

Dec. 9, 1947.

See (51a)

Y40 x W-1.

Plate V. dilute on EMB lactose (B₁). and on EMS Mal ± B₁.

Look for sectoring. Count only clearly scored in unscorred portions from 20-100/plate. Yields much higher on Mal.

M(O).	+	-	sector.
1.	2	32	0
	1	38	0
	4	32	0
	3	25	1
	12	117	1
	22	244	2
			288.

v. clear sector. (1)

2. M(B₁). 15 285 5

seems to be fairly frequent among Mal +. However, plate is too crowded for accurate estimation.

3. Lac B₁. Yields lower than on Mal. (Mal contaminated with amae, etc?)

	+	-	Sec.
1	1	9	0
1	17	32	0
2	16	16	0
3	17	27	0
	6	33	0
	6	18	1
	12	21	0
	9	14	1
	9	16	0
	0	3	0
	99	189	5.

2? 1? { Not clearly duplex. Maybe all lact. Almost certainly not contain. v. clear (⊕)

Streakout mosaic.

Test remainder of population to get complete score on lac, Mal + V,

Supplementary Recombinants -
Maltose Segregation.

5/2

Dec. 9-14, 1947.

Y4/O x W-1.

lac, Mal ∇ I^R segregation.

Plates very dilute on EMS agars. & look for sectoring. Scored by inspection of plates.

1. M(O).	Mal+	Mal-	Sectoral	Sum
	22	244	2	268.
2. M(B ₁)	15	285	5	305.
Sum.	37	529	7	573
Mean.	.067	.9250	.012	100.00.

About 8% of colonies carry Maltose+.

3. lac(B ₁).	lac+	lac-	Sec.	Σ
	99	189	5	293
\bar{M}	.338	.646	.017	

(B) Sample colonies from lac to Mal & Mal to lac.

B.	From 1. to Lac.	lac+ R	lac+ S	lac- R	lac- S.	Σ
1	Mal+	4.		12		16
2	Mal-	45	1	66	7	119
	From 2. to Lac.					
3	Mal+	10		6		16
4	Mal- No tests.					
	From 3. to Mal.					
5	lac+	1	0	66	3	70
6	lac-	0	2	89	21	112.

Phage scores probably unreliable from appearance of ~~+~~ sensitive. Not too good a fit with 3AD. 39% lac+

Compare R/S with 82!

Scores on 51 segregants.

S1A.

Maltose, ~~Agar~~ agar, minimal.

Phage scores uncertain.

Maltose +. 12 Lac- V_1^R
 4 Lac+ V_1^R .

Maltose +. B. agar. 10 Lac- V_1^R .
 6 Lac+ V_1^R .

Maltose +. 22 Lac-
 10 Lac+

Maltose -

Lactose B. agar.

Lact.	Malt+ R	Malt+ S.	Malt- R	Malt- S.		
	1		14	1		
			7	1		
			9	0		
			10	0		
			2	0		
			8	0		
			9	1		
			7	0		
	1	0	66.	3		70
Lac- →	0	2	89	21		112.
			7	1		
			8	1		
			6	0		
			6	3		
	1		4	1		
	1		7	0		
			2	0		3
			15	3		15
			8	3		6
						0
						4
						5

M -	lac+R	lac+S	lac-R	lac-S:
	12	5	5	
	3		2	2
	3		1	
	5		3	1
	6		9	
	3		9	
	4		7	
	4		4	1
	4	1	7	1
	2		10	
	3		8	1
			1	1
	45.	1	66	7

Phage success un-
reliable

Accumulative data on maltose:

	-	+	
a +: -	272	15	
	244	24	
	285	20	
	801	59	860.

$$M+ = 6.8\%$$

Lact: -	in Mal +:	15 :	13 Lact +:	9 Lact -
			22	10
			35 Lact +	19 Lact - / 54

The 2.4% triples compared to 4.4% singles imply a map distance ca. twice that found on the basis of the Lac, V data. A crossover in the Mal region may, by interference (a) ~~conclude~~ favor additional crossovers to make unrecoverable that chromosomal, or (b) argument the relative frequency of triples.

