin
Madison, Wisconsin

Dear Dr. Lederberg:

Unfortunately, your recent visit to our laboratory was of such short duration and our conversation was so inadequate, that I have decided to take the liberty of writing to you. I should like to tell you of my experiences with the citrate-utilizing (C+) mutant of E.coli (strain K12) up to the present time, and of what experiments I intend to do in the near future.

As I told you, my initial attempts to obtain a C+ mutant of E. coli K12 were fruitless, whether I tried heavy inocula into standing citrate media, or irradiation followed by plating on citrate agar, or inoculating into citrate plus limiting glucose medium (the latter method to encourage enough C- growth to make probable a C+ mutation, which might then overgrow the culture). It was not until I inoculated C- cells from standing glucose plus G&T salts medium into rolled-aerated citrate medium that I obtained adaptation to citrate-utilization. The adaptation time depends upon the medium employed: adaptation occurs between 24 and 48 hours with Koser's citrate; it takes somewhat longer (about 12 hours more) and growth reaches a lower final level in citrate plus G&T salts medium. The presence of glucose may prevent adaptation (in 2 of 15 cases).

The C+ mutants are small, thin, Gram-negative rods, morphologically similar to E. coli and A. aerogenes. They give a non-lactose-fermenting reaction on Endo's agar, however, and growth is very feeble on this medium, moreover. A complete testing of the C+ mutants for coliform characteristics is in progress. I have found that 0.25% citrate plus G&T salts medium gives maximal growth under aerated conditions, but that growth is much slower in this medium than in Koser's citrate. I am presently investigating the possible influence of the trace elements used in G&T salts in depressing the C+ growth rate. (I already know that buffering action in Koser's citrate is no better than in G&T salts-citrate medium.)

In preliminary experiments, I have learned that C+ growth in citrate is characterized by (1) increase in pH proportional to amount of growth, (2) autolysis immediately following end of logarithmic phase of growth; the decrease in optical density of the culture is correlated with the maximal growth level: the greater the maximal growth, the greater the decrease in optical density, (3) a more or less stationary phase following the abrupt autolytic period, (4) production of gummy material in the late stationary phase. If viable C+ cells are desired from liquid culture, they must be taken from the logarithmic phase of growth. The longer the culture remains in the stationary phase, the fewer viable C+ cells will be obtained in

samples taken from it. Furthermore, inocula from stationary phase-adapted cultures into citrate media will result in either no growth or delayed growth. The possibility of C- mutants predominating in the stationary phase and which inhibit C+ cells will be investigated.

The C+ mutants obtained thus far can utilize citrate but cannot utilize glucose as a source of carbon. Howver, a recent case of a "C+ G-" mutant adapting to glucose-utilization has been noted, and the biochemical characteristics of the mutant parent and the "adapted" cells will be studied. Wild-type E. coli K12 is, of course, citrate-negative and glucose-positive. Furthermore, despite the fact that C+ mutants are obtained only from aerated citrate cultures of C-, C+ can utilize citrate in standing cultures and in aerated cultures. Final level and rate of growth of C+ in citrate are higher under aerated conditions, however.

An interesting case of what I call "plate selection" has been observed in mixing C- and C+ cells on glucose agar: C- colonies prevent C+ colonies from appearing after layering with citrate agar. If mixed on citrate agar, C+ colonies appear first; then after layering with glucose agar, complete C- recovery is obtained.

I intend to work this "citrate-utilization" locus for all that it is worth, and I hope that it will merit being the major part of a Ph. D. dissertation. My planned experiments include:

(1) doing a variance analysis on C- cultures plated on citrate, in the expectation that citrate does not induce the C- to C+ mutation but acts only to select those C+ mutants regularly present in C- cultures;

(2) Mapping the "C" locus, to demonstrate the genic basis of citrate-utilization;

(3) checking the utilizability of all the obtainable substrates involved in carbohydrate metabolism in C- and in C+, with subsequent experiments to determine the nature of the action of the "c" locus. (If a trace element is found to be responsible for the decreased growth rate of C+ in citrate, it may provide a clue as to the "sensitive" reactions in the citrate metabolism of C+);

(4) investigating all the possible angles of C- and C+ interaction and selection in standing and aerated, in glucose, citrate and glucose-citrate cultures.

Planned also are experiments to obtain C- mutants of A. aerogenes by the penicillin method.

At first a sub-project in the investigation of the mutability of the biochemical characteristics which distinguish E. coli from A. aerogenes, the investigation of citrate-utilization is becoming a full-time project in itself. I would appreciate hearing from you concerning suggestions or questions about my work. I would especially like to hear if your non-glucose-fermenters are citrate-utilizers, and what some of the characteristics of your non-glucose fermenters are, and how obtained.

I hope that the "C+ G-" culture I gave you will be useful to you. I should have remembered to remark on the "gumminess" or "stickiness" of the C+ growth on either citrate agar or nutrient agar slants. This "gumminess" increases with prolonged incubation of freshly-prepared slants, and makes

transfers very difficult. I suggest making stock transfers at least once a week, even if stocks are refrigerated.

I expect soon to publish a note on the preliminary experiments and early findings, and I shall be glad to send you a reprint of it. In connection with reprints, may I remind you of my desire to possess reprints of the publications of your extremely interesting and significant work?

Sincerely yours,

Arnold W. Ravin

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