

Segregation of Viral Resistance

Prepare inocula P21. Plate P22 - also surface

A 24: - pick from colonies to minimal agar to avoid contamination
also test 15 directly.

	T1	T3	T5	T7	Lac B- M-	\times TLB, Lac-
A	✓1 R	S	S	S	T ₁ T ₃ T ₅	
	✓2 R ?	R	R	S		
	✓3 R	R	R	S		
	✓4 S	R	R	S	R S S	2.
	✓5 ?	R	R	S?	R R R	4
	✓6 ?	R	R	S	S R R	3
	✓7 S	S	S	S	S S S	2.
B	✓8 S	R	R	S	-	
	✓9 R	R	R	S	-	
	✓10 R	R	S??	S	R R S?	1
	✓11 R	S	S	S	-	
	✓12 S?	R?	?	S	-	?
	✓13 R?	R?	?	S	-	2
	✓14 S	S	S	S	-	

as demonstrated by Lac test!!
A1 and B1 were confused
should be:

A ₁	S	S	S	S	-	
	R	R	R	S	-	
	R	R	R	S	-	
	R	R	R	S	-	
	R?	R	-R	S	+	
	R?	R	R	S	-	
	S	S	S	S	-	

and there is only one
possible discrepancy! A₅.
dashed - R.
otherwise: 10R/14.

A ₂	R	R	S	-		
	R?	R	S	-		
	R	R	S??S	-		
	S	S	S	S	-	
	R?	?	S	-		
	R?	?	S	-		
	S	S	S	S	-	

Virus - Resistance segregation

357.

Y61 x Y53.

From same plating as 359:

	T1	T8	T5	T7	Lac		T1	T3	T5	T7	Lac
1	R	R	R	S	-	B	R	R	R	S	-
2	R	R	R	S	-		R	R	R	S	+
3	S	S	S	S	-		R	R	R	S	-
4	R	S	S	S	-		R	S	R	S	-
5	S	S	R	S	-		R	R	S	S	-
6	R	R	S	S	-		R	R	R	S	+
7	S	S	R	S	-		R	R	R	S	+
8	R	R	R	S	+	A	R	R	R	S	+
9	S	S	R	S	-		R	R	R	S	-
10	R	R	R	R	-		R	R	R	S	-
11	R	R	R	S	-		R	R	R	S	+
1	S	S	S	S	-		S	S	S	S	-
2	R	R	R	S	+		R	S	S	S	-
3	R	R	R	S	-		S	S	S	S	-
4	S	R	S	R	-		S	S	S	S	-
5	R	R	R	R	-		S	S	S	S	-
6	R	R	R	R	-		S	S	S	S	-
7	R	R	R	R	-		S	S	S	S	-
8	S	R	S	R	-		R	S	S	S	-
9	R	R	R	R	-		R	S	S	S	-
10											
1	R	R	R	R	R	R	R	R	R	R	R
2	R	R	R	R	R	R	R	R	R	R	R
3	R	R	R	R	R	R	R	R	R	R	R
4	R	R	R	R	R	R	R	R	R	R	R
5	R	R	R	R	R	R	R	R	R	R	R
6	R	R	R	R	R	R	R	R	R	R	R
7	R	R	R	R	R	R	R	R	R	R	R
8	R	R	R	R	R	R	R	R	R	R	R
9	R	R	R	R	R	R	R	R	R	R	R
10	R	R	R	R	R	R	R	R	R	R	R

R↓?

22R | 40. all T_1^R lac + $T_3^R T_5^R$

T_1^R lac - T_1^R lac + T_1^B lac - T_1^S lac +

21 11 18 0

Varic resistants

360.

strains sent to purify:

After Y-plateings, test again 12/10.

		T ₁	T ₃	T ₅	T ₇	Morph.	#
Y63	Y53/Muc from A.	Y57	R	R	S	Y53/3	M
Y64	Y53/1 from A.	Y58	S	SR	S	Y53/7	SR
		A	Y59	R	S	Y10/1/7	SR
			Y61	RS	S	Y40/7	SR
			Y62	S	S	58-161/3	SR
Y65	Y10/1/7 M from C.	58	S	S	S	✓	SR
Y66	Y53/3, 1, 5, 7 _M from D.	Y63	S	S	S	"Y53/1"	SR
	Y53/3, 1, 5, 7 _S from D.	Y64	R	R	S	Y53/1	SR
		B	Y65	R	R	Y10/1/7	SR
			Y66	RS	R	Y53/3..	SR
			Y67	SS	S	Y53/7	M
			Y68	S	S	Y53/7 S	M
Y67	Y53/7 M from E						
Y68	Y53/7 S from E						
						also ≠ T4, T6 R. probably contaminant.	