Dec. 9, 1947.

Spread W-1 and T1 on EMB-Maltose plates to select for spontaneous T1 resistant mutants.

Numerous, well-defined smooth W-1/1 found.

Streakout one such colony to provide (W-54) Reptile. (1)

Test 70 others. all ~~are~~ resistant to TS.

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W-45 (Lac₂- B₁+ Lac₁+ x Lac₂+ B₁- Lac- Mal-)
x
W-1.

Plate dilute on B, EMS Lac.
Numerous Lac+.

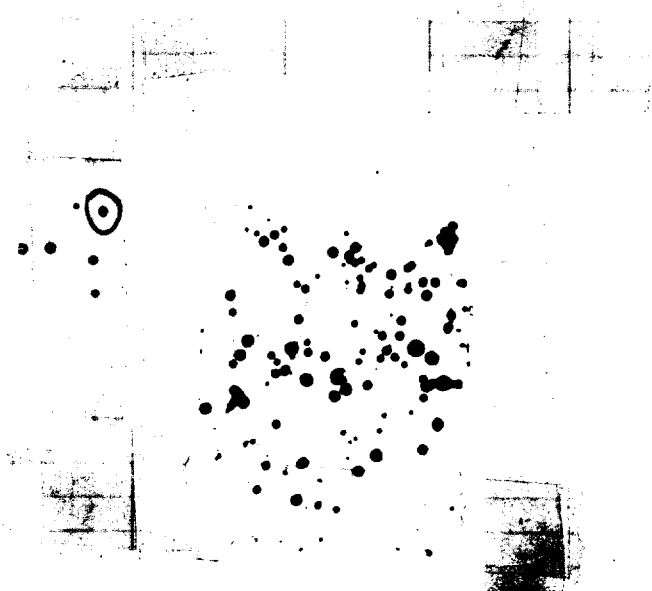
+ 32 - 68. | 100

plates still rather crowded in some cases.
Some probably valid recombination noted.

Streak out lac- on lac agar to look for
irreversible lac- types.

Pick single colonies to maltose
plates to score Mal and to
provide cells for reversibility
test.

of 42 Lac- tested, #41 Mal-
1 Mal+



Test all of these for Lac reversibility on lactose .05% medium.
All reached variable turbidities with heavy inoculum. Test by
loopful streak on Lac EMB. All+ except number 5.

Keep as (53-5) If this is irreversible, regard as Lac₁- Lac₂-
and test by recombination tests.

Streak out, on EMB lac

all produced papillated colonies, although only one
rec. colony of W-45 was a papillate.

53-4

53-5

53-6 - note that two colonies were non-papillogenic. Retest!

453

W45




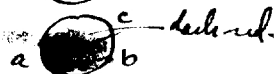
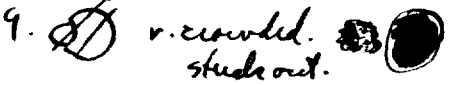




53-5
revert!

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Irradiate .1 ml Y40 per EMB plate, on plates 10 sec. u.v.
Hanover lamps




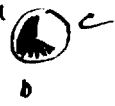






Irradiation by D.G.

Lac. 59 plates. ca 200 readable colonies/plate = 12000.

1. Crowded plate  maybe juxtaposition. 8. 
2.  v. clear sectoring.
3.  9.  v. crowded. stuck out.
4.  small colony.
5.  v. small crowded plate
6.  both pure white. separated.
7.  v. small.

Mal. 45 plates.

= 9000

11.  Thin sector, scarcely distinguishable.
12.  Mutant sector "wh" at best. Streak out entire colony
13.  Clear, small sector. Streak out mainly of sector.
14. sector indistinct
15.  16.  17.  sector indistinct.
18. intact white. 19. intact white 20. intact white
21.  22. Like 12. 23.  24.  intact white
25. 

Classification of Mal + Lac mutants -

54A.

