874/377.
377
1251

(This Y64 stock is substantially L)

as B_1 | $\bar{-}B_1$, ca 690:302: 2.25

Lac, V_1.

Compare 524
184:75 ~ 2.42

$\chi_1^2 = < 1.$

T(0).

-R	-S	+R	+S.	
7	8	1	4	
5	13	0	7	
5	8	1	10	
5	13	2	4	
22	42	4	25	/93

no difference in distributions

T(B_1).

8	6	3	7	
10	12	1	4	
5	17	1	4	
2	3	0	3	
7	13	2	5	
32	41	7	21	/101.
54	83	11	46	/194
in %: 29	43	6	23.	

(30 u.)

B_1	ϕc	Lac	V
−	+	−	R
+	−	+	S.

↑ +S 23 ↑ −S 43 −R 29.

−S/−R should be same as before. ✓

Colony description

May 28, 1947.

Streaked various cultures on EMB:

58-161	A24 (16h.) L+S	ca 1:5	→ all S. → all L.
Y40	(do.)	purify to Co^L and Co^S lines.	
Y87	all S. ??	→ S.	
Y53	all L? Pick 1 large	→ all L? some S??? →	
Y10	L+S	ca 10:1. Purify.	→ L OK. → S not sharply different from L.
Y64	(all?) large.	Exc.??	L → OK. Pick for stock. S? → somewhat smaller than Y64L
Y46.	(all?) large.		
Y94.	Predom. L.	Some small?	

L = large "rough"

S = small, "smooth?"

Y40 S from BM synth. all S.

on EMB, Lac+ S do not show a gum sheen
Lac+ L do. ; particularly in (Y10)

Take L colonies for new stocks. label as Co^L and Co^S respectively.

Y40/6. all small.

Y87/6. large (somewhat mucoid) and small. →

(and Y87L). Ac empaco Y87S × Y10L with Y40S × Y94L
58-161S × Y64L.

Some produce less sediment, more pellicle in broth

Effect of colony dimorphisms on segregations

May 29, 1947.

A) Y40·Co^S × Y53.[Co^L] B) Y40·Co^L × Y53. [Co^L].

all T_1^S. This argues for an error in the setting-up of the experiment! Test Y40 suspensions which were kept!

A. large colony selections:

Lac−	Lac+
17	6
20	4
9	2
20	5
17	5
11	6
84	28 / 112

small colony.

Lac−	Lac+
7	5
14	8
17	5
41 38	15 18 / 56.

81	84	31.	28	/ 112
122		46		168

together: 122 : 46 / 168

$\chi^2 = 9\left(\frac{1}{38}+\frac{1}{15}+\frac{1}{31}+\frac{1}{81}\right)$

= .026
.067
.032
.012
9 × .137
= 1.2

p =

b).

9	8	16	3	
17	6	14	7	
13	8			
14	8			
12	11			
16	6	30	10	
19	2			

b) 104 100 49[45] / 149. $\chi^2 < 1$.

p = ...

A) 118 122 46[50] / 168

222 / 95 317

May 3[illegible], 1947

A. 58 161L × Y64L.

B. 58 161S × Y64L

C. 58-161 S × Y64S.

[D.] 58-161L × Y64S.

Stock parents:

58-161 S } indistinguishable!
58-161 L }

Y64S } occasional L.
Y64L } ca 1:1 S:L.

Test parents as T1. [. also "Y40" from exp. 536.

a : a' ca 1:10 in frequency.
(T(0) : T(B1)).

B : B' do. lower frequency of prototrophs. May due to suspensions.

C very few recombinants. (S×S)

D. same. [Y64S v.g. for recombination ???].

June 3, 1941.

Y100 x 58-161. [Y53 - V_{1T}^{R} x 58-161 V_{1T}^{S}]

plates of V-test today forward first. n. g.

T(0).

-R	-S	-R	-R	-R
-R	+R	+R	-R	-R
-R	-R	-R	+S	-R
+R	+S	-R	-S	+S
+R	-S	-R	-S	-R
-R	-S	-R	-R	-R
-R	+S	+R	-R	+R
+R	-S	-R	-R	
-R	-R	-R	-R	
+S		-R	-R	
+R	Put on.	-R		
-S		+R	-R	
+S		+R	-R	
-S	6S:3R.	-R	-R	
+R		-R	-R	
-R			-R	
-R			-R	
			-R	
			-R	
			-R	
			-R	
			-R	

V_{1T} is therefore either 30 rel. units to the left of TL, just right of V_{15}, or to the right of TL. This could be settled by studying interaction ; Lac. This favors the V_{15} - TL position. Or, in the cross BP x Y100, a

B	Lac	P	change in V_1

segregation would situate an intercalary location.