Prepare plates for:

58-161/3

58-161/7

Compare $\frac{B^-}{B^+} = \frac{50}{212}$ with $\frac{5}{20}$ on p. 364.

but much better information.

5 hour cultures, washed, mixed, and plated into various media.

Turbidity Medium. Colony ts. Mean m. d. Excess. R/pot.

±	O O O O O	217 193 279 234 137	{ 212 ± 34		1.00
++	B. B.	760		548	2.58
-	B	100 282	c.u.u. note thin agar layer	<u>50?</u>	≤ 1.
±	L	421 367	{ 389	177	.85
+	T	304 395	{ 350	148	.65
++	M.	0	Does not seem to be so turbid that growth should be inhibited !! Repeat w/ added proteolipids.		
+	BB.	764.		0.	
	BTL			-	
	ML	0		-	
	MT	0		-	
+++	BLB.	v. small cols.		?	
	BTB.	+++		-	
	MB,	0		-	
	MTL	0		-	
	MTB,	0		-	
	BL			-	
	BT.			-	

Y40 x Y53

263.

Reversion controls

Y53m:	T.L.	0	sub.
	T_B , L_B	12 2	++ + +

Y40	14	0	++
	13	0	+

Conclusions:

Plate count determinations may be in error due to variable numbers in cell density. B_1 seems to be a limiting factor in synogenesis. (Try it in $aB^+ + bB^-$).

B_1 independent, or linked to: $B^+; M^+$

B linked to M .

L independent?

T. independent or linked to L.

$\therefore B^-$ should be linked to Lac

and in this case, one may find that the B^- are ped.
lac + compared to B^+ .

Similarly $\in B^-$

$5/20$ B^- Exp. 10.

$$\begin{matrix} 5 & 15 \\ 10 & 10 \end{matrix} \quad \chi^2 = \frac{25}{10} + \frac{25}{10} = 5$$

$$p = .025. \quad \text{Need more data!}$$

Test colonies on A, B, B₁; T; L medium appropriately + segregate together various single mutants for lysis & form. tests.

10/31 - L - ?? -

		T_1^R Lac-	T_1^R Lac+ T_1^S Lac-	T_1^S Lac+			
46/48	B ₁ -	17 26	9 2	13 20	1 0	17 100	9 55
✓ 2130	L-	2	0	0	0	100	50
✓ 5/2a	A- B-	2	2	0	1	all recotypes	
✓ 4/78	** T-	2	2	0	0	of B ₁ , lac, T ₁ ^R .	

Prototrophs: See 362.

Prototrophs.

Ser 1.

4 - 4 - 6' 0

Ser 2.

25	17	15	0
24			

28 21 20 0.

G. Summary.
G. 357:

51	23	11	4.
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~~B₁- may be
deficient in T₁^R Lac+ class (parental type).~~

79	44	31	4
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G. 359

21	11	18	0
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49	55	41	4
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150	50		
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Preparation P24.

Plate Y55 (lactose - from Y53 + Y40) into lactose - minimal.

10^4 colonies too high

Gelatose mutant

366

Incubate 58-161 P24. Dennis. Nov 2nd 1948. Quad 1 min
Dennis.

20-24,000 colonies examined.

No typical gel - colonies. Several sectorial colonies + some rather
mucoid gel - were seen.

Sticks out on ~~lectose~~ EMBS.
~~gelatose~~

No mutants.

1 mucoid form.

Y53 x Y54. grow separately. Pick prototrophs

1. fermenter.

2-16 var. fermentans.

give to Tuffus.

Y10/1 x Y53 x 58-161.

"3"

368

November 24, 1946.

(Nogrowth) test prototyphs.

38 T₁^S Lac-

16 T₁^R Lac+

No { T₁^R Lac+
 { T₁^S Lac+

Sex conditions. Y4C x Y53.

369.

26 NOV 1940

a) Synthetic medium preparation ($T + B, B4$): -

much more turbid; no prototrophs. Occ. on surface. ca 10^8 ; ...

b) YB. 10^{-7} on surface. assuming in deep agar.

suggests YB better than synth. However, must be repeated!

plan

Inhalage:

11/24/46.