Dec. 13, 1947.

11. Dark + dull blue colonies noted. Streak separately in effort to find destruction
12. Some colonies light brown in transmitted light. As above.
13. Mostly - W56 Same + W-57. Also some v. light ±
Restrict these.
14. Some definitely ± compared to stand. Re.

54-15 Revi.

54-16 Revi.

17 No marked variation.

18-20 Revi.

18: W60
19: W61
20: W62

22. As 11.

23. As 11.

25. Mostly - . A few + in sectors. - W58
+ W59. Restrict.
+ 's in form of sectors.

Dec. 15, 1947.

54-11 Reticulate. Reject.

54-12. No difference noted.

54-13. 3 types noted.
 - W56 } Repeat comparison
 + W57 }
 ± W63 }

54-14. a) Mal +

b) Faded in part of streaks. Reject.

15. Turned Blue. Reticulate ~~Mal~~ Mal - like a. Reticulate W71.

16. Reticulate.

21 Reticulate

22. Reticulate.

23 Reticulate.

24. W64.

25 W.59 (Mal+). No sectors. Pick to slant.

1. + and - colonies. - W65
 + W66

2. 2+ 2- sets. - W67
 somewhat systematically - W68
 contaminated. + W69
 + W70

3. Indistinguishable parts

4. ~~Foot?~~ + and - - W72

5. ~~Foot?~~ + and - - W73
 - W74 + W75

~~6. a + b. W76 W77. Maybe sets.~~

7. + and - Mostly - - W76
 + W77

8. Reticulate.

9. all +.

Dec. 16, 1947.

- 16 two types of colony.
a. + W79
b. - W78

22- a. + W81
b. - or ± W80

+ weals? Definitely less than a.

8 - a. Mostly + colonies. W84
b. = Apparently - colonies } Pick all of these W83
c. " " " " W82

23. Indistinguishable.
1. Indistinguishable

15. b. = H alt. W85.

a. + and - . Probably mixture. - W71a.

c. = W71

Dec. 8, 1947. and earlier.

Streak out various Lac- strains on synthetic lac agar to select for reversion. Streak from reversion colonies directly into β -galactoside and read after 48 hours. Original responses from previous data.

#	Strain	ori.	Reversion		Original responses from previous data.							
			a	b	c	d	e	f	g	h	i	
1.	Y53.	+	+	+	+	+						
2.	W35	-	±	±	+	±	±	+	+	±	±	
3.	W30	+	+	+	+	+	+	+	+	+	+	
4.	W36	-	±	±	±							
5.	W40	+	±	±								
6.	Y87.	+	+									

Isolate on Lac EMB the W35 series.

- 2: a. Only an apparently "weak" positive on lactose. Keep.
 b. Lac++
 c. Mostly ++. Some Lac-
 d. Lac++
 e. Lac-
 f. Lac++
 g. Lac++
 h. Lac-
 i. Lac-.

Types.	a	b	c	β gal	Lac
	±	-	+		-
	-	+	+		+
	+	+	+		+

Compare:

	β gal	Lac
S8-161 W35	+	++
W-35 W35	-	-
a	±	±
b	±	++
c	+	++
d		
f		
g		
h, i		

libre b
 libre c
 libre c

Keep on slants.
 Also compare \bar{c} W-35 on
 lac ~~can infect EMB.~~

Dec. 15, 1947.

Y132: on Arginine T(0) +:

Proc. P15.

- | | | |
|-----|-----------------|------|
| | | A16. |
| 1. | - | - |
| 2. | - | - |
| 3. | NEA | - |
| 4. | EAA | - |
| 5. | N+EAA | - |
| 6. | HC | - |
| 7. | Y _{ox} | +++ |
| 8. | V, TS | - |
| 9. | HC+V, TS | - |
| 10. | Glycine | - |

Yeast Extract Mutant?? try N₂ case, Nucleic acid.

U5-1, 2, 3 on: Valine T(0) +

1. -
2. EA
3. NA
4. V, TS
5. Pur+Py.

