Dehydrogenase Media.

2/18/48.

Make up ½% Ca (Casamino acids) in .2% aq. Ammon. Molybdate. + Agar

A.- B. Add 2% Sodium Succinate.

After autoclaving, A is blue; B is lt. yellow.

K-12. N.G. on A. Colonies on B.

Analysis of W-236.

W-236 x Y40. On EMS.

Ca. 5-10% Lac- Therefore W-236 is not Lac1+. Call the gene "reverting" in W108 – W117 Sl_1+ (Suppressor of Lac_3). Call the differential between Y40 and W-236 Sl_2+. If Sl_1+ ≠ Sl_2+ then some of the Lac+ recombinants will be Glu- and v.v.
Compare on Lac + Glu S.:

(A) { 9 cultures were Lac – (±?) but glucose +.
10 cultures were Lac + Glu +

(C) 23 cultures were Lac – Glu –

(B)

Streak out samples of each type on Lac + Glu EMB.

		Lac	Glu	
1	L-/+	+±	–	This type suggests that the mutation
2	L+/+	++	++	differentiating W117 from W236 is at a distinct locus
3	L-/-	–	–	from the one between W108 and W117
4.	L+/-	–	–	

Cross-Test Glu Mutr.

	A-W108 × A	~~B-W453 × B~~
1. W239	++	
2. W243	++. Also ± and − (on cross-plate. ± not verified)	
3. W245.	++	

Knockout parents.

108 R/S variation; 1 ± / > 200 + revertants in streaks.
239 < 5% reverted. Natural colony − darkening around +
243 all − OK. Thin colonies.
245 50% reverted.

Crosses in conclusion!, etc.
243?

Gal Mutation Run.

Y10 80 secs Watson's Sterilamp.

70 plates × 200 cols. = 14,000 scored.

Very few entirely – found.

 ++ and – 254.

 ++ and – 255

0 – (and ++) 256 ±

Also, 9 cultures recovered which are not = but ± :

24h. ⊖ Pick 2 for study: 257
258

58-161 80 secs. Watson's steriamp

50 plates × 200 = 10,000 scored.

W-253

About 10 others picked were not mutant. Pick to gluconate broth.

Lac Mutation Rem.

February 21, 1948.

4/10 80 secs. Watson's Lamp EMB.

100 plates x ca. 1500 cols. = 150,000 (very rough estimate) colonies

68 mutants = 1/2000

			Glu	Mal	Lac	Sucrose	Gal	Mgal.
W-	259	v. slow ±	+	+	±	+	+	slow Lac −
	260		−	−	−	±	+	108
	261		−	−	−	±	+	108 +
	262		+	+	−	+	+	Lac + −
	263		+	+	−	+	+	Lac + +
	264		+	+	−	+	+	Lac + +
	265	slow??	+	+	−	+	+	Lac + +
	266		+	±	±	+	+	slow Lac + +
	267		−	−	−	±	+	108 + ÷
	268		−	−	−	±	+	108 + ÷
	269		−	−	−	±	+	108 + ÷
	270		−	−	−	±	+	108 + ÷
	271		+	+	−	+	+	Lac + +
	272		+	+	−	+	+	Lac +
	273		+	+	−	+	+	Lac +
	274		+	+	−	+	+	Lac +
	275		+	+	−	+	+	Lac +
	276		+	+	−	+	+	Lac −
	277		+	−	−	+	+	108 +
	278		+	+	−	+	+	Lac +
	279				−	+	+	
	280	+ diffuses.	−	−	−		+	108 +
	281		+	+	−	+	+	Lac +
	282		+	+	−	±	−	(145) −
	283		+	+	−	+	+	Lac −
	284		−	−	−	±	+	108 −
	285		−	−	−	+	+	108 ÷
	286		+	+	−	+	+	Lac −
	287		+	+	−	+	+	Lac +
	288		+	+	−	+	+	Lac +
	289		+	+	−	+	+	Lac +
	290		+	+	−	+	+	Lac ?
	291		+	+	−	+	+	Lac +
	292		−	−	−	+	+	108 −
	293		+	+	−	+	+	Lac +
	294		+	+	−	+	+	Lac +
	295		+	+	−	+	+	Lac +
	296		+	+	−	+	+	Lac +
	297		−	−	−	±	+	108 +
	298		−	−	−	±	+	108 −
	299		+	−	−	±	−	(145) −
	300		+	+	−	+	+	Lac +
	301		−	−	−	±	+	108 +
	302		+	+	−	+	+	Lac +
	303		+	+	−	+	+	Lac +
	304		+	+	−	+	+	Lac +
	305		+	+	±	+	+	slow + + +
	306		+	÷	−	+	+	Mel − Lac − +
	307		−	−	−	+	+	108 +