Y40 x Y53. Plate is growth with B₁.Pule to H₂O; test on B₁+, B₁-.

$$33/39 = B_1 -$$

$$\underline{6/39 = B_1 + = \cancel{5\%} \cdot 15\%}$$

Test B₁- for lac, T₁.

12/3/46: Tests:

T ₁ ^R lac	T ₁ ^R lac+	T ₁ ^S lac	T ₁ ^S lac+	T ₁ ^R	T ₁ ^S	lac+ lac-
1	3	3	—			
6	3	1	—			
2	1	2	—			
4	1	2	—			
<hr/>				13	8	0
						6 4

3-way crosses, etc.!

370.

26 NOV 1946

a) BT_L

BT } 0. 0
BL } B 10^2 turbid.

BT } 0 1 !!
TL } T \neq 0

BL } 0 0
TL } L 10^2 ? T

BT+TL+BL } 0 0 plague??
 } 0 0 These plagues??
 } B 10^2 turbid
 } T 0
 } L 0 ?

Plagues are probably a bacterial infection

bubbles

b) BB_B

BB } 0
BL } B ? turbid.

BB } 0 0
BL } B 0

BL } 0 - clear not turbid
BL } L 1?

3 } 0 0.
BT+TL+BL } 0
 } B 10^2 1 plague?
 } B 0
 } L plagues??

BB_B x TL. 0.

December 4, 1946.

Y53 x YO in YB. (5 growth) Plate in various test. Compare itNB } 0. ✓ 10² just as good as YB. do. grown in Nutritive Saline Glucose.

YB:

O
B
B
B
B,
B,
B,
T
T
T
T
L
L
L
L

BLT

BLT

BLT

BLT.

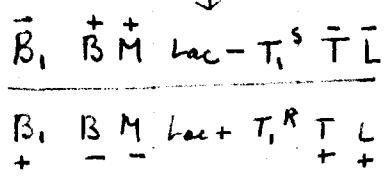
cont! ✓✓ colonies not so large as BLT.

B₁ B⁺ M⁺ lac- T₁^S T LB₁ B M⁺ lac+ T₁^R T L
+ -- + +Look for types which are B₁⁺M⁺, i.e. cross-over at lac operon.
and study progeny.

December 5, 1946.

$$10^3/10^9 = \underline{10^{-6}}!$$

YB. a) Plate Y40; Y53 on B₁ plate. Select colonies and plate entire undiluted culture into BMTL. If any colonies appear they may be either BM or the complementary recombinants.
PS. Test for leucine, any ... L- should be tested thoroughly. Use detection procedure?



complementary type is $\bar{B} \bar{M} \bar{T} \bar{L}$ and may have any lac, T, configuration, particularly lac + T_1^S .

b). Assuming that M is relatively far from L or T, so that (in 4-strand) 2 double exchanges can be expected to occur in this region, plate out for such exchange (e.g. -M, -T or -M - L [BB,L; BB,T] and examine for heterogeneity in lac or T_1 (particularly the former).

$\beta\beta B, L$: as above + v. turbid. do not use!
 below (37)

Lysisage -

Campase $B(P) \times Y53$ in (P) B_1 (cancel P- with proline)

and $BB_1 \times TL$.

12/5/46. $BB_1 \times TL$.

Expect. 12/9/46.

A. $BB_1 \times TL$.

O ~~#~~ 3 colonies. ?? coli.

B_1 No prototrophs. #

B. $B \times TL B_1$.

O }
 } No colonies.
 B_1 } rotten turbid!

~~bamboo~~. 4 strand fast

374.

December 9, 1946.

Y40xY53: into BB,L.(A) and BB,T (B)

h.g. like 375

bivalve

375

12/9/46.

Y40xY53. into PTL.

Cultures ca. 8 hours.
(too old????)

ca. 10 colonies. Latter inhibited by hybrid growth.

6315 x Y53.

376

December 9, 1946.

Y53 x 58-6315. (Biotin - "D-alanine?" + cysteine, isop.)

Gave a very high frequency ($5 \times 10^3 / 10^9$) of prototrophs; ca. same number of colonies on a D-alanine plate. To Earl

Find prototrophs on T₁-lac plates.

Earl - found ♀ - ... + eggs indicating separability of D, cyst. sp.

Complementary type

10 December 1946.

8 Dec. plate colonies of (453 x 440) from B₊ agar, into BLTM agar.Most plates have 1-200 colonies, & many non-proliferating B₊ in between.

12/10/46. Pick colonies a) to BLTM; BTM small tubes 10 tubes x 10 plates.

b) to BLTM large tubes (for detection plates)

Tests. (only BLTM+ BTM- or? recorded).