1st reading A16

- | | |
|---|-----|
| 1 | ± |
| 2 | +++ |
| 3 | + |
| 4 | ± |
| 5 | ± |

not coli

3
-
-
-
-
-
-
} No growth on any of these!
see 45. W-1.

no mutant. Grows on T(0), + all T(+ amino ac.) except isoleucine which seems to inhibit it! ^{ess.}

U-1A. on T(0) +

1. Y_{ox}.
2. V, TS.

- | | |
|-----|---|
| A16 | inc. at 22-28° (surface of water bath at 37). |
| - | A18 |
| - | ++ |
| - | ++ |

U-1' on T(0) +

A16
++

1. -
2. 2.5, 25 µg/ml CAB (2-chloropal from Strandkov)

+ ~~++~~

3. do. + methionine 100 µg/ml.

+++

4. do. + palb .1 µg/ml

+

YNA. Preparation. Dissolve 25g. Schivany Nucleic acid in 125ml H₂O + 11 ml 28° NH₃ water in 500 ml flask. White stopper and autoclave at 15 lbs.

Dec. 15, 1947.

Test the following on trehalose EMB. 16h.

	Tre.
Maltose + : Y40.	+++
Maltose - : W1	+++
W3	+++
W21	+ weak.
W60	+++
W61	+++
W62	+++
W56	+++
W64	+++
W58	+++.

Trehalose is, therefore, attacked by Maltose negative mutants. Cross adaptation should be checked! ✓

Dec. 18, 1947.	Tre	Mal
W-63	+	+
W-71	+	-
W-78	+	-
W-80.	+	+

all maltose-negative mutants so far found are Tre- +.

Dec. 17, 1947.

T(0) +.

		A17 (16h.)	A18.
1. Y. Ex. .5%		+++	✓
2. " .05%		++	✓
3. " .005%		±	✓
4. " .0005%		-	✓
5. Y.N.A. .5%		-	++
6. N2 Case .5%		±	+
7. -		-	-

N2 Case is much less active than yeast extract.

YNA has some activity - only ca. .1 - .01 of yeast extract.

~~Try Casein, Acetate, other protein hydrolyzates, e.g. gelatin; lactalbumin; fat-solubles. incl. Lecic acid.~~

T(0).

1. -
2. Y. Ex. .1%
3. N2 Tone .5%
4. N2 Case .5%
5. N2 Amine B .5%
6. N2 Amine A .5%
7. Casein .5%

~~Suspend 10g. Y. Ex. in ca 30ml CHCl3. After 1 hr. filter. Evaporate CHCl3 from extract and take up in # 20ml H2O. Do residue, taking up in 200 ml H2O.~~

Lac-1 and Lac-2 mixtures

Dec 18, 1947.

Make up 10 ml tubes of lactose 1% BCP broth.

Add .5 ml inocula of :

Set up 2P16

		6 P 16	10 A 16	18	A18	*
1	W-45	-		18	++	*
2	W-45	-		18	++	
3	W-54	-		18	-	
4	W-54	-	The Same		-	
5	W45 & W54	-			++	*
6	W45 & W54	-			++	
7	K-12	+++				
8	K-12	+++				

Therefore mixtures of Lac-1 and Lac-2 are unable to utilize lactose, although recombinants are able.

* Streak out on lactose. Probably reversions
Mostly + colonies. Streak ~~out~~ out to get W-45^R for allelic tests.

Dec. 17, 1947.

Harvest ~~the~~ W-45 (Mal~~/~~ Lac-1~~/~~ Lac-2-~~)~~ and W-54 (Mal-Lac-1-Lac2~~/~~ V₁^r) from fresh YP cultures, and mix at a conc. ca 10^{10} /ml each in water. Store overnight in refrigerator. Dilute to 10^3 / ml. and spread .1 ml on EMB-Lac (NZCase) plates to detect~~ed~~ possible Lac1~~/~~Lac2~~/~~ recombinants.

