Fermentation tests. 434
Mutants.

Spread out mustard-treated 440 (see 426) on EMB-lactose + a few maltose, sucrose, glycerol plates and examine for mutants ca 400 per plate.

Lactose: 36. # of plates. 1 mucoid found

∴ ca 15,000 colonies examined.

no fermentation mutants.

~~Maltose~~ Mannitol 2 plates + 2 streak plates.
ca 10200 examined.

2 very small M- colonies noted. Streaked out on fresh medium.
all M+.

Glycerol - slow utilization. — compare passages on glycerol & peptones.

Sucrose - very slow but definite utilization

Fermentation mutants – enrichment cultures.

Feb. 25, 1947.

Inoc 50 ml NB + 2% sugar + Brom Tresol Purple A25. Y53.
symbols ind. acidity.

	A26	A27	A3	A4	P5
glucose	+++				
glycerol	+	+++	Y81.	growths heavier than in glucose	sm % black on EMB
lactose	–	–	+	++	* +++ [illegible]
sucrose	–	–	–	–	– all white colonies!

* growth mic. on standing at rm tm.

Streak out some apparently Gly – (a) colonies from 434 + compare with a typical Gly ± – (b).

	Y84.	
a)	–	Y80.
b)	+	

Compare a, b + gly + enrichment culture above.

	EMB	
Y80	–	No evidence of papillae.
(from glucose above) Y53 gly ±	+	
Y53 gly +	+ or ++	different from Y53?

enrich.

on BCP –, medium is not changed in color, cells show slightly different shades (+, ± pinkish, – more translucent or violet.)

on Gly-BCP broth – Gly –, Gly ± and Gly + all show slow acid + gas.

(see over)

1. Enrichment for gly+

A5 - streak from gly tubes to new gly EMB

A8 - scoring OK, as before!

Resistance mutants - cross test.

436

Streak out susp. of on NAagar + Mal Prem. 10^{-4}.
Streptomycin 5 u/ml

	M.G.	str.	B.G.
Y77	++ *	—	— !
Y78	—	++	

* dye is decolorized.

after 3 days, several hundred colonies appeared on the streak. Y79.

streak Y77 over Y88/MG. to determine if decolorization reverses effect of dye. No evidence of stimulation of previously streaked (Mg^{3}) culture. Probably due to pH change.

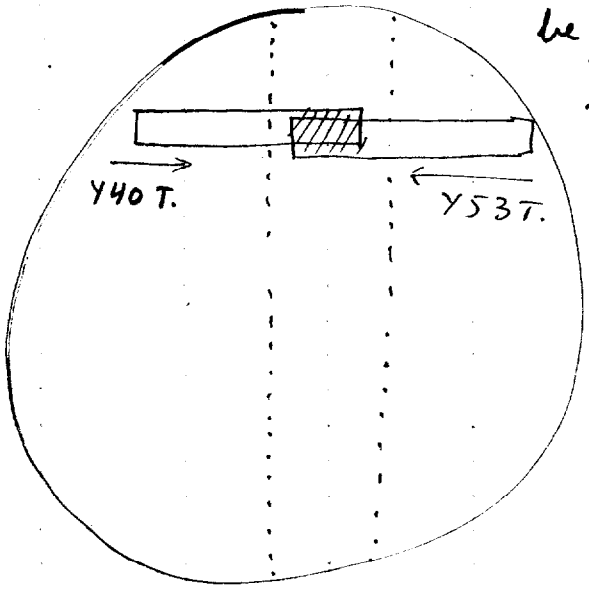
Inversion Detection

See 437a for summary.

1 MAR

Streak out Y40, Y53 (T from 426) on NA plates.

P1. Pick single colony and streak on NA, overlapping a streak of the other type in the center of the plate, and mix well in center. This can then be picked after growth and inoculated into minimal.



P3. Test combinations by suspending growth from center in 1 ml water, ~~and streaking~~ pouring ion minimal plates. (i.e. 84 tests!) ca 10^9 inoculum.

Tests. log prototrophs

1	1+	13	1+	26	2	39	1
2	2	14	1	27	1+	40	1
3	2	15	1+	28	2	41	1
4	1	16	1+	29	2	42.	1
5	1.5	17	1+	30	1		
6	1.5	18	1+	31	1+		
7	2	19	1	32	1+		
8	1	20	1.5	33	1		
9	1	21	1+	34	1		
10	1+	22	1+	35	1		
11	1	23	1+	36	1		
12	1	24	1+	37	1		
		25	2	38	1		

1-6 also tested by streaking 1 loopful on T(0) plates.

	colonies
1	0
2	3
3	1
4	2
5	7
6.	10
7.	1
8.	1

84 tests - no inversions

Streak technique not as reliable as desired!