T₁^R lac - T₁^R lac^t T₁^S lac - T₁^S lac^t

Plate no:	1	0
	2	0
	3	0
	4	0
	5	0
	6	1
	7	1
	8	1
	9	2
	10.	2

378-1
-2
-3
-4, 5
-6, 7.

/

c) Pick colonies to EMBS lactose (1 plate):

	15 +	(8)
	4 -	(9)

1	B _M
2	B _M
3	B _M
4	B _M
5	B _M
6	B _M ?
7	

December 13, 1946.

Plate following arindri.

- A 1. Y53 x Y40.
 (Shoma)
- B 2. Y64 x 58-161
- C 3. Y65 x 58-161. (Y106/7 x 58-161).
- D G.
- E 4. Y67 x Y40
- F 5. Y53 x 58-161 Y68.
- G. 6. Y67 x Y68.

most morula too large

Best method: surface spreading!

A: Yield rather low! B: too turbid. CTC OK but \gg than o.

B: also too heavy. v. low yield.

C: (0. none β_1 : ca 20 \pm very wide zones of stimulation)
 $\underline{\text{all dep.}} \quad (\text{certain?})$

D 0 when no. enough venoc.

E $\approx 10^2 - 10^3$ colonies. Not very much like coli, but test on EMB lac all mucoid, lac +

F O.

December 16, 1946.

- 12/16. Use B_1^- / BMTL plates of exp. 378. Pick colonies from fettered plates to EMB-lac to eliminate bac+ which from 378 are probably B-14-.
Streak out bac- colonies on EMB-lac to obtain pure cultures & avoid pitfall of *Syntrophus*. Test on: ~~BMTL B,MTL B,BTL B,MTL B,BMTL~~
~~BMTL, MTL BMB, TLB~~

A. 14/15 +

iac- ~~m~~

B. Norwegian

$$C. \frac{8}{8} = \boxed{1}$$

D. 1/1 -

$$-1 \rightarrow b_{\alpha\beta}$$

F 8/8 -

1

G 44 -

1 (vac⁺)

H 4 /12/

J. 111 -

4

K 6/6 -

1

L

M 1/5 -

8

Page 1

380 - D1

380 - E2 (an, o)

380 - F2

378-8

378-9.

See 380.

Small colonies.

	-	Lac	-
C1	B ₁	-	-
C2	B ₁	-	-
*	D1	M(T)	✓
*	E1	B ₁	-
*	E2	(T)	-
*	F1	B ₁	-
*	F2	(B) M	-
*	G1	B ₁ M	-
*	H1	B ₁	-
*	J1	B ₁ M	-
*	K1	B ₁	-
*	M1	B ₁	-
378-8	B M	-	-
378-9.	(B?)	-	-

Pick small colonies to colic ∞ , v. $\frac{1}{10}$

albat

check on D1

E2 μ
F2.

	T	F	C
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			

$$- - - + + + + - (+)$$

January 5, 1947

1 ml, 36 hour broth cultures in YB agar

+ff = unif. turbidity.

Y40	1 ml	A
Y40	.1 ml	B
Y55		C
Y53	.1	D

Peflavin

1:10 ³	A	B	C	D
1:10 ⁴	+ff			
5	"			
6	"			
7	"			

n.g. still from broth

Crystal Vol. 1:10³ do. do. ca 10³ cols.

4	+ff		
5	"		
6	"		
7	"		

Peflavin is n.g. under these conditions

Survival & crystal violet is OK in range 10⁻⁴ to 10⁻³.

This should be extended. Unwashed cultures?

January 9, 1947.

Incubate in flask, varying times. Dose YB-1 ml Also plate on EMB .01 ml undiluted cultures

	S 1/10	S.	pS.	
0	+++			
15 sec	+++			
30	++±			
60	can $\times 10^3$	10^5	#9	3?
120	cap $\times 10^3$	10^5	#10	4
300	0.15	10^3	6	non-papillate?

P5/10 - ca 1/15 sec.

i.e. after 15 sec. kills

10% of the variants
survive.

non-papillate?

P10 Dilute 120 sec.: 1:10⁷ on EMB plates + spread.

P14: Pick colonies which seem to be non-papillate. Sample is not clear-cut because plates are crowded and entire population could not be screened. Estimate ca 5-10 $\times 10^6$ tested to fresh EMB for further test. 50,000

Pick 6 colonies to YB slants which seem to be non-papillate.
1 is mucoid.

Jan 9/47