12/18 PM. 111 plates x 357/2 totalling ca. 40,000 colonies examined.

None were Lac~~/~~. This is a control on the reversion of both Lac-1 and Lac-2. The recombination rate under these conditions is apparently too low.

Dec. 18, 1947.

dec. P19

Inoc. into T(A) \neq

	A19.	P19.
1. Y. Extr. .5%	+++	✓
2. Y. Extr. .05%	++	✓
3. YX Residue .5%	+++	✓
4. YX Residue .05%	++	✓
5. YX Extract .5%	±	✓
6. YX Extract .05%	-	✓
7. Gelatin Hydrolysate .5%	±	+
8. Tomato Juice .5%	++	* ✓
9. Casein .5%		
10. NZTone .5%	+++	✓
11. NZAmine A .5%	+	++
12. NZAmine B .5%	±	±
13. YNA intact (NaNucl.) .5%	±	±
14. YNA hydr. .5%	-	±
15. YNA hydr. .5% \neq YX .5% test for inhibition.	+++	+++
16. ---		
16. --	-	-
17. NZase .5%	±	+++ (adaptation? or activity?)
18. Citric acid .001%. <i>Fine acid still at surface.</i>	± - +	turbidity <u>not</u> due to bacteria.

inactive in neutral extract.

[(Used acid extract of fresh yeast!)] [Maybe in NZase?]
 Try Timmer, Na oleate, etc.

10g. Yeast Extract Difco extracted with 40 ml. CHCl₃ in flask. Separate, evaporate extract and take up in water. Expressed in terms of original yeast content. (Very little material was extracted, perhaps 1-5mg. at most.)

45-3 in T(V) + P19

1. -		
2. YX .2%	++	++
3. MC+V	+	++
4. NZase	-	++
5. YNA		

2-Chloro-4-aminobenzoic acid
Inhibition and resistance mutations

Dec. 15, 1947.

Prepare plates of T(0) agar with 25 mg% CAB. Do. (0) agar.

Spread ca 10^2 cells of K-12 on both, incubate 72 hrs.

A) T(o) agar: 400 colonies noted

b) CAB: ca. 42 colonies noted. However, direct microscopic observation and smear impressions show a large number of "micro-colonies", probably equivalent to the difference between CAB and T(0) plates. Each colony contains, as a guess $10^4 - 10^5$ cells.

(This suggests that Strandskov's observations can be accounted for on the basis of spontaneous mutation and selection among the relatively large numbers of cells in the micro-colonies.)

Dec. 16, 1947.

Harvest from YP and cross W-55 x W-54, heterozygous for Lac₁, Mal, Sal, B₁ as well as V₁^R. Cross on EMS-maltose with .002% glucose added. +B₁.

A20. a) Estimate frequency of maltose⁺, and of sectored colonies. Score only those where the sectors could be scored accurately.

Proportion of Mal ⁺ (including sectors). Count sectors as 1 ⁺ and 1 -.

	+	Sec.	-	
1	1	2	57	$\frac{\text{Mal}^+}{\text{Total}} = \frac{17+5}{512+5+17} = \frac{22}{534} = 4.2\%$
2	4	0	139	
3	3	1	117	
4	2	0	77	
5.	7	2	132.	
	17	5	512	

Proportion of sectored to plus colonies: (Score under conditions stated above)

Plate # ↓	Sec.	+	#	Sec	+	#	Sec	+
	2	6		1	5			
	0	3		2	3			
	0	2		1	4			
	1	6		0	3			
	1	1		1	1			
	0	2		2	1			
	0	4		0	2			
	1	1		1	4			
	0	2		3	3			
	0	1		1	7			
	1	3		4	1			
	0	4		0	2			
	2	4		1	4			
	2	3		0	3			
	1	3		1	2			
	4	2		0	2			
	1	4		2	2			
	1	3		2	3			
	1	3		2	3			
	0	1						
	0	4						
	2	2						
	0	3						
	0	2						
	0	1						
	2	3						
	1	1						
	2	2						

52. 130 / 172.
 30% of the Mal⁺ colonies also have a Mal⁻ segment.

a20

Score Mal- segregants re Lac and V_1 . Also score Mal+

Mal- Lac+ V^R Lac+ V^S Lac- V^R Lac- V^S
 0, 1, 10, 9, 5, 4 4, 2

Mal + Lac+ V^R Lac+ V^S Lac- V^R Lac- V^S

(Not scored well on Lac)
 too heavily contaminated
 with parental to score on
 EMS. Recover Mal+ from
 these plates & test on EMS.