Inversion Tests: Summary.

exp.	material.	tests:			Cumul. Tests.	Yield
426	MN_2 trt.	Y40 × Y53T; Y53 × Y40T, by st. tech., O + B,		20 tests.		0
433.	MN_2 trat.	do.		17	37.	0
437.	"	Y40T × Y53T. in O only, X on agar;	2 × 42 =	84	121	0
508	X-ray	Y40T × Y53T. in O only. on agar.	2 × 14 =	28.	149	0

Trans of prototroph initiators.

Pour Y40×Y53 plates in T(0). To 4p. add also 10^{-6} K-12 cultured + washed similarly in order to compare rates of colony development.

See 445.

Y65, Y68 test for inversion.

Plate Y65 × Y40

Y68 × Y68.

in T(0) + T(3,).

no prototrophs at all!

∴ not due to suppression of X in B, - B4 region.

Segregation of Gly –

March 4, 1947 –

Y80 x Y81. cf. 435.

$B_1^- > B_1^+$ as usual. Pick 4 plate on EMB lac + EMB gly agar.

	lac – R	lac – S	lac + R	lac + S
gly –				
gly +				

B_1: lac – R – S; + R; + S.

Gly segr.

Y80 x Y81.

A. in min.

Gly	Lac	V
+	−	R
+	−	S
+	−	R
+	−	R
+	−	S
+	−	R
+	−	S
+	+	S
+	−	S
+	−	S
+	−	R
+	−	R
+	−	S
+	−	R
+	−	S
+	−	R
+	−	S

B.

	Gly	Lac	V.
T(0)	+	−	R
	+	−	R
	+	−	S
	+	−	R
	+	−	R
	+	−	R
	+	−	R
	+	−	R.
T(B₁)	−	+	R
	−	−	R
	−	−	R
	− ?	−	S
	−	−	S
	− ?	−	R
	−	−	R
	−	−	R
	−	−	S
	− ?	−	R

[scoring maybe inadequate]

When real again 4 hours later, series B had gotten considerably darker!

C.

	−	−	R
	−	−	S
	−	−	R
	−	−	R
	−	−	S
	−	−	S
B₁	−	+	R
	−	−	R
	−	−	S
	−	−	R
	−	−	R
	−	+	R
	−	−	R
	−	−	S
	−	−	S
	+	−	R
	−	−	S
	−	−	R
	−	−	R

D.

	−	−	R
	−	−	S
← [	? +	−	S
		+	R]
	−	−	R
	−	−	S
B₁	−	−	S
	−	−	R
	−	−	S
	−	−	S
	−	+	R
	−	−	R
	− ?	−	S
B₁	−	−	R
	−	−	S
	−	−	S
	? −	+	R
	? −	−	S

Note: B_1^+: 25/25 Lac+ Gly+ close linkage? These results are difficult to reconcile with the map!

$B_1^+ + B_1^-$: 44/46 Lac+.

Lac, V segregation.

March 6, 1947

Lac− V^R Lac− V^S Lac+ V^R Lac+ V^S

B_1^+: ~~15~~ ~~9~~ 0 1 25 Faulty segregation of Lac+?

all Gly+.

B_1^-

Gly− 24 19 5

Gly+ 1 (1?)

This scoring of Gly, done at 2 da. is not borne out by readings at 3 days, when in both groups, all were Gly+, $\frac{VS}{c}$ some variation in intensity.

Note: 24 Lac− : 1 Lac+ in B_1^+ (95%!)

46 Lac− : 5 Lac+ in B_1^-.

1) B_1^-/B_1^+

25	24	1	25
41	40	~~4~~ 5	45
64	6		70

$\chi^2 = .04 + .02 + .04 + .03 = .13$

compare ī standard.