T(B_1).

June 13, 1947.

T(0).

-R	-S	+R	+S.
9	7	1	4
2	3	2	1

T(B_1).

1/2	8/9	0	2/5

Total:

-R	-S	+R	+S
14	22	3	12

S = 36/51 = 70 %

R = 30 %.

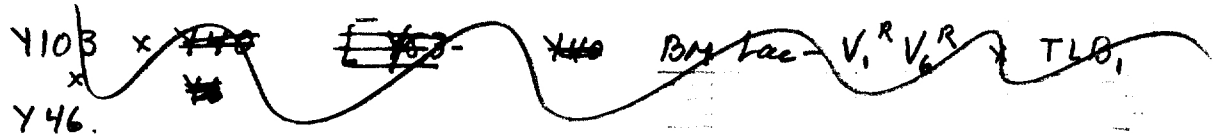
B_1	BM	Lac	$V_{15}^{R.}$	V_{1T}	TL	V_{1T}
	−	+		T	+	.....
	+	−			−	.... V_{1T}^{R}

June 3, 1947.

Reversion of B/1. Tryptophane requirement.
Plate ca 10^9 cells/plate of B/1 into T(0)

on one plate, ca. a dozen colonies on surface. [~~probably~~ contaminations].
~~Reconstruct with other phages~~ ∴ no revertants

Segregation of V_6, V_1 539



Y103 x Y10

no T_6 sensitives seen; phage probably s.g. (see text) $m_{G_1 G_2}$

for T_1 and Lac:

	−R	−S	+R	+S.	
T(0)	2	0	4	6	
	2	0	2	7	
	2	0	4	4	
	6	0	10	17	
T(B_1)	2	0	3	8	
	2	1	8	3	
	0	0	4	6	
	0	0	0	4	
	1	0	3	7	
	2	0	5	2	
	2	0	3	2	
	13	1	26	32	
	19	1	36	49.	

OK.

Sex activity of smates.

Prepare Y40, Y53 suspensions as usual in ~~Y53~~. saline. Treat in sonic apparatus for 1 hour. X = Hd; C = control. Add ½ + ½ + plate ½ ml.

a.) Y40X in N.A.
do. 1:100 $> 10^4$

b). Y53X in NA.
do 1:100 $> 10^4$ "

c). Y40X × Y53C ++

d). Y40C × Y53X ++

e) Y40X + Y53X ++

f.) Y40C × Y53C. +++

g.) (Y40C × Y53C) + (Y53X × Y40X) [illegible] ++

V_2^R.

5-4-1

June 4, 1947.

T2R – stock received from Luria. titre = 10^9 when made.

plate covered 5 colonies

Cla - titrate

Plate 58-161 on NA + Cla:

		12h.	24h.
1.	0		
2.	200 r/ml	no inhibition	
3	400	colonies somewhat smaller; no papillation	v. sl. inhibition:
→ 4	600	marked inhibition; no papillae	large and small colonies.
5	800.	very marked inhibition. colonies pin point minute	all single colonies are small; papillae in streak.

36h.

400

600 colony dimorphism ◌ ○. visible papillation

800. colonies very large or very small. ○. distinct papillation.

Range between 5 - 700 r/ml probably optimal. Use mutagens??

June 6, 1941

Rec'd from A. Boivin, lysogenic C_1's and C_2's, recorded as Y105, Y106 respectively.

a) Test on Cla - agar: papillae found; streaked to purify and test for aerogenesis.

b) Test on T(0) - grows well both on liquid + solid T(0).

c) Lac - both strongly Lac+

d) Phage reactions:

	T1	T2	T3	T4	T5	T6	"T7"
Y105	R	R	R	R	R	R	R
Y106	R	R	R	R	R	R	R
K-12	S	S	S	S	S	R	R

! cf. Hershey!

∴ available phages are n.g. find more?