Obtain new sample of Mal- from ^{cross-} plates.

Pick 57 apparently sectored colonies to water N20. Store in refrigerator for
 later separation.

Streak out on Mal.

Scores on Mal p/m components of maltose sectored colonies.
Lac p/m and V₁ r/s

Colony Mal p Mal m Scoring very clear except where total lysis may have obscured fermentation reading in 22

1	ms.	ms.
2	ps.	ps.
3	ms.	ps.
4	ms.	ms.
5	ps.	ps.
6	ms.	ps.
7	ps.	ps.
8	ms.	ms.
9	ms.	ms.
10	mr.	ps.
11	ps.	ps.
12	ps.	ps.
13	mr.	mr.
14	ms.	ms.
15	ms.	ms.
16	ps.	ps.
17p	ps.	ps.
18	ms.	ps.
21	ps.	ps.
22	ps.	?s (m)
23	ms.	ms.
24p	ps.	ps.
25	ms.	ms.
26	ms.	ms.
27	ms.	ms.
28	ms.	ms.
31	ms.	ms.
32	ps.	ms.
33	ms.	ms.
34	ps.	ps.
35ms	ms.	ms.
36	ps.	ps.
37	ps.	ps.
38	ms.	mr.
39	ps.	ps.
40	pr?	ps.
41	ms.	ps.
42	ps.	ps.
43	ps.	ms.
44	ms.	ms.
45	ms.	ms.
46	ps.	ms.
47	ps.	ps.
48	ms.	ms.
49	ps.	ps.

Totals: 45 sects.

	Mal+	Mal-
-S	23	
-R	20	
+S	20	
+R	1	

	Mal+	Mal-
-S	22	21
+S	20	22
-R	2	2
+R	1	0

M- →
M+ ↓

	-S	+S	-R	+R.	Σ
-S	17	4	1	0	22
+S	4	16 16	0	0	20
-R	0	1	1	0	2
+R	0	1	0	0	1
Σ(M-)	21	20 22	2	0	45.

Compare - and + only.

M+L-	M-L	M+L+	
19	12	5	24
M+L+	4	17	21
23	22	45.	

$\chi^2 = 16.2$

$p \ll .001$

∴ There is a definite correlation between the Mal- and Mal+ components of sectors in re-lac segregation. Recover pairs to Mal plates.

(154 x 155) Mal-lac, -V₁^R x Mal+Lac, +V₁^S.

Lac, V₁ scores of intact colonies:

A) Mal /

-R	-S	+R	+S.	
5	4	0	7	
2	7	0	9	
3	4	0	10	
2	4	0	1	
12	19	0	27	58.
21	32	0	47	

B) Mal -

5	7	1	10	
7	5	0	10	
5	6	2	5	
4	7	0	10	
4	3	0	11	
25	28	3	46	102
25	28	2		

37. 47 3 73 160
 [23.2] [29.4] [1.9] [45.7]

$\chi^2 = .85$ $p = .6$

Compare with Table 5 of Knott's paper.
 Retids were.

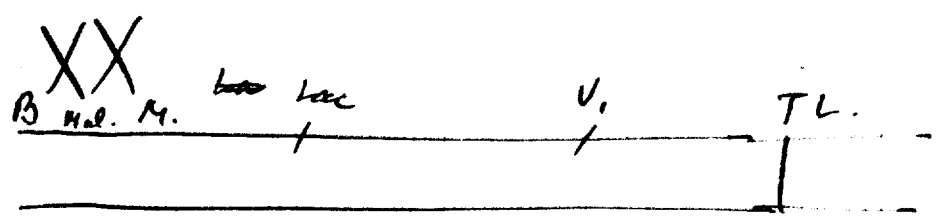
Is this medium's better for?

