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\textsuperscript{+}) 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\textsuperscript{+} 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\textsuperscript{-} growth to make probable a C\textsuperscript{+} mutation, which might then overgrow the culture). It was not until I inoculated C\textsuperscript{-} 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\textsuperscript{+} 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\textsuperscript{+} 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\textsuperscript{+} 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\textsuperscript{+} 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\textsuperscript{+} 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\textsuperscript{+} cells will be obtained in
samples taken from it. Furthermore, inocula from stationary phase-
applied 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. However, a recent case of a "C+ C-"
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 genetic basis of
citrate-utilization;
(3) checking the utilizable 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+ inter-
action 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 bio-
chemical 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 character-
istics of your non-glucose fermenters are, and how obtained.

I hope that the "C+ C-" 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