18	24	7	25
51	~~6~~ 19	6	70

$\chi^2 = \frac{13^2}{51} + \frac{13^2}{19} = 3.3 + 8.9 = 12.2$

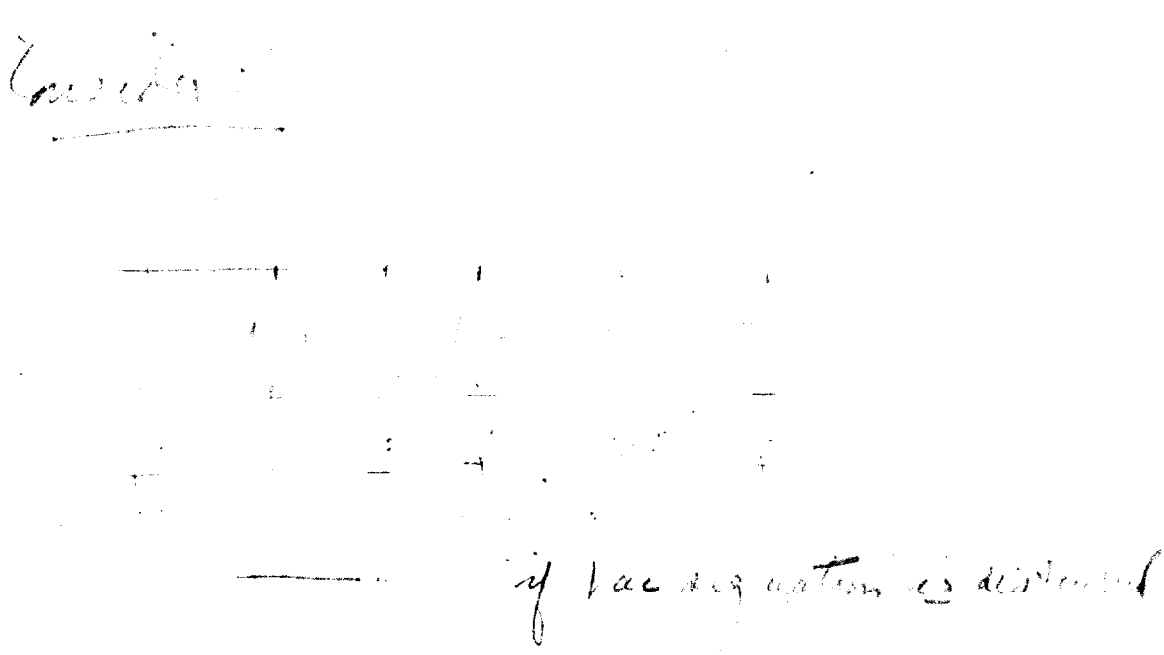
a) All data: 72% Lac−

b) new data 64% Lac−

$\chi^2 = \frac{36}{18} + \frac{36}{7} = 7$ $P = < .008$

efficiency of Lac+

Peculiar segregation may explain peculiarities of Gly segregation.



Segregation of drug-resistances

A ~~Y79 x Y80~~ Y77 x Y78. – in T(0) OK!

Streptomycin 5 u/ml
Malachite Gr. 100 r/ml

B Y53 x Y78 OK! ‡6 Lac – 1 Lac +.
dis standing [illegible] 17 h

C. Y40 x Y77 OK! no ‡

A. Y77 x Y78.

	Lac	V	Str	M.G.
	+	R		R
‡	+	R		R
	+	S		R
	–	S		R?
‡	+	R		R?
‡	+	R		R?
‡	+	R		R
	–	R		R

‡ peculiar dup blue color

? probably S.

scoring uncertain due to selection of resistant residue

	Lac	V	Str	M.G.
	–	R		R
	–	S		R
	–	S		R
	–	R		R
	–	S		R
‡	+	R		R
‡	+	R		R?
‡	+	R		R?
	–	R		R?
	–	S		R.

do.

M.G. resistance 10^{-4}. ~~Lac~~	Lac+MR	Lac+MS	Lac–MR	Lac MS.
C.	2	1	~~[illegible]~~ 8	2
total.	0	2	16	3
Lac+S x Lac–R.	2	3	24	5

scoring not certain

ind. cont.

not exc. of resistants. If valid, indicates linkage to BM.

should score on minimal plates to avoid selection for resistant contaminants.

Selection of recombinants with drugs.

P3. – mix YB cultures of Y77 (Mg^r) and Y78 (Str_5^r). 30°
also use mixture of nutr. plating technique of 441.

			A6	A8.
1.	Mix culture	in Mg + Str	0, 0	1 colony;
2	Y77	"	0	2 colonies; also as 3.; 4 cols.
3	Y78	"	0	1 colony ca 100 minute clearings, micr cols.
4	Y77 + Y78	"	ca 10 v. small	A – as 3. B > 10 large colonies innumerable minute clearings

A6 – incubate at 36°.

Indeterminate whether the mult. resistant colonies represent recombinations.

Compare 4 (cross) c̄ 2 mutations of $Sm^S Mg^R$ to $Sm^R Mg^R$

There may be some synergism in view of the long lag before colonies are detectable!

[Use Y84 × Y79. and plate in brilliant green. + streptothricin.]

Resistance steps; resistance to streptothricin.

443

March 4, 1947.