Mal must be between M and B. (no interactions with Lac).

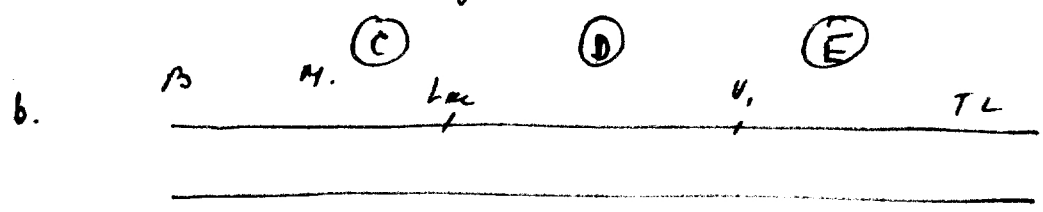
Compare with sectored colonies:

Intact.	37	47	3	73	160.
Sectored	44	43	1	42	90.

Note: Absence of -R and deficiency of +S.



a) a double crossover in B₂A₁ region does not interfere with chiasmata to the right - homogeneity of H₂+ / H₂-.



if a double takes place, it tends to be both in region D or, ^{both} in region C. Very few doubles are recovered involving region E.

Killing curve.

Ded. 24, 1947.

Spread .1 ml. young (ca. 10^9) 58-161 culture on EMB-Lac plates.
Irradiate at 25 cm.

Lamp borrowed from Stauffer. operated in horizontal position, with 10 mins. warm-up allowed. This lamp has section of glass cut out to allow unfiltered uv radiation.

Time	Surv.
5s	37L + 33S = 70. ca. 10^4 ✓
* 10s	ca 100L + do.S. 200. ✓
* 15s	52L + ca 100S = 130. Including 1 <u>white colony</u> . Streaks out + test with T1
20s	17L + 26S = 43
30s	13L + 9S = 22

Are these curves really non-linear? What about resistance of residuals?

Large and small colonies noted. Restreaks for mls. determination.

* Keyit as probable contaminant: not lysed by T1.

The difference between large and small colony noted on the above survival plates breeds true on the first restreaking on EMB-Lac. Transfer to slants as 67-1 and 67-2 for the large and small respectively.

Dec. 25, 1947.

Use same cells of 58-161 as in Exp. 67. Expose .1 ml culture per plate to 15 secs. UV from GE AH-4 lamp, as in 67. *EMB - Trehalose plates*

A). 9 plates.

Most plates, due to faulty pouring, had pitted surfaces and were very unsuitable for scoring. 1 plate, prepared earlier was more satisfactory. This had many (5-10%) colonies which had radial striae suggesting numerous variations affecting intensity of fermentation. Hold plate in refrigerator for later testing.

1/3/47. No mutants found.

Dec. 25, 1947.

Set up as in 68. EMB Mal plates.

Ca. 11 x 200 or 2200 colonies. 1 sector noted. Pick to slant as 69-1 for
later verification. =

large and small colony types seen as above.

Jan 2/48




all Malx.

Streakout on EMB ~~Mal~~ Mal

No mutants

Dec. 26, 1947. Set up as 68. Lac-EMB plates.

Killing variable. Ca. 200 scoreable survivors per plate average
65 plates, or ca. 13,000 colonies examined. 5 possible sectors.

- 1 Mucoid
- 2 Not sectorial
- 3.  No mutants. All lact
- 4.  + and -. - W87
 + W88
- 5.  No lac mutants. Many mucoid.
Pick to start

Jan 2, 1948. Strains out on lact EMB.

Dec 27 1947.