Test Cla^R types on glucose fermentation tubes. (pick 5 colonies / plate streaked ē a Cla^R papilla

gas →

105	A	+	+	+	+	+	
(C_1)	B	−	−	−	−	−	Y106 = C_1 Cla^R S
106	A	−	−	−	−	−	Y105 = C_2 Cla^R S
	B	−	−	−	−	−	Y104 = C_2 Cla^R S.
(C_2)	C	+	+	+	+	+	

d) Sucrose: no definite fermentation by either. Both show slight papillae in region of second streak. When these are streaked,

Transformation Expts.

June 10, 1947.

a) Prepare extract + sterile filtrate of a 24 hour culture of Y105 in YB.
Filtrate: sediment cells in centrifuge; sterile-filter.
Extract: Suspend cells = ca 200 ml in 10 ml H_2O. Treat sonically for 2 hours at 0°C. Sediment debris + emulsify supernatant with benzene overnight. Remove sediment + excess benzene; remove benzene in vacuo. Should leave a sterile preparation. —
add 1:10 to YB tubes for assay.

b). Inoc Y107, 108, 109 into glucose-gas tubes + 1:1 filtrate.

24h.	107	—
	108	—
	109	—
	—	no acid

c) Inoc 107, 108, 109 into YB + filtrate. grow 24h. Use this to inoculate heavily gas tests:
6h.

(Transformation?!)	107	—
	108	—
	109	±

d. Streak out 109 (da); 109/Fb (db); 109/Fc (dc); 109/Fc/test (dd) on Cla plates to detect sensitive colonies.

ca 20% sens. ~~Sens. not!~~ sensitives noted!

e. Sucrose — all sucrose — on second transfer on EMB plates.

June 13, 1947.

		gas	
Y109.	in glucose broth	–	
		–	
		–	
		–	
Y109	in YB.	–	
	then glucose	–	
Y109	in YB + 1:1 filtrate of Y105	–	see previous test for +.
Y109	in YB + 1:10 extract of Y105	+	
		+	
		±	
	1:10 extract Y105	–	no acid.

∴ It appears very likely that a transformation has been effected from Ac^- to Ac^+.

Lactose mutants of Y106. 546.

June 1/, 1947.

Inoculate a washed suspension of Y106 in saline: ca 4 cmm. from 1 ml into YB; incubate 24 hours. Plate out on EMB lactose.

ca 500 x 58 = ca 30,000 colonies examined.

No clear cut, smooth, Lac- seen. (possibly due to transformation - reversion.)

About 8 possible, small-colony Lac-? were marked for retest.

Also pick a number of small colony types in hopes of finding an R_2 transformable to S_1.

Pick 40 small 10 large colonies to small tubes of YB, and sort on basis of "autoagglutination".

a) large colo: all dispuse 10/10.

b) small colo. 23 clumped 17 dispuse. discard dispuse and incl. clumped types into large YB tubes for further tests.

In general, growth of these types is poorer.

of 23 clumped in 1st test, 15 do not show dispuse growth on second in large tubes.

4 good roughs 546- 1, 2, 3, 4

Tatian in S_1, S_2.

Treat washed suspension of Y106 with 4.5% Na deoxycholate (DX) for 3 hours at room temp. Slight lysis observed. Wash & inoculate into 10 ml YB broth + 1 mg. deoxyribonuclease. Incubate overnight and plate for mutant detection P21.

A23 – look over for preliminary small colonies. Overlay with: agar 1.5%, N Z case 2%; Y. Extr. 1% 11 A23. The colonies of this form are extremely uniform, and less than .1% of the original sample are abnormally small, e.g. ● ● normal. <.1% are ≤

○ red.
11 picked

Examine for small colonies.
ca 250 colonies/plate × 26 = 6,500 tests.

P23. X red 16 picked
A24. 1 red 8 picked. 6 small YB.

Grew:
○
X
Δ.

Test on T/O)

Spread treated suspension on EMB lactose. 82 × 100 cols. = 8000
examined

		T(0)		
Δ	1	−	Y110	
	2	−	Y111	
	3	−	Y112	
	4	−	Y113	
	5	−	Y114	valine ; isoleucine
	6	−	~~Y115~~	
	7	++		
	8	−	Y116	
X	11	−	Y117	arginine
	12	±		
	13	++		
	14	++		
	15	−	Y118	arginine.
	16	−	Y119	
	17	−	Y120	valine isoleucine
	18	++		
	19	±		
	20	−	Y121	cystine
	21	−	Y122	
	22	++		
	23	±		
	24	−	Y123	
O	31	++		
	32	−	Y124	
	Y106	++		

otherwise grew on minimal or second best on rot E. coli by L. R.

