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PDS

F-disinfection experiments involving stocks other than 58-161

K-12

Details on experiment #5320 are not very complete. K-12 was subcultured massively from slant to gelatin motag. Swarms appeared as readily as with 58-161. After 12 passages, highly motile K-12 was crossed with W1817 and W1177 via SRP. Cultures were not mixed prior to plating on streptomycin. Prototroph recombinants appeared in neither cross, although recombinants did appear in simultaneous cross of K-12 (non-motile) x W1177, but not in K-12 (non-motile) by W1817. After 16 passages, streaked out and single colony used to initiate W2285.

58-278

In experiment #5334, considerable difficulty was had in obtaining motile variants of 58-278 and S^r derivatives of same. This was in part due to inadvertant use of high-% agar. However, one fairly motile culture was still F/ (passage number, confused by the preceding difficulty). Even upon reinitiation of passage on known 0.4% agar, however, motility was obtained with difficulty and motile strains were not obtained which seemed appropriate for testing F-status.

W1817

As observed elsewhere, W1817 and W1177 swarm less readily than 58-161 and do not appear to reach as high a degree of motility. Further, growth aberrations of W1177 appeared upon repeated passage on motag. These factors may be related to the inability to obtain F- W1817 by swarming, so far (tho, cf. W2202).

Exp.#5301: Subculture from broth to motag. At 24 hr. swarm radius (sr) 15 mm. Subculture. At 66 hr. new swarm with sr of 11 mm. At 90 hr. sr 21 mm. Subculture. At 114 hr. subculture. At 117 hr. sr 1.5 mm. At 139 sr 21 mm. Subculture. At 158 hr sr 20. At 160 hr sr 23. At 169 hr sr 30. Subculture. At 176 hr sr 2. At 177 hr sr 2. At 191 hr sr 15. Subculture. At 203 hr sr 24. At about this time, crosses were attempted among W1817, W1177, 58-161 and W1607 (motile and non-motile of each). Although the F- nature of motile 58-161 was first detected here no significant effect on the fertility of W1817-motile could be seen. The prototroph yield was slightly lower in all crosses, including that x58-161 F/.

Exp.#5331: After 23 passages of same line described above, it was apparently as motile as it would be, though still less than had been observed for 58-161. Crosses with 58-161 and W1607 gave 2000 and 1000 prototrophs resp., whereas simult. crosses of non-motile W1817 gave only 1000 and 100 protos. Thus, if anything, fertility was enhanced. This motile W1817 slanted as S8.

Exp.#5386: S8 reexamined, after almost 2 months. Still motile; now gave exactly comparable crosses with 58-161, W1607, and 58-161 F- as did W1817 non-motile. So if enhanced fertility was real, no persistence.

It appears that this line was the only one that I followed exhaustively; perhaps a repetition starting with another isolate would be successful.

W2202

Exp.#5337: considerable difficulty was had in getting this TLB_1 strain to swatm. By the eighth passage, however, it moved 8 mm in the first 7 hours and appeared to be incapable of crossing with W1607 in a rather unsatisfactory test. After 10 passages motility was somewhat greater and a satisfactory set of crosses revealed it to be F- vis-a-vis W1607. However 2000 prototrophs resulted from crosses with 58-161 (mostly mal-) as compared to only 200 prototrophs in a simult. cross of W1817 x 58-161 (mostly mal/). Samples of eigth and tenth passages were saved, I believe, as W2235 and W2234 respect. Esther has further data on crosses of these. Thus, the phenomenon applies to some TLB_1 strains at least.

W1896

F-removal. In expt.#5343, repeated inoculation of motag with separate colonies of W1895 verified a tendency to swarm readily, more so than 58-161. This could be significant. Rapid appearance of what seem to be sequential mutants on one plate. History of one extended passage: Inoc. on motag from single colony (M1). By 24 hr

3 sequential swarms. Pick from fastest and subculture (M2). By 24 hr sr 32 mm. At 28 hr subcult. (M3). At 25 hr. sr 29 mm. Subc (M4). At 28 hr sr 42. Subc.(M5) At 4.5 hr sr 6. At 26 hr covers plate. Subc. (M6) and inoc for test cross. Still Hfr. Subcultures M7-M12 confused by use of too conc, agar. Upon final return to 0.4% agar, swarms readily. M14 moves 47 mm in 12 hr. Subc. (M18) At 14 hr covers plate. Subc (M19) and inoc for cross. Cross x W1817 yields 4000 protos; cross x W1177 gives none. Str ak out and isolate 3 colonies. Cross each. Two verify mass cross, third does not cross with W1817 (but moldy). Save one apparent F- as W2284. Subsequent cross of W2284 x W1817 gave only 500 protos, but simult. W1895 x W1817 gave only 1000.

Reinfection. Expt.#5396: W2284 (F-Lac+) was grown with W1817 (F+ Lac-) and W1941 (Hfr lac-) separately, for 6½ hr. Loop inoculum from slants, grown until turbid. Five lac+ colonies from each mix were pooled and crossed with W1817 and W1177. Both pools gave results identical to W2284: 500 with W1817, none with W1177. Thus no evidence of reinfection. But no 58-161 x W1177 control; concurrent W1895 xW1177 gave Hfr results, but only 1000 colonies with W1817. Repeated process again with same essential results.

In expt.#5398, a repeat of preceding, three isolates from W1941 mix and four from W1817 mix were tested vs. W1817 and W1177. Again all crossed with latter, not former giving mostly lac+ protos. Hfr crossed with W1177 but control 58-161 x W1177 cross did nto go. Follow up by J.L. (#1055).

Crosses. In expt # 53107, W2284 was crossed with W2057 (Hfr T-L-Mal- S^s) and gave only 7 prototrophs (sic). Three were mal-, four mal+. protos were purfied and crossed by SRP with W1177 and W1817. None gave recombinants with W1177, but no adequate control since 58-161 did not cross with W1177 at the time. 150-500 recomb. in crosses with W1817. Most of the latter were lac+ suggesting that the proto parent had been F-. 10-30 lac- recomb where proto parent had been mal+; o-2 lac- recomb where proto parent had been mal-. Repetition of cross using two mal- protos showed no fertility with W1177 in spite of simult. fertil. of latter with 58-161.

Het stcks.

In expt#5349, considerable difficulty was had in obtaining motility in W478, W1325, and W1590. No g od swarms were ever obatined from W1590. The other two were passed repeatedly (passage numbers meaningless since some were on harder agar). After 13 passages, at least the last two on 0.4% agar, W478 was tested adequately and found to be F-. Saved as W2210. After 11 passages (mostly on 0.4%) W1325 was tested and found to be F-. 800 protos vs. W1817, 0 vs. W1177. No 58-161 x W1177 control recorded however. Saved as W2301.