Non-sutrial Mult. Cartl.

		Glucose	Maltose	Lactose	Galactose	Gluconic	Hegal	
308		−	−	−	±	+	108	+
309		+	+	−	+	+	Lac	+
310		+	+	−	+	+	Lac	+
311		+	+	−	+	+	Lac	+
312		−	−	−	±	+	108	+
313		+	+	−	+	+	Lac	−
314		+	+	−	+	+	Lac	+
315		+	+	−	+	+	Lac	+
316		+	+	−	+	+	Lac	−
317		+	+	−	+	+	Lac	−
318		+	+	−	+	+	Lac	+
319								
320								
321								
322								
323								
324								
325								
[redacted]								
[redacted]								
248	1	+	+	−	+	+	Lac	+
249	2	−	−	−	±	+	108	+
250	3	+	+	−	+	+	Lac	−
~~253~~	4	− ✓	− ✓	+ slow-variable.	− ✓	+ ✓	Glucose	+ + + +
254	5	+ ✓	+ ✓	+ ✓	− ✓	+ ✓	Gal − !	−
255	6	+ ✓	+ ✓	+ ✓	− ✓	+ ✓	Gal − !	−
256	7	+	+	+	−	+	Gal −	−
257	8	−	−	−	±	+	108	+
258	9	−	−	−	±	+	108	÷
259.	10	+	+	slow	+	+	Lac slow.	+
S. para A								inhibited
Lardas 27								−
dublin 37								−
E. coli ML								

Lac Mutation Runs.
Spontaneous control.

February 23.

Dil. 1/10 suspension used in 132 to 5×10^{-6}. Use 1 drop (= .05+ cc) per Lac EMB plates. 20 plates.

ca 800/plate = 16,000 Test all suspicious cultures.

10 examined. No mutants.

Cross-Test Lac, + Lac4

February 24, 1948. EMS-Lac 8 plates each.

A. Y53 × Y87. 0/400 0/400 0/2600.
Lac1- 6-11-T+L+ 0/400 0/200 B+M+L+
from T-L+ Lac1- 0/400 0/400 0/400

B. Y53 × W67 1/300, 0/200, 0/300 0/300 2/2000
Lac1- Lac4- 0/200 0/200 0/200 1/200.

C. W128 × Y87 0/300 0/400 0/200 0/200 0/2400
Lac1- 0/400 0/400 0/200 0/300

D W128 × W67 400 0/200, 0/100, 0/200, 0/100 0/1300
Lac4- 0/200 0/200 0/200 0/100

E W120 × Y87 0/400, 1/300, 0/400 0/500, 3/3200
Lac4 Lac1- 0/500 0/300 2/300 0/500

F W120 × W67 0/400, 0/400, 0/100, 0/200 0/2000
Lac4 Lac4- 0/300 0/100 0/100 0/200

Parents:

Y53	0/>>1000. +	Lac.	Y53, Y87 (W128)
W67	"		
Y87	"	Lac4	W67, W120, (W128).
W120	"		
W128	"		

W128 may be a deficiency for both loci, or a double mutant. Anor. heavily into T(m) + Megal!

Methyl galactoside [illegible]

February 23. Short streaks on 1% Megal EMB. 50/plate.

