

March 10-12, 1942.

Y10 x Y45.

A) T(B₁) plates.
Streak on lac S agar.

+	-	
18	2	/ 20

7 tested were all T₁^S as expected.

B). lac S B₁ plates.

Hold. A11.

+	-	
41	1	
72	2	
32	1	
145	4	149.

Recount A12.

LB₁

+	-	
100	8	
121	13	
71	3	
117	9	
409	33	442.

lac- = 7.5%

Compare with 8.6% of p. 42.

Lac (o)

31	2.	
35	2	
66	4	/ 70

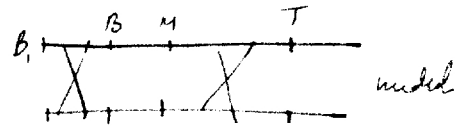
5.7%.

March 8, 1948.

Cross on Lac (O) Agar, W ~~22321~~ 337 with the following.
3 plates each (.1 ml each.)

W45.

No colonies.



W35 8 Lac - colonies all told.

W72 3 lac - colonies all told.

Y87 9 Lac - colonies.

Crosses should be repeated.

Mix up T(m). BHTLB, + equivalent of .05% glucose in 5cc volumes.
 inoculate legittly with : P10. [Filter - Sterilized].

		Y10	W-108	W-327
1. K. glucos-1-phosphate (hardy)	P11	-	-	-
	A12	-	-	-
	A12	-	-	-
	A13.	-	-	-
2. Glucose.		++	-	-
		✓	-	-
		✓	-	-

Mantel⁹, 1948.T(B₁) 410 + 487. Measured drops dilute suspension.

A) + 4 ml H₂O / plate B) 4 ml H₂O + $\frac{700 \mu \text{B}_2 + 35 \text{ mg Glutarate}}{100 \text{ ml medium}}$.

A. P10 (ca 364.) 33 / 7, 12, 4, 5 m = 8

B. 34 / 4, 11 (2 drops), 6, 13 m = 8½.

No pronounced effect of B₂ + glutarate.

(More colonies may appear later).

12 appeared altogether.

See 155.

March 8, 1948.

1. Y-87 X Y-10
2. Y-53 X Y-40
3. W-183 X Y-46

1A On EMS (-B₁) plates.

a) Readings from plates.

+	-
13	5
13	5
10	4
<u>22</u>	<u>14</u>

b.
S.O.
T₁-lacs'

-R -S +R +S.

S.O. counted twice

x WYS according to label!

36	14	77.
<u>63</u>	<u>14</u>	
64	18	82
<u>36</u>	<u>18</u>	

a' Repeat A12. :

52. 14 3 27 8

1B. On EMS (B₁) lac plates

a. direct counts.

1S.

17	4
16	8
16	3
30	15
30	5
16	3
22	11
28	11
19	3

Total: 6 sectors.

a' repeat A12

1S.

194	63	257.
<u>227</u>	<u>101</u>	
		338.

1C. From T(B₁) plates.

See page following for raw data. Totals of all experiments this page are:

	-R	-S	+R	+S
S. 445	131	6	207	101
	(.294)	.013	.465	.227

Cf published results:

13 211 103

1a. Scored originally as Lac+.

	-R	-S	+R	+S.	
	0	0	14	3	
	0	0	13	<u>3</u>	
As Lac-	14	3			
	14	3	27	8	52:
	.269	.058	.519	.154	

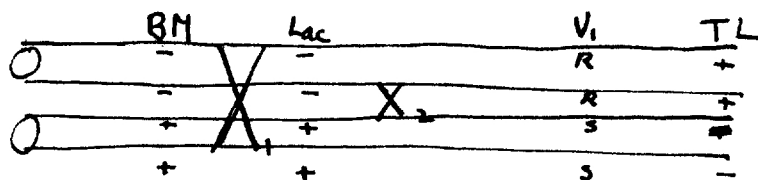
1B. As Lac+

	+?				
	1	1	29	13	
	0	0	33	21	
	0	0	15	8	
	0	0	12	9	
	0	0	16	6	
	0	0	13	7	
	1	0	13	5	
	1	0	15	5	
As Lac-	49	1	0	0	
	39	1	0	0	
	91	3	146	74	314 ✓
	.290	.095	.465	.236	

1C. (plates = b_2 , glut).