Recd. 127,000 u. streptothricin from Woodruff, Merck. non-sterile ampules.
Suspend in 2.7 ml 95% alcohol for 3 hours. Add 10.3 ml sterile H_2O → ca 10,000 u/ml in 20% alcohol. Dilute further as required.

u/100 ml = u/ml

100 u	1	
Y78		T
Y53		T

500	5		
Y78		T	streak Y78 on 5u agar for —
Y53		1-200	Y82 - streakout on 5u agar

1000	10		
~~Y78~~		50	Y83 - streak on 10u agar
Y53		1	Y84 — do.

10,000	100	
Y78		0
Y53		0

Y78 — Streptothricin on 5u agar gives colonies but not so large as Y82.

Y83, Y84 OK on 10u agar.

See 452.

Y40 × Y53. Mix cells in agar pour 5 ml each plate.

A).

O:	B:	B_1:
11	12	124
15	11	136
9	14	
10	12	m = ~~10.~~
15	9	130
60	13	more turbid.
	6	
	12	
m = 12.0	m = 11.1	

B) 20 B tested: 18 B^+
2 B^- (Lac −R; Lac +R)

5/20	364
2/20	445
5/50	452
12/90	13%

C. Segr. of Lac, V_1^R in O, B_1:

	−R	−S	+R	+S	Σ
O:	24	~~[illegible]~~ 11	9 ~~[illegible]~~	0	44
B_1	22	~~[illegible]~~ 15	14	0	51.

	−R	−S	+R	+S
O	17	10	9	0
B_1	16	12	13	0
	~~12~~	~~11~~	~~10~~	

Anone O plate, streak out 11-12 on surface. These colonies appeared at same time as prototrophs (24 hours) and were of comparable size.

Lac – V segregation.

March 6, 1947.

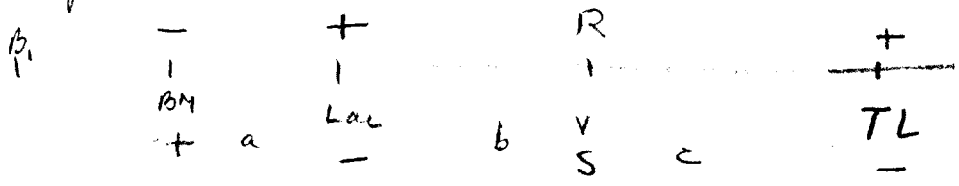
Use colonies from Expt. 437 (grown together – chance for selection) [Y40 x Y53 (T) by plate number].

	Plate #		Lac – R	Lac + R	Lac – S	Lac + S.	Σ	* V scoring not dependable – phage apparently very low titer.
	9	10	2	5	1	0	8	
	20		4	4	2	0	10	
–	* 27	40	9	6	0	0	15	
–	* 22	40-50	8	10	0	0	18	not significant by itself
	* 3		8	3	0	0	11	
	8		3	1	0	0	4	
	3		7	3	3	0	13	
	42		1	2	1	0	4	
	6		6	2	2	0	10	
	4		4	2	0	0	6	Lac – = 135/214
	14	20	6	1	0	1	8	= 63%
	40		1	1	2	0	4	compare ± 70% pur.
	21		2	1	1	0	4	V^R = 190/214
	7	40	6	3	2	1	12	= 85%
	23		3	2	0	0	5	comp. ± .74 pur.
	2		13	7	1	0	21	
=	25		5	14	2	0	21	
	11		1	3	0	0	4	
	10		11	1	3	0	15	
	19		1	1	1	0	3	
	17		5	1	0	0	6	
	29		7	4	1	0	12	
			113	77	22.	2	/214.	

The agreement of the Lac+R; Lac – S classes with exp from previous expts is very poor. Reexamine crosses of aberrant cultures.

There is a shift from Lac − S to Lac + R

In map basis



β_1	−		+		R		+
	BM		Lac		V		TL
	+	a	−	b	S	c	−

$<$ Lac −   bvc $<$ $>$ a

$>$ V^R bva $<$ $<$ c

($<$ c. [illegible])

a = + R
b = − R
c = − S.

[− R would not be augmented by the diminution of c?].

---

or, another interpretation, is that ~~[illegible]~~

the previous stocks were reverted for T or for L, c a larger distance in the interval V − (T or L)

∴ compare types c a short or long interval (i.e. low or high − S) biochemically.

contd. 446.

