

LONG ISLAND BIOLOGICAL ASSOCIATION

COLD SPRING HARBOR, LONG ISLAND

THE BIOLOGICAL LABORATORY

November 15, 1953

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Dear Josh:

I'm afraid I must plead distraction rather than difficulty.

I can offer no ready explanation of the discrepancy in our findings on the motility of line 28. However, I am sending complete data on motility on all lines, except special information on K-12. More information on other subjects will follow.

The following comments about the line 28 situation may be helpful:

1. These tests were made on 0.4% agar plus gelatin. Was your medium different?
2. I confess to ambiguity in the report you have. As you will see from the data, two of the positive mot tests were by microscopic examination, and one of these was on the same slant isolate which was tested on soft agar.
3. As to the identity of the organism I used, the tests were made 2/22/52 from stock slants in the refig. at that time. At about the same time, line 28 was listed in the WG stock book as W1258 from Cavalli, lac⁻ mal⁻.
4. Strangely enough, as the data show, line 28 was the fastest swarmer on motag. Altho I don't have a record of the fact in my notes, I remember that the fastest swarmer (i.e. line 28) had moved out very rapidly in the form of a faint, expanding ring. There was no macroscopic evidence of cells behind the moving front. This was one of the most extreme instances of disparity between vigor of swarming on motag and % motility in broth.

I'll try to summarize as briefly as possible, what I've done and plan to do here.

1. B x K-12 crosses are being studied with considerable success in collaboration with Bryson and Szybalski. A little recent history is desirable here, I think, in view of the fact that I have recently obtained the impression that Cavalli and/or cohorts are making something of a project of the same cross. I knew of course that the cross was possible before I arrived. Szybalski tarried in Italy to get the straight scoop from Cavalli. He returned with the discouraging information that the cross worked only with one or two strains of B and there, recombinants were too infrequent to be of much use. Bryson had been especially sanguine about the possibility of using the inter-strain cross to analyze mutants of B (cf. below) and was consequently esp. disappointed. We began to prepare stocks for analogous intra-K-12 study. Meanwhile, I took a flyer and tried the cross: B/r (proto mal-) x 58-161 (M- mal⁻) on EMS-mal. Whereas, the B/r control plates exhibited a solid sheet of mal- growth and eventually mal-, fast-growing papillae, the cross plate contained 300-500 mal⁻ prototroph papillae which bred true. These same results have now been repeated several times and the following crosses have also been fertile to almost the same degree: B x 58-161 on EMS-mal, B/r x 58-161 on synthetic medium with maltose as sole carbon source and no dyes, B/r lac- x 58-161 on EMS-lac, several B auxotrophs x 58-161 on DNA, and W1177F⁻ x B/r on EMS-mal (male not allelic apparently).

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The prototrophs appearing in all of these crosses have not been followed up carefully as yet. I have information on a number of obvious next questions, but not enough to hazard an opinion as yet. Suffice it to say that a transductive mechanism has not been ruled out. Incidentally, I am filtering furtively. Very little if any segregation of unselected markers has been observed and one filtrate of 58-161 may be effective. I'll let you know immediately if this thing becomes too Morsian. Meanwhile, I'd be interested in your comment. I think that the W1177 x B/r and auxo x auxo crosses are critical, but the prototrophs ~~have~~ are only now being verified for these. The thing that puzzles and worries me is that whereas Cavalli got only low frequencies of recombination, I am getting either high (relatively) frequencies or nothing at all, depending on the cross. Despite my assurances, I'm sure Waclaw thinks I'm surreptitiously using a top-secret, high-power Hfr.

This lab is a natural for B x K-12 crosses of course. Specifically, the following studies are projected. Bryson, as you know, has a large number of induced mutants to T1 resistance which simultaneously underwent auxotrophic mutation. He wants to see if the associated changes are genetically separable. If the appropriate B x K-12 crosses can be made, the problem can be attacked directly. However, if this does not pan out, intra-K-12 crosses are planned. He is preparing, he hopes, analogous double mutants in 58-161. To study these, we will need a crossable stock that is T1 sensitive, preferably the Y10 line that is best marked.