∴ at least 18/24 mutants.

18/8000 = .5%.

Requirements identified by L. Rodriguez

Later:

133 Lysine
138 leucine
139 histidine
} from 118 ∴ arginine −

141, 142, 143: arginine from 121 ∴ cystine.

S_1 and S_2 Bovis

[June 20]

Chemical test for iodide:

Take 10 ml. suspension of S_1 + S_2; sediment and suspend in 60% HCl + 1:10 1% alcoholic napthoresorcinol. Boil 1 min. Let cool; add 1 ml ether shake + examine.

C_1 - red color at interface

C_2 - no color.

Both show a green fluorescence in aqueous phase.

Test for Sucrose fermentation

a) on plates (EMB). - C_1 + C_2 both negative

b) in liquid - C_1, C_2, Y109 all negative after several days.

Acetate utilization:

after 4 days - Y109 ∓

Y106 +±

C, 1P. 549

DX- alcohol procedure.

June 23, 947.

36 hour culture of Y105 : 600 ml YB in 1 liter flask.
Shake at 30°.

Sediment + suspend in 20 ml 4.5% DX. Add benzene and shake at 25° from 1230 P23 to .

P23. Sediment and remove debris + ~~[illegible]~~ benzene phase by filtration.

~~add 10 vols. 100% Alcohol.~~ Collect sediment in a ~~[illegible]~~ tube.

Sedimentation required ca 5 hours, due to thick emulsion. Possibly pH too low. Supernatant collected.

same DX in solution.

Upon addition of alcohol, a thick fibrous ppt. formed. Probably consists largely of desoxycholic. Sediment and resuspend in alcohol to dissolve desoxycholate. Sediment (easily done in centrifuge). Supernatant ppts in aqueous 6.8 buffer. ∴ probably desoxycholate

Try dissolving sediment in H_2O. OK - very viscous solution.

R_2: In second ppt attempt, add a few drops of NaOH to prevent ppt of NaDX & alcohol.

Test R_1; R_2 on Y109. no gas + produced.
sterility - ok.

medium.

(Repeat, omitting DX)

Transformation.

June 26, 1947.

Y109 in YB + 545 extract.

tests on glucose tubes.

sterility	—
Y109	—; —
Y109 + TP	+ +

transformation OK. sterile acid on Cla.

Preparation of TP: alcohol procedure.

Autolyse 500 cc ≙ Y105 in 15 ml N/5 CC .9% + 1 ml benzene at 30°.
Sediment + separate extract.
550 - X1 aliquot centrifuged free of cells. Shake with benzene & store overnight at cold room.

550 - X2. Add 6 vols. 100% alc. to extract. Ppt ca 5-10 mg of material.
sediment transfer to sterile tube; ~~sediment~~ resuspend in 100% alcohol for 3 hours. Sediment and redissolve in H_2O. 1 ml ≙ ca. 25 ml culture.
Add 1:10 to YB tubes to test for sterility and activity.
Inoc with Y109. After 16 hours, add 1 2 ml culture to glucose tests.

1. Y109	-
2. Y109 + X_2	+
3. Y109 + X_2	++
4. Y109 + X_2 + 1 mg DRNase	-
5. Y109 + X_2 + 1 mg DRNase	+
6. ~~—~~ (sterility control.)	

all were inoc ± X_L and 109!

inoc. ± 109 in exp. ±

June 27, 1947.

1. Y109
2. Y109 + X_2
3. Y109 + X_2
4. Y109 + X_2 + 1 mg DRNase
5. Y109 + X_2 + 1 mg DRNase
6. Y109 + X_1
7. Y109 + X_1
8. X_1.
9. X_2.

Preparations from 550.

June 21-28 1947.

Prepare extract from 500ml of Y105 by alcohol ppte. method, after toluene autolysis 3½ hours. Add extr. to 10 ml YB.

		2-3 hours test
1.	109	+++
2.	109+ X	+++
3.	109+ X	+++
4.	109+ X + DNase	+++
5.	109+X + DNase	++
6.	X.	

not sterile? – non very slow growth on transfer to glucose test.

P 28. Add 10 vols alcohol to remainder of X to sterilize. 1 pt + store sediment in 70% alcohol.

"109" inoculum?? probably in error. · Letters: