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Dear Josh,

The people who have come around for your strains are the f:

∴ Margarite Vogt (Delbrück's person who has been attempting to repeat my stuff) for 679-680 because her nutrient agar counts don't correspond to counts on leucine threonine supplemented medium (mine do)

Derek (sp?) Rowley, ex Fleming's lab, ex Heidelberger's lab, pro-Hinshelwood, medical bacteriologist. He is looking for penicillin sensitives - normals are penicillin resistants to him (50 OU/ml vs 0.01 - 0.1 OU/ml). He has surveyed all my stocks - your Y strains (all 2_x^+), W1205, all my 2^- strains, & found none but has produced two by screening:

OV, plate → NH + bacteriostatic (to sensitives) concentration of penicillin, let grow, mark the resultant normals, knock out penicillin with penicillanase, re incubate, new ones are pen sensitives

unfortunately he gave them to me to test for auxotrophic requirements, one is & one isn't, and ~~neither~~ both

are Endo negative (i.e., not merely lac⁻ but non-growing completely).

Vogt gave a private talk on her work — she is attempting to find out when the zygotes are formed by killing the strep. sens. parent + zygote by strep at different times after mating. Unfortunately @ her methods are somewhat wiped off — she grows the strains together in nutrient broth "in oscillation" — i.e., $10^8 \rightarrow 2 \times 10^8$/ml, adds 1 ml to 1 ml fresh nutrient broth $\rightarrow 1 \times 10^8$ + start all over again (no controls to show cells are in log phase continuously), dilutes in phosphate buffer 1/10, 1/50, 1/100, 1/200, 1/500 + plates in minimal A medium (washed again but differential carry-over of nutrient broth whose growth potential is not exhausted — + she wonders, ① why dilutions don't correspond, and ② why prototrophs begin appearing at 2, 3, 4 days) with + without strep. In S⁺ plates only ~10% of prototrophs in S⁻ plates — pinning her down dicted the fact that her figures on the board given in prototrophs/10^7 cells as 50, 240, 4120, etc represented respectively 2, 5, 100 colonies. She gets an increase with time that they sit in minimal medium on the plates before the layers — 0, 1, 2 hrs being used — hence

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believes that "sensitization" occurs (they are trying to bring her around to believing that the real affair is segregation). That is about all I can make out of that.

As for s^+, s^- I'm working on the biochem with powdered cells. The genetics is screwey but as ff for genealogy:

(1) All Y stocks tested, + 679-680, 58-161, are (by serial reversion or test of prototroph) capable of growth in or on fumarate + malate hence are s^+ in phenotype. Since they may represent several loci I call them s_x^+; i.e, Y53 is s_{Y53}^+, 679-680 is $s_{679-680}^+$.

(2) Ryan's K12 from which we prepared auxotrophs is s^-, i.e, incapable of growth in ~~succin~~ fumarate and malate.

(3) Once + only once an "adaptation" to the s^+ condition occurred - s_0^+. A diauxotroph - s_0^+ $bip^=$ (histidine ~~p~~ alanine) was prepared from this.

adapted from (2)

Now - as for crosses:

$2^+_x \times 2^+_x$ (1/53 × 1/24 for instance) give only 2^+

$2^- \times 2^-$ give only 2^-

* $2^+_0 \times 2^-$ give mixture of $2^- \times 2^+$ (unless cross is made directly to K12 2^- on primarate to select out only 2^+)

$2^+_0 \times 2^+_x$ give only 2^+

$2^+_x \times 2^-$ give mixtures of $2^- \times 2^+_x$

* Formerly only - 30 prototrophs and only 1 cross were tested + all were 2^+ - now, in other crosses, 2^- is also thrown. Incidentally - is the Lac^- marker in 1/53 Lac^-_4? And does it segregate independently in crosses with 1/24? I found it to do so - also with $2^+ Lac^-$.

I now have W 1177 + will try crossing directly with 2^+. Azide was NE - I have several ~~an~~ 2 meth tryps $\equiv Az^R_n$ which wouldn't cross by 2^+ (at least no 2^+ were thrown). One Az^R is mucoid colony form - same locus or closely linked with other Az^R non-mucoid.

Further work on 2^+ will be, besides biochemistry - survey of other peoples' K12 + dominance test if I can squeeze out a bet - should be dominant, that is, $2^+ > 2^-$.

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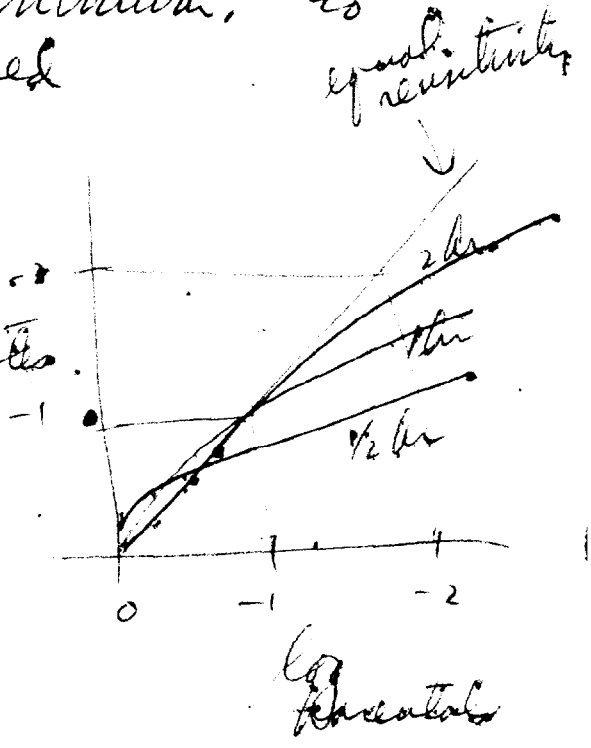
Ya I have been using in isolating single putative zygotes - by Bisson using 2 sets of markers one for screening the other for determining cross-over relationships. Of course I can recover only one "strand". Little data on this yet - the experiments get too big 400 tubes minimum. To see whether zygotes are formed immediately in liquid or later I have used UV assuming resistivity of zygote would be less than parentals if killing by lethal induction occurs (probably doesn't).



Of course there are troubles, differential killing of parentals (controlled by plating for totals in medium in which one parental gives large, other small colonies) screening in clumps causing release of nutrilites (added previously irradiated parentals in reconstruction).

My data are few but the accompanying graph gives an idea - times are mixing times, previous to OV - concentration is 10X at best, haven't carried titling down to fx, no protos left.

In agglutination I've been trying to reduce iso agglutination in culture tubes + washing prior to mixing + increasing hetero agglutination on mixing - not much luck.

Thanks for the rain check offer - I may take you up on it. There was a possibility of a position at UofW in industrial urology but it fell thru. I am applying for a VAF to stay here next year. as affected my fellowship was only extended thru the summer. Maybe I had better investigate gov't jobs - the draft age is being increased.

Sincerely,

Gene