Szybalski is interested in ~~stopping~~ knowing whether mutagen stable and mutagen unstable deficiencies for the same nutritional factor are allelic. Several of the pertinent stocks seem to be fertile with 58-161. We would therefore be interested in any K-12 stocks which are deficient for the same factors: histidine, tryptophane, and proline. I don't know how many independent mutants of these types you have, or how much trouble it would be for you to dig them out. I suspect that it would be just about as easy for us to isolate some ourselves.

For several purposes, e.g. the experiments above it would be nice to cross B x B. What do you think of the following screening technique for F⁻ B? Assume that B is infectable by F, but at an extremely low frequency. Then, mix W1177F⁻ (T-L-B₁-) with B (T-X-) and B (Y-L-) and plate massively on synthetic. If the T and L mutations are allelic and X and Y are two different deficiencies, only progeny of the B x B cross would be recoverable, and these in the event of the rare infection of a B with F. F⁻ persistence could then be checked by an SRP cross.

2. The disinfection work has yielded little that is new. I repeated the experiment with one 58-161 isolate and have a store of isolations made during the course of the experiment which I plan to examine carefully. I'm also repeating the experiment in 0.4% agar minus gelatin. I hope to be able to run several lines through Demerec's chemostat soon. I'm glad to hear that the frequency of disinfection was so high in your experiments. I suppose it would be advisable to test one isolate from each successful disinfection for re-infectibility. I think it would also be well to verify my observation that reinfected cells are still highly motile.

3. Nothing has been done, as yet, with 58-278. Demerec seems quite

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excited about the problem. Treffers will be here soon to give a seminar, so I will be able to discuss the situation with him at that time. Meanwhile, I would appreciate a culture of 58-278.

4. I have been trying to define the "step-wise" motility mutants and am a little discouraged by the apparent complexity of the system. It seems that maximal adaptation to gelatin motag does not adapt a culture maximally to 0.4% agar minus gelatin. Adaptation to the latter doesn't adapt to 0.5% agar minus gelatin. A series of apparently mutant swarms appear upon such seemingly trivial changes in medium composition.

5. Bryson is pressed by Detrick to study genetic factors involved in resistance to lyophilization, and has asked me to do something about the problem. Bryson feels that there are definite strain differences in resistance to storage on dry beads and that this may parallel differences in response to lyophilization. He has had a lot of trouble with K-12 on beads. He suspects this may have something to do with clumping. I haven't really thought very much about the problem yet, but would appreciate any suggestions you might have from your experience with K-12.

I've had to practically set up a new lab here, which is a time-consuming but worth-while experience. Have had no trouble with EM media. Bryson seems to be a very nice person to work with, although his procrastination at times approaches mine in magnitude. New this year is Joe Hepler, who has been with Tatum. A biochemist, he is set on finding lysogenicity in Mycobacteria. He's paid by TB. Witkin was asking about use of the penicillin technique for selecting fermentative mutants. I couldn't think of any objection, except that it was about as easy to select on indicator medium. She claimed that mutant growth was effectively nil in the system she was interested in. I've seen Norton several times; he has a lot of unanalyzed kinetic data. It seems that genes are incorporated into phage at the last minute. His tracer experiments with Garen were inconclusive.

Linda and Steve are fine, altho Steve fell down a flight of 22 stairs today and then fell 3 feet off a chair and gashed his chin wide open. We are living in spoiling luxury in a 4-bedroom house in Port Washington. We pay only \$100 per month, heat furnished - friends of the family. The honeymoon ends in March however. The trip east was uneventful except for a stop at Hawk Mountain, Pa., where we watched hundreds of hawks migrating south along the Kitatinny Ridge.

Best regards to Esther and the rest of the lab.

Yours,

Dave

Dave Skaar

(over)

In summary, the following strains would be appreciated:

1. a Y10 strain that is V_1^S and has the most markers possible
2. 58-278
3. W518 to test for λ in $\theta \times K-12$ prototrophs.
4. W677 for crossing with 58-278 + derivatives

The following would be appreciated but are not necessary:

1. W1817
2. W1607 ($M^+ F^+$) } as standard testers (I have comparable stocks)
3. Any separate histidine⁻
tryptophan⁻
proline⁻

P.S. I'm enclosing the retirement form. I already sent one of these to the retirement office.