1	W-45	−
2	W-35	−
3	55a	?
4	55b	?
5	55c	?
6	122	−
7	124	+
8	127	+
9	131	−
10	132	−
11	134	−
12	135	−
13	136	+
14	137	−
15	138	−
16	139	+
17	140	−
18	141	−
19	143	−
20	144	±

W-190 series.

21	190	+
22	192	+
23	193	+
24	194	−
25	196	+
26	197	+
27	201	+
28	202	−
29	205	+
30	206	+
31	208	−
32	209	+
33	211	+
34	212	−
35	214	−
36	216	−
37	217	−
38	218	+
39	219	−
40	222	−
41	223	+
42	225	+
43	228	+

Check Stocks.

Feb. 24, 1948
Streak out NA stocks on glu + Lac EMB:

	Lac	Glu.
W- 108	all-, papillating	
188	~~all~~ 1+/1000 - (pap.)	- pap.
239	colonies large, smooth, ±	v. small colonies. (bacch?)
243	++	all ±
245	~~all~~ large +, small -	all -, 2 colony types
251	+++	- glossy.
252	++ rough	all rough
327	++ rough.	all -

253: slow + on glucose, majority for lactose response. pH effect??
papillating v. strongly anglucose

Specific Revisions.

February 24 ff. 1928

1. W-35. Resuscitate 55-a + 55-b; Retest on lactose, Megal.
   55a (E) Relabel W-
   55b (E) " W-

2. Test W-253 papillae from glu, gal & mal on all three media + & T1.
   5 + on all. T_1 - sensitive.

3. Incr heavily into T(ws) + .05% sugar

Incr. P14.		Lac	Mal	Megal	RGma.	Sucrose.
24h.	W.45			—0—		
48h.				—		
P29.						
	W.145	± ++ all-	+++ all-		— +++ ++ clusters Lac, Mal.	
	W.243	— +++ Mostly+	— +++ Mostly+		W327:	— — —
	W.125	+++* +++ all+ clusters Megal		++* +++ all+ on Megal.		
	W-128	=	=			

4. Test W45/Lac papillae on Megal. 9 +. 0 -

a) 37 125 Lac+R. test on Megal. All +.
16 Megal+R tested on Lac All +.
1 128 Lac+R " " Megal +.

33 W.145 Sma+R on Lac All +
29 " " " Mal " +

15 W.188 Glu+R on Lac All +
42 W.243 Lac+ on glu all +
53 " Mal+ on glu all +.

Test 6-120 papillae on Megal. Megal- streaked out on Lac.

6 all- 2 + and -. Test ± on Megal.
(apparently) ——— =

Both are Mg +.

No specific reversions noted.

Nitrogen sources

Febr. 25, 1948.

Prepare, −N, per l.:

glucose	1
NaCl	5
$MgSO_4$	.1
K_2HPO_4	3
KH_2PO_4	1

and autoclave 50/125 flasks.

Incc K-12 dil. suspension into:

			A27.	P29
P25.	A.	—	—	—
	B.	NH_4Cl 5% (.2%) 2.0 cc	v. dense +++	++++
	C.	Urea 20%, ster. filt. (.2%) .5 cc	±	±
	D.	Glycine, 15% (.5%) 1.5 cc	++	++.
	E.	Asparagine, 5%. (.2%) 2.0 cc	++±	+++++! v. dense!

Final solution is ca. N/15 N.

This medium seems to be satisfactory for crease plating.

Cross-Test Lac Mutants.

~~124~~
138

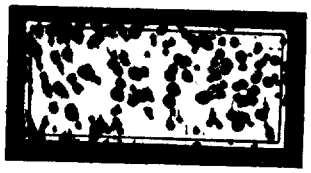
Reversions.

February 27, 1948.

~~+ x W-45 B. x Y-87~~

(1) Test ē W-45 for Lac-2 the fungal-ait of the current lac-series. 3 plates each.

276	+++ ✓ ✓	None of these are Lac_2-.
283	+++ ✓ ✓	
286	+++ ✓ ✓	
287	+++ ✓ ✓	
313	+++ ✓ ✓	
316	+++ ✓ ✓	
317	+++ ✓ ✓	



(2) Test 327 & 329 with Y10 + W236 on Lac & Mel for suppressors.