#	Lac-R	Lac+R	Lac-S	Lac+S.	$\bar{c}$
15.	3	9	1	1	14
33	2	1	2	0	5
34	5	0	0	0	5
30	6	2	2	0	10
31	7	7	4	0	18
24	3	3	1	0	7
32	7	3	1	0	11
35	7	2	1	0	10
1	6	4	4	0	14
36	4	2	2	0	8
16	12	10	3	0	25
26	14	9	3	0.	26
83-76.	52 53	22 20	1	/ 151. ~~214.~~	
106-113	77 76	22 27	2	/ 214	
189	129	44	3	/ 365	

cf. 445.
compare $\bar{c}$ remainder of expt.
These samples agree.

$$\chi^2_2 = \frac{49}{83} + \frac{49}{106} + \frac{1}{53} + \frac{1}{76} + \frac{9}{20} + \frac{9}{27} = 1.86 \quad p = .4.$$

Homogeneity ??

.59 | 1.86
.46
.02
.01
.45
.33

analysis of
446 vs 359 summarized.

−R	+R	−S	+S.	Σ
189	129	44	3	365
100	55	50	4	209
289	184	99	7	574

$$\chi^2 = \frac{25}{105} + \frac{25}{184} + \frac{144}{67} + \frac{144}{117} + \frac{196}{36} + \frac{196}{63} + \frac{1}{4} + \frac{1}{3} \ldots$$

$= .2$
$.1$
2.2
1.2
5.4
3.1
6.33

8.5 by this component. $p = .005$.

∴ it is the difference in the frequency of −S which differentiates the distributions.

$12.7 = \chi_3^2$

$\frac{8.5}{4.2} = \chi_2^2$ $p = .04$

Compare

194 - 189	129	124	318
95 - 100	55	60	155
289	184		473

should be 194:124
95:60

$\chi_1^2 = \frac{25}{189} + .25 + \frac{25}{124} + \frac{25}{60}$ = ca 3.

Heredity of segregation types.

March 7, 1947

Recover 446 – 22, 25 + 27. in order to ascertain heritability of disproportions in ratios. Compare segregation of Lac + V c̄ Y40, Y53 stand. In previous test:

A	22:–	8:10:0:0	
B	~~27~~:–25	9:6:0:0	
C	~~25~~:–27	5:14:2:0	Lac, V.
D.	Y40 × Y53.		

	–R	+R	–S	+S
A	47	19	9	1
	44	20	7	1
B	26	12	16	0
	[illegible]	13	16	0
C	7	5	7	1
	80	36	32	2
D. –	30	~~21~~ 22	19	0
O_2	10	7	5	0
N_2	6	5	6	0
M.B.	4	4	6	0
S.H.	2	1	2	0
D. total:	52.	38	39	0
Total	132	74	71	2	279.

This is homogeneous with the previous tabulation: 100:55:50:4.

$$\chi^2 = \frac{100}{37} + \frac{100}{62} = 4.3$$
$$+ \frac{4}{21} + \frac{4}{36} = .21$$
$$+ \frac{64}{18} + \frac{64}{31} = 5.6$$
$$\chi^2_2 = 10.1$$
$$p = .007$$

Compare A c̄ D..

47	19	9 + 1	0	76
52	38	39	0	129
99	57	49		205

See over.

Compare R & S.

58	66	18 10	76
98	90	31 39	129
	156	49	205

$$\chi_1^2 = 64\left(\frac{1}{58} + \frac{1}{98} + \frac{1}{18} + \frac{1}{31}\right)$$

$$= 64(.017 \quad .010 \quad .055 \quad .032)$$

$$= 64(.114)$$

$$= 7.3 \qquad p = .007.$$

Therefore the total discrepancy is due to a difference in the proportion of V^R cultures. In practice, this means a deficiency of $-\underline{5}$ cultures in the new group.

	−R	+R	−S	+S.	
A. 22	~~44~~	20	7	1	. This is the "abnormal" destribution;
B. 25	25	13	16	0	1 plate showed 13:14:0:0.
C. 27	7	5	2	1	(perhaps scoring of V^R was not proper.' Recheck!
	~~76~~	38	~~25~~	2	

D.	—	30	22	19	0	
	O_2	10	7	5	0	
	N_2	2	1	2	0	
	SH	6	5	6	1	
	MB	4	4	5	1	
	$\bar{2}$.	52.	39	37	2	4·7
		69	38	31	2	

Plate by plate

A	6	2	0	0
	6	14	6	0
	8	1	3	0
	17	3	4	1
B	4	1	6	0
	12	10	8	1
	9	2	2	0
C	7	5	2	1

compare +, −

89	41	130
100	40	140
189	81	270

Caution! Beraufel of scoring V^s.

Interpretation of 447.

Compare $T^- \rightarrow T^+$ in 447 Lewis + standard.