Grow W-55 (58-161 Salicin pos. mutat,) in YP broth, harvest and conc. to ca. 10^{10} /ml. Irradiate 3 ml. in quartz flask ca 5 secs. rotating at front of Hanovia UV lamp, and inoc. .5 ml samples into 50 ml T(m) with .05% sugar.

1. Unirr.

- | | |
|-------------------|-----|
| A. Salicin (.1%) | 3+ |
| B. Cellobiose | - |
| C. b-Me Glucoside | 3+/ |
-
- | | |
|-------------------|----|
| 2. B. Cellobiose | - |
| C. b-Me-Glucoside | 3+ |

Incubate 37°

Examine Jan 3 48.

Streak out the b-MeGl cultures on similar ~~low~~ EMB plates. This may be slow rather than mutative utilization, as has been observed before.

No rapid utilization indicated on EMB - b-me glucoside plates. Growth assumed to be due to slow continuous utilization.

Raffinose tests continued from previous experiments; some possible diversity in progeny of repeated selections. Compare streaking of a - and ± colony.

A8. (3 da.) + types are somewhat more purplish than -. Buds true on this streaking and all colonies are more or less scoreable. Inoc. into .15% T(m) - Raffinose to continue selection. No growth.

A15. 1B (Cellobiose) noted to have reached +± while 2B is still ±. Streak out on EMB Cellobiose agar to isolate possible mutant.

A13 inoc melibiose TP (BM) = 58-161 to select more rapid Heli+ types. Streak out and compare with standard.

Jan 4 1948

OK. W-1 x

Parentals.

A8 (proportion +).

All -.

✓
✓

1	W-56	-	
2	W-58	+	ca 1:10 Malt
3	W-60	+	ca 1:20 "
4	W-63 (±?)	+	< 1:10. Parent Malt.
5	W-71	+	ca 1:50
6	W-78	+	ca 1:50 Malt.
7	W-80 ±	++	
8	W-20	+	ca 1:50

W-63 x Y53 all+ Parent +.

W-80 x Y53 all+

(Crossed with Y53 x Y53.)

a) W-1 & W-56.

b) W58, W60, W-20, W-71, W-78.

all + recombinants checked and definitely ++.

check also as parentals.

	-	+
W1.	OK.	
W56.	OK.	
W58.	OK	
W60.	OK.	
W71	OK.	Numerous papillae. streak out single colony.
W20.	OK.	Many mixed. W-20 may be faulty ±.
W78.		1 Malt+ colony / 200 Malt -. May not be fruitful. streak out.

Jan. 5, 1958

Irradiate Y-53 on Tre-FMB plates, 10 sec. under Hanovia UV lamp.
10 plates.


10 x ca 150 = 1500 colonies.


2 colonies showed fairly distinct sectoring.



Retreated.

① and ② both give two colony types:

a.  Extensive darkening; no stem W-89 and W-~~89~~ 91

b.  central spot only. (This may be due to hydrolysis.)

W-90 W-92

2. colonies showed radial striation suggesting quantitative variations.
This is correlated with green sheen and from 1 colony, sheen+
and sheen- colonies noted. The original population is very
variable in this character.

Test on Maltose:

89 ++

90 ++

91 ++

92 ++.

Retest on Tre EMB. (study Trehalose by filtration).

Jan 5, 1948.

3P. Inoc. Y105 into YP broth to obtain calls.

Lamp Broke Down

Use Stouffer's lamp. Irradiate 15 sec. (Y105 is apparently more sensitive to UV than is Y10)

Mal EMB 37 plates x ca 20 / plate. = 700 colonies. ~~No mutants~~

Lac EMB 38 x ca 20 = 750 colonies.

1 white colony noted. Struck out on Mal and Lac.

Mal - OK. Lac+ and morphologically identical with other types.

W-94.

Nutrition of W-93

Jan. 9 1947

Inoc from fresh slant into:

T(Val) plus:

48h.

1. -	-
2. HC	+
3. Vits	-
4. HCVits	++
5. NZVits	+++
6. HCV/ YNA	-
7. Y. Extr	++++

~~8. Vits~~*look for sp. vitamins.*