	16	0	21	12	
	10	0	13	7	
	26	0	34	19	79.
	.329	0	.430	.240	
	131	6	207	101	445 ✓
	.294	.013	.465	.227	✓

A total of 6 sectorial colonies were noted. These were purified and tested with T1. All 12 cultures were V_1^R .



No X_1 $+R$ X_2
 $-R$ $+S$

In calculating p , the chances of X_2 being in $lac - V_1$ $\therefore V_1 \neq TL$ only should be considered. X_1 is almost completely fixed in region \textcircled{D} as $-R$. An expectation of 4:2 is not signi. different from the experimental value of 6:0.

Test on B_1 for requirement.

A $lac - B_1$ $+$.

- ~~A~~ B
1. B_1
 2. $+$ B_1
 3. B_1 B_1
 4. B_1 B_1
 5. B_1 B_1
 - 6.

Also, test Y10 on pyrimidine + thiazole:

1. TL -
2. TL B_1 +++
3. TL P_1 +
4. TL Th ++
5. TL Th P_1 ++
- 6.

Species Rowsoni.

#64

April 8.

Streakout W-108 on EMB glucose, mannose, fructose. EMB

Apr 17. No papillae seen on these plates.

March 31, 1948.

Test strains on lactose, epi-lactose, neolactose & galactosan received from N.K. Richtmeyer. 1% - EMB (small plates).

	Str.	lac	Neolac	Epilac	Galactosan	[Megal]
1	+ K-12	+ ^P	-*	+	-	+
2	+ Y10	+	-	+	-	+
3	lac ₁ W53	-	-	-	-	+
4	lac ₂ W45	-	-	-	-	-
5	lac ₃ W-108	-	- ^P	-	-	+
6	lac ₄ W-126	-	-	-	-	-
7	lac ₅ W-145	⊕	-	-	-	+
8	lac ₆ W-125	⊕!	-	±	-	-
9	lac ₇ W-133	- ^P	-	-	-	-
10	sl, W-117	- ^P	- ^P	-	-	-
11	sl W-252	+	±	+	-	+
12	sl W-328	+	-	+	-	+
13	Gal- W-254	+	-	+	-	-
14						

[5/16/50 -
Note that
W-252 is recorded
as neolactose-pos!]

* Papillae to form showing
v. considerable utilization

Galactosan - all.

Neolactose all -

epilactose follows lactose.

Strains out papillae of K-12 / Neolactose n lactose. Test colonies
on neolactose. 8+ 3-. Isolate + as W-341. Still lac+

See over.

Inoculate 58-161^R into 20 ml T(m) + Melactose 25%.
+ galactosan

Delayed growth on melactose.

Streak out and test on melactose EMB. 11- 0+.

Repeat streaking.

per 10 liter bottle.

Use technical grade chemicals.

NaCl 50 g.
 K₂HPO₄ 30
 KH₂PO₄ 10
 (NH₄)₂SO₄ 50

Sugar 150 g. sterilize separately.

Grow K-12 24h. aer., nonstirred, mixed, with sucrose.

Collect 44g. cells. Divide & incubate each portion for 3 1/2 hours in 100 ml 1% peptone + 5% lactose or glucose. for adaptation.

Sediment after 3 1/2 h. + resuspend each in 50 ml 1/100 Na citrate under toluene + autolyse. P8 - P10.

Autolysate volume after bacteria are removed is 50 ml each.

The autolysates give very high blanks on Bradford's mouse, so they cannot be directly assayed.

To 10 ml samples add 3.5g AS + sediment. Assay ppt redissolved in 1/100 saline citrate. use HNO₃.

G alone < 1 drop
 .1 ml G + 10mg lac .90
 1.0 " " .41

L alone < 1 drop
 .1 ml L + 10mg lac .90
 1.0 " " .33

lactose 10mg. 1.14 [Blank].
 Glucose + galactose 10mg. 19.06
 " " 1mg. 1.97

163 B2 + lac. 5.42
 " (blank) < 1 drop

Neither preparation hydrolyzed lactose beyond the blank (ca 6%).

$$\frac{5.42 - 1.14}{19.06} = \text{ca } 22\% \text{ hydrolyzed in 20 mins.}$$

April 9, 1948.