March 7, 1947

Using cells from Exp. 447, plate the Y53 components at a 10^{-2} dilution into LB$_1$ agar.

Y53	turbid	10 c.c.
(Y53-Y27)	clear	30 c.c.
25		10 "
22		1-2

There is no stable diffuse negative, but apparently due to scoring difficulties.

Plating medium for recombination

March 7, 1947

Factors: buffer: phosphate, citrate, acetate, phthalate. More alkaline.

R_H : thioglycollate; ascorbic ac.; pyruvate-lactate; O_2; Methylene Blue. Indophenol??

B_1 ? — Try on BM x TP.

T(0) — Y40 x Y53 mix in agar + pour on pre-poured plates.

1. Controls

2. "O_2 atmosphere" least turbid plates

3 N_2 atmosphere turbid plates; colonies v. small, countable ones are rare.

4 Na thioglycollate 2 mg –
.2 mg –

5. Ascorbic ac. 1% no colonies (pH 4-5) No turbidity

6 Methylene blue 100γ Turb. [illegible]
10γ " "

The number of apparent colonies is correlated inversely c̄ the gross turbidity, and was least in O_2, most in N_2. SH had no apparent effect, however. There is, however, no marked increase of colonies in the O_2 atmosphere plates, but those which do appear are larger.

See 441. Test Y78. on EMB; Lactose. cf. 453.

-also 441A - Lac Blue - on Lac → ; glycerol white + black colonies + some c̄ blue haloes

	Lac		Gly.	count as Lac —
Y78	(58-161 Sm^R)	+++ grsh.	+++	- gum sheen !
58-161			-	(± ??)
Y80	(58-161 - V_1^R - Gly -)		-	

Note, Y80 on v. long incubation turns a faint violet color, à la sucrose from previous expts + now

Y53	++
Y81	++
Y40	++
Y78	++

? relative amts.

Y80	—
58-161	±

58-161 is slow, but wilfscore vs. Y80. see 453.

Y80 x Y81.

Gly. segregation.

March 9, 1947.

A. Repeat 440 See 451A.

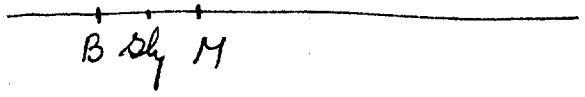
Y80 x Y81.

T(0).	16/16 Gly+	12/14 Lac−	86%
T(B,)	13/13 Gly+.	56/72 Lac−	78%

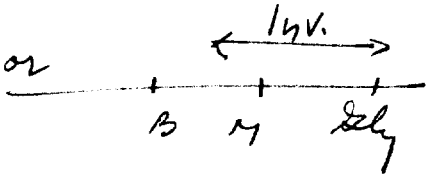
Note, this denotes absolute linkage of Gly to BM. This is predictable either as the mapping:

written Y80 x Y53
not Y81 x Y40
seems to be disturbed!

typo!



or



Note (also) the segregation for Lac may be disturbed. Comparing ē accum. data for Y40 x Y53: (71% Lac−) $p = .06$

ē 440	$p < .025$
(This + 440) ē 71%	$p = < .001$
440 ē 71%	$p = .008$
B_1^+ / B_1^-	$p = .3$

Glycerol segregation.

March 9, 1947.

These plates were allowed to stand 3 das. before testing

B. Y80 x Y53. Test B_1^+ + $(B_1^- + B_1^+)$ on Gly; Lac.

a. B_1^+ 27 Lac− : 9 Lac+ 35/35 Gly+

b. B_1^- 50 Lac− : 16 Lac+ scoring uncertain. 1++ ; 30+/31

77 Lac− : 25 Lac+ ca 75% Lac−

C. ~~AsB~~. Y81 x Y40. Test on Lac

B_1^+ 104 Lac− : 43 Lac+

B_1^- 28 : 13

132 : 56 188 70% Lac−

Gly 16/16 + (not [illegible])

This experiment is not homogeneous with the earlier Y40 x Y53 ones.

D. Y53 x Y40.

	−R	−S	+R	+S	
B_1^+	33 32	28 25	14 18	0	75
B_1^-	18 19	12 15	13 10	1	44
	51.	40	27	1	119

→ stand $\chi^2_2 = 3.2$ $p = .2$ data not adequate

→ $\chi^2_2 = 3.5$ $p = .2$

cf. standard. $p = .06$

91 : 28 76% Lac−

Segregation of B−

March 9, 1947.

See 445

Y40 × Y53 in Biotin medium. Pick colonies to H_2O, + moi. loopful in T(O) + T(B). 50 isolates.

50 isolates. At 12 hours, 5 def. B⁻ ~~6~~ did not grow in either. 5 at first.

Lac, V sgr.

	Lac	V
1	+	R
2	+	R
3	−	R
4	−	R
5	−	R.

P13 − 5 grew on B, not O. Antest, 1−4 contained primarily −S, E +R as well. 5 was uniformly −R. see 456.

To summarize: B⁻

~~Lac −R −S +R +S.~~

−R	+R	−S	+S.
~~IIII~~ 1	~~IIII~~	0	1

∴ 0−S/12.

	6	5	0	1	12
A (Lac)	6	3	3	.36	
B (M−B−)	1.4	9	.8	1.4	

$\chi^2_2 = \frac{4}{3} + \frac{9}{3}$ 4.3 $p = .14$

$\chi^2_2 = 5 + \frac{16}{9} +$

$= 7 +$ .03

∴ maps

B. 9 B 13 M

Glycol reactions, various strains.

March 11, 1947.

P11. Streak on a single Glycol plate (EMB - 2%) the following:

		A12	P12	A13	4P13	A14
1	K-12	−	+	++	+++	+++
2	58-161	−	−	−	− + stands.	
3	Y40	−	−	±	± + stands.	
4	Y10	−	−	±	+	
5	Y53	−	−	+	++	
6	Y46	−	−	+	++	
7	Y64	−	−	±	++	
8	Y78	−	±	++	++	
9	Y77	−	−	±	++	
! 10	Y80	−	±	±	± translucent, creamy shade, not opaque purple.	
11	Y81	−	−	+	++	
12	Y82	−	−	±	+	
13	Y73	−	−	+	+	
14	Y74	−	−	+	+	
15	Y82	−	−	+	++	
16	Y83	+	++	+++	+++	
17	Y84	−	−	+	++	
18	Y79	−	−	+	++.	

— resistance of bacteria

4/11/47

An various plates, streaks: Serratia marcescens 1
Salmonella 20 2 Incubate
" 21 3
Phytomonas tumefaciens 4
Staph aureus 5

			1		2		3		4		5	
✓ or u./ml. Malachite Green.	M.G.	1	++	✓	++	✓	++	✓	±	++	—	✓
		10	+	++	++	✓	++	✓	—	✓	—	✓
		50	—	—	—	—	—	±	—	—	—	—
			—		(— cols)		++		—		—	—
		100	—	✓	—	±	±	++	—	✓	—	✓
Brilliant Green.	B.G.	100.	—	✓	—	✓	—	✓	—	✓	—	✓

Streptothricin	Sth	1	++ R	✓	+	✓	+	✓	+	++	++ Y	✓
		5	+	+ cols.	—	—	±	± papillae	±	++	— colonies.	✓ OK.
		10	— cols.	good cols.	—	—	—	—	±	+	—	
Streptomycin	Sm	1	—	—	+	+	+	+ papillae	±	+	— cols.	✓ grow large.
		5	—	—	—	—	—	—	—	±	—	✓
Penicillin	sic!	10	++	✓	—	✓	—	✓	—	✓	—	✓
	sic!	100	++	✓ R	++	✓	++	✓	±	++	++ Y	✓
Control	N.A.		++	++ R	++	✓	++	✓	±	++	++ Y	—

Incr 10P11. Readings 1st. 1130A12.
6P12
9A13

Concl. — [krh. of selective conc.]:

	M.G.		B.G.	Sth		Sm		Pen
Serratia	10-50	—	<100	10	10	<1.	—	10-100.
Sal 20	50	50	<100	5	5	1-5	>1	
S21	>100		<100	>5	>5	1-5	>1	
Phyto	1-10		<100	>10		>5		
Staph	<1		<100	5	5	1	1	

order of activity:

M.G.	Sm	Sth	Pen.
Staph	Serr.	Staph	
P.t.	Staph	S 20	
S.m.	S 20	S 21	
S. 20	S 21	Serratia	
S. 21	Phyto	Phyto	Serratia

↓ incr: resistance

Use studies following papillae on higher drug conc for higher steps:

S20/MG 50 on B.G.100
M.G.100.

S.m/Sth 10

S20/Sth 5

S21/Sth 5 on higher Sth.

Staph/Sth 5

S20
S21 | Sm on higher Sm.
Staph

Resistance mutants

4.54a

Staph.	M.G.	1	turbid.
—		5	ca 10 Mg^R/cc; gd. inhibition of residue
—		10	perfectly clear!
—	Sm	1	ca 10^4 Sm^R.
—		5	ca 10 Sm$_5$^{R}
	Proteus	100.	Turbid.
		10	clear at 48h.
— Serratia	M.G.	20	clear at 24 hrs.
		50	a. ca 20 Mg^R [ctd?]
—	Sm	1	clear. no Sm^R seen. ; some v. small Sm^R.
—		5	~~ca. 10 appr. colonies~~. 1 Sm^R? white!
—	Sth	100	ca 5 Sth^R/ml col. v. small
—		20	ca 100 Sth^R/.1 ml Wide range of color.
	Prof.	100	turbid
— S20	Bg	100	clear, no Bg^R!
	Sm 1		turbid
—	Sm 5		turbid; but some possibly Sm^R, v. small ✓
	Proff 100		turbid.
— S21	Bg 100		clear, no Bg^R.
	Sm 1		turbid.
—	Sm 5		as S20. inhibition incomplete.
	Proff 100		turbid.
Phyto.	M.G.	10	inhibition incomplete
	Sth	50	inhibition incomplete; some selection
	"	100	clear! ca resist.
	Sm	10	incomplete inhibition ca
		50	clear! ca 100.
	Prof	100	clear! — ca 300

available:

~~Staph~~: Sth 100
Phyto Sm 50
Prof 100

St: Sm 5
Sth 10
Mg 5

S20 B.G.
Sth 10
Sm 5

S21 Bg
Sth 10
Sm 1

Serratia Sth 20
50
100
} color variation

Sm 5
Mg 50

Reversion detection

4/11/47.

As above on N.A. and T(0).

photographs show up as papillae in T(0) streak, justifying this technique. Throw out NA plates.

T(0)	colonies in center zone
1	++
2	++
3	+
4	++
5	++
6	++
7	++

Streak out further 48 hr. colonies of 440T + 453T.

e.g. too irregular. NA mix + plate is more reliable.

"Syntrophic colonies?" 456

See 452 for sources.

Colonies from 440 x 453 on Biotin were picked to water + T(0), T(B) inoculated. Those reported are 5/50 which grew on T(0) only after 2-3 days. The T(0) and T(B) tubes were both streaked on Lac-V agar: (↓ no turbidity)

	T(0)	T(B)
1	−S ; +R	−S ; +R
2	−S ; +R	−S ; +R
3	−S ; +R	−S ; +R
4	−S ; +R	−S ; +R
5	+R	+R.

Since −S and +R are the parental configurations, the delayed growth [and the original colony formation] might be due to syntrophism ?

∴ streak out T(0) tubes on EMB lac to purify.

Test − (a) and + (b) colony of each on B, O mediums.

	O	B
1a	−	−
1b	−	−
2a	++	++
2b	−	−
3a	−	−
3b	−	−
4a	−	−
4b	−	−
5a	−	−
5b		−

Expl. 1. Syntrophic colony

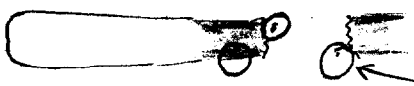
2-Colony not picked; only interstitial growth in agar. Requires repeating.

Mucoid variation.

19 MAR 1947

Streak Y53 across T1 on EMB agar.
Suspend slimy growth from "[redacted]" intersection of bacteria + phage in H_2O + streak out.

Note, at intersections of bacteria + phage, a zone of coloration of the bacteria as if there were there some enzymatic activity.



← mucoid colonies. Y53M.

N21. Pick from red region + from mucoid colonies to water and streak out on EMB lac. (Y53/1).

Y53/1 same growth (probably resistants; ~~both~~ lac − + lac +) (standard type).

1. Y53M – all mucoid. pick one colony + test on T1 V^R
   also streak out →

2 $Y53M_2$ – all mucoid. P23. Streak out →

3 $Y53M_3$ – all mucoid. Pick to slant and label for subsequent analysis. Y86

Selection: Y40 x Y53.

Plate Y40 x Y53 as usual. At. 48 hours pick 5 largest (>) and smallest (<) colonies from each of 7 plates + compare the distributions

−R	−S	+R	+S.
4	1	0	0
4	0	1	0
3	0	2	0
2	2	1	0
2	1	2	0
2	0	3	0
1	0	4	0
18	4	13	0
27	12	30	1

In large, +R > −S.

In small +R > −R.

$\chi^2_3 = 5.14$ $p = .16$.

But. compare all 3 groups,

$\chi^2_4 = 12.61$

$p = .013$

selection may play a role.

Random selection from these plates gave:

27	13	13	1.
221	136	142	6

is cumulative data.

Note that both in in large + small selection types, there is a marked deficiency in −S as compared to random selection + cumulative data!