327 x Y10 on maltose Synthetic. 5 plates. No colonies!

Lac EMB 1 plate No colonies.

(3) 329 x 236. (W-35 Lac+ Rev. Mg- x W108 Sl.+ Lac_3-).

Many ++, --.

329 x Y10 on Lac. Apparently all +.

5 x 500 = 2500 colonies tested. Therefore, there are at least 3 alleles at the Lac_{35} locus: +, -, and a = lac+ fung-.

[Test 329 for mutation to +, and W-35 for relative frequencies of mutation to other states.]

→ Test prototrophs on Mg S. 71 all +! Should have been ca. 8% -.

B. globigii

138a

Feb. 25, 1948.

"Constant" yellow strain from PW Wilson.

Preliminary irradiation: 1 drop broth culture / ~~Nutrient~~ GA plate.

40 secs. T

80 secs. T

120 secs ca 1000

108 x 243, 188.

Feb. 25, 1948.
Anglucose - EMS'.

W-108 x W-188.

Yield very low. 0/5. 0/5 0/16 Total: 0/41.
0/9 0/6.

W-108 x W-243. 0/13 0/7 0/10 ①/13 ①/50.
0/7.

The + recovered might be a reversion. Cross should be repeated on a large scale.

Test Reversions of Lac –

March 1, 1948.

Cross with Y40: 4 plates each. Lac S.

A3. W-235. Two classes noted: ++ and ±. (Allele?

P3. All ++. –: 0/200, 200, 200, 200. ~~Streak ± ++/++ and purify~~

W-233 –: 0/300 0/200 0/400 0/400. ? Streak out.

W-232. 0/200, 0/300 0/300 0/300.

W-234. 0, but hold. 0/1000 –

W-231 0/200 /300 0/300. ? S.O.

W-33. 0/150 0/150 0/200 0/100 ✓

W-34. 0/1000, 0/300 0/300.

W-327 × [redacted]. Y40. T–L–L_3–B+M+ × T+L+L_3+B–M–

~~T+L+/L– B+M+ Lac~~

Lac S: 17+ : > 100 –. ∴ L_3 is linked to BM [redacted]

Mal S: 7 plates. ca 300/plate. some probable Mal– noted.

Streak out. ✓ 9 Mal– recovered. Test these on glucose. All –.

Extract Lac_2 - .

Cross W-45 × W-34 on Lac, Mal's + Leucine or + Threonine.

Lac : leucine. Very few Lac - only one recovered.

Threonine ca 5% Lac - only four Lac - recovered

	T(o)	(B_1)	(T+L)	(T+L/B_1)
Streak out on EMB Lac & test purified clones: 1:				
2:	-	+	-	+
3:	-	+	-	+
4:	-	+	-	+
5:	-	++	±	++

Mal Mostly - L. agar needs doubling then T.

Test some + and - on Lac EMB. Fuchsin. 7 found.

Threonine: Purify the Lac - 's.

Mal - 5/32 are Lac -

Mal + 2/16 are Lac -

		T/o)	B_1	T	T B_1
M+	1	-	+	-	+
M+	2	-	+	-	+
M-	3	-	+	-	+
M-	4	-	+	-	+
M-	5	-	+	-	+
M-	6	-	±	-	++
	7	-	+	-	+

141-G

See over

Test more Threonine Mal− segregants:

All Mal−.

Lac+	Lac−	?(Motility −)	
46	8		
42	5	3	
59	4	4	
50	3	8	61
42	7	4	
	4		

Streak out prob. Lac − and test mutation.

		O	B_1	T	T B_1
	1. B_1				
	2. B_1				
	3. B_1				
W-338	4. (T B_1)	−	−	±	++
	5. B_1				
	6. B_1				
	7. B_1				
	8. B_1				
	9. B_1				
	10. B_1				
	11. B_1				
	12. B_1				
	13. B_1				
	14. B_1				
W-339	15. (T B_1)				
	16. B_1				
	17. B_1				
	18. B_1				
	19. B_1				
W-337	20. Prototr.				
−6	21. (T B_1)				
−6.	22. (T B_1)				
	23. B_1				
	24. —				
	25. B_1				
	26. B_1				
	27. B_1				
	28. B_1				
	29. B_1				
	30. B_1				
	[illegible]				

2/28 T B_1

1/28 Prototr.

