FEDERAL SECURITY AGENCY
U. S. PUBLIC HEALTH SERVICE

IN REPLYING, ADDRESS THE

Tbc. Research Laboratory,
411 East 69th St., New York 21, N. Y.

December 1, 1949.

Dr. Joshua Lederberg,
Department of Genetics,
University of Wisconsin,
College of Agriculture,
Madison 6, Wisconsin.

Dear Joshua:

I should have answered your letter of November 16th earlier, but I have been trying to see whether I could plan to help solve this nuisance problem that my earlier observations have raised. I obtained the results described (very little recombination in plates) in three experiments, and had no experiments which yielded good recombination in plates. I did send you the composition of the complete media; one of them contained casein hydrolysate (NZ Case-Sheffield), and the others contained Difco yeast extract. It seems very unlikely that the composition of the complete medium has anything to do with the case; I would rather look further into the role of the composition of the minimal medium in the plate. Recently Gordon Allen has been getting perfectly good plate recombination in minimal medium here, but we meanwhile have somewhat changed the composition of our minimal medium. My suggestion would be that I take the responsibility for retesting the amount of recombination taking place on our old minimal medium and on variations of it.

You might be interested in knowing the composition of our new minimal medium, which represents the result of quite a ~~good~~ bit of systematic work. It consists of the following:

K_2HPO_4	7 gm.
KH_2PO_4	2 gm.
Na_3citrate $\cdot 5H_2O$	0.5 gm.
$MgSO_4 \cdot 7H_2O$	0.1 gm.
$(NH_4)_2SO_4$	1 gm.
Glucose (autoclaved separately)	2 gm.
Distilled H_2O	1000 ml.

This medium is so highly buffered that exhaustion of the glucose rather than lowering of the pH is the limiting factor with 0.2% glucose, and it yields much heavier turbidity in tubes than did the old medium. By increasing the glucose to 0.5% still further increase in turbidity can be obtained, but a growth limiting pH (4.5 to 5) is reached unless still more buffer is used. It seems desirable on general principle to stock our bugs in a medium in which they have not reached growth limiting acidity. I might further mention that the sodium and chloride ions were found to be without detectable effect; the lowering of pH from 7.6 to 7 permits more regular initiation of growth with minimal inocula; the lactate was not found to prevent the development of limiting acidity, and it had no other obvious beneficial effect so we dropped it. The citrate does not serve as a carbon source for growth, but it does improve the initiation of growth by small inocula, presumably acting as an ion binder. Finally, this new medium has eliminated the irregular appearance of mottled agar plates.

Our several mutants that were considered to require an unknown vitamin in yeast extract, and which responded poorly to cozymase, have turned out to respond to purine plus thiamine. Esther told me you have several Coenzyme I requiring mutants. I would be curious to know whether they similarly respond to adenine plus thiamine. If Gordon tells me the biotin plus methionine strain you sent seems to grow on methionine alone in our medium. Since our biotin-less mutant does not grow on this medium, this growth cannot be accounted for by impurity in the medium. I think we will impose a second deficiency on this bug before setting up more recombination experiments.

Sincerely,

Bernard D. Davis

BDD/hl