In neolactose tests it was noted that W-125 and W-145 were positive or slow positive on lactose. When streaked out again as controls on outcrosses, this was noted again, and suggests the need for reexamination.

Streak out on lactose EMB and compare:

W-145 stock slant < 1% Lac - colonies. - colonies quite small.

W-145, lyophil tube All Gna -, Mal -, Lac -. Recover to slant.

W-125. Numerous fairly good sized colonies that might be considered slow. Streak out must to good +.

[It seems that ^{slow.} 145 colonies near + are more likely to be lact+ than those further removed. This suggests a pH or redox effect.]

Apr. 9, 1948.

Inoculate 58-161 or Y10 heavily into T(m) TLB₁BM with 0.1% sugar.

25 ml.

		24h.	48h.	
1. Lactositol	Y10	±	++	Apparently lactitol mutants can be selected for.
2. "	Y10	±	++	
3. "	58-161	±	+++	
4. "	58-161	±	+++	

100 ml.

				A28	
5 Galactosan	58-161	-	-	-	Throw out
6 Galactose	58-161	+++.	-	+++	

A28. Streak out 1 and 3.
on lactitol which was +.

1 was sterile. (3) gave 1 colony

A29. S.O., side by side W-349 and 58-161.

W-349 is pure hol+, but relatively weak; 58-161 is definitely -.

LACTITOL

170a.

EMB - 1% (from Wolfram, dihydrate)

K-12 -

Y10 -

Y53 -

W45 -

W-108 -

W-145 -

W-125 -

W-126 -

W-133 -

K-12 Neol+ - ~~1860~~

58164 Neol+ -

see p. 170 for selection of Lol+ mutants.

April. 9/9/8.

410 5. minus 40 Hanover.

L-Arabinose EMB. ca 2000 /plate unevenly spread + difficult to score.
 36 plates = ca. 70,000 colonies.
11-30. 20 "mutants"

d-xylose EMB. 50 plates. ca 5000 scoreable colonies per plate
1-10 ca. 50,000 colonies

10 "mutants"

W -		Xyl	Arab	Lac	Mal	She	Ena	Sal	TI
351	1	-	-	+	+	+	+	+	
352	2	-	-	+	+	+	+	+	
353	3	-	-	+	+	+	+	+	
354	4	-	-	+	+	+	+	+	
-5	5	-	-	+	+	+	+	+	
	6	-	-	+	+	+	+	+	
	7	-	-	+	+	+	+	+	
	8	-	-	+	+	+	+	+	
	9	-	-	+	+	+	+	+	
L 360	10	-	-	+	+	+	+	+	
361	11	+	-	+	+	+	+	+	
	12	+	-	+	+	+	+	+	
	13	+	-	+	+	+	+	+	
	14	+	-	+	+	+	+	+	
	15	+	-	+	+	+	+	+	
	16	+	-	+	+	+	+	+	
	17	+	-	+	+	+	+	+	
	18	+	-	+	+	+	+	+	
	19	+	-	+	+	+	+	+	
370	20	+	-	+	+	+	+	+	
	21	+	-	+	+	+	+	+	
	22	+	-	+	+	+	+	+	
	23	+	-	+	+	+	+	+	
	24	+	-	+	+	+	+	+	
	25	+	-	+	+	+	+	+	
	26	+	-	+	+	+	+	+	
	27	+	-	+	+	+	+	+	
	28	+	-	+	+	+	+	+	
?	29	-	-	-	-	-	-	-	S
380	30	-	-	-	-	-	-	-	S

29 + 30 are probably contaminants, but mutations should be checked.

EMB \pm 1% glucose +. Read at 24h.

1. 2% F. no growth.
2. 2% + G no growth.
3. 1% F. Inhibited growth; some papillae?
4. 1% F G Small translu. colonies.
5. .5% F Moderate colonies translucent.
6. .5% FG large colonies. Milky or blue. \leftarrow good selection level.
7. .1% F Moderate colonies translucent.
8. .1% FG large, purple colonies.

9. 1% oxalate + .4% glucose
10. ~~1% oxalate~~
.4% glucose.

For formic "decarboxylase" selection medium, use
.4% Na formate, 1% glucose EMB.