25/28 B_1 −

W-306.

March 1, 1948.

Streaks out on Mal, Lac EMB. P29.

3/2/48. Lac-, rather alkaligenes. No papillae

Mal- (faint slow purple); Numerous papillae. Test on Lac+ Mal.

Papillae are alkaligenes. ~~Slow~~ Still all Lac- Mal-.
(111) (8)

3/3/48. W-306 x 58-161 on Lac's

3/4/48. Papillae noted in 306/L. Picks to Mal to check specificity. All seem to be Mal- or Mal±. Streaks out on Lac. Test purified Lac^R+ on Mal.

March 3, 1948.

W-306 x ~~W-46~~ 58-161 on Lac's.

T+L+ B-M-#Lac+ × T-L-B+M+Lac-

5 plates. ca. mean Lac,.

Lac+	Lac−
11	14
9	8
9	17
4	8
4	10
37	57 / 94.

Test Lac+, Lac− on Mal.

Lac+: #1 Mal++ 29 Mal−

142-aa

test by streaking.

1. Mal- Lac+
2. Mal- Lac+
3. Mal+ Lac+
4. Mal+ Lac+

142-ab. Lac− 2 Mal++ 27 Mal−.

Test by streaking

1. Mal+ Lac−
2. Mal+ Lac−
3. Mal− Lac−
4. Mal− Lac−

∴ W-306 is a double mutant, Mal_x^- - Lac_y^-.

Hug Lac^R (Lac+ Mal−) as LU−

~~81 plates~~. March 7, 1948.

1 drop 10^{-5} dil. Y10/YB culture spread on each of 81 plates.

ca > 1500/plate. About 160,000 colonies scoreable

some plates > 2000.

9 suspicious colonies streaked out. All intact.

5 mutants recovered.

W- 331
W- 332
W- 333
W- 334
W- 335

[Compare with 68 mutants recovered from about the same number of colonies in Exp. 132).

Antisera to C1 + C2. 144

March 3, 1948.

Immunize Rabbits against Y105 + Y109.

Prepare antigens from broth cultures, wash in H_2O. Estimate cells.

386 F6	Y105.	$3/3$ 10^9
387 F6	Y105	10^9
383 F5	Y109	10^9
385 F3	Y109	2×10^9

All rabbits died in 12 - 20 hours. No post.

Fermentation tests.

3/4/48.

EMB:	W-108	Y10	W-118	W-119	
Mannose 1%	–	+++	+++ *	+++ *	
Sorbose 1%	*	–	–	–	v. Poor growth (nils.?) 108 should beat growth.
Glucose 1%.	*	++	++	+++.	

* Indistinguishable. Compare Maintal.

Melezitose 3%	K-12	Y10	W-306	W-55	
	–	–	–	–	No detectable utilization

3/11/48. Ethylene glycol-β-glucoside (any more?)

18h.			
	K-12	+ weak	++
	W55	++	+++
	W-108	–	–
	W-145	+	++
	W-[illegible]1	+++	+++
	W-327	–	–
	W-328	–	–

	145	K-12	W-55	W45	W108	Y53	327
Methyl β-l-arabinopyranoside.		–		–	–	–	
Methyl α-d-xylopyranoside		–	–				
Methyl β-d-xylopyranoside		–	–				
Ethylene glycol-β-d glucoside		±	++				
Melibiose.	±				–	±	–

328+!

weakness ambiguous!

Urease; Glycine utilization.

3/3/48.

See 137 for "N" medium. Inoc K-12 lightly or YIO heavily into: B3.

		Inoc.	A5.	A7	
N(TLB,) Urea	1. Y10	+	+	+	
"	2. Y10	+	+	+	
"	3. Y10.	+	+	+	
"	4. K-12	-	+	+	N from amino acids!
N(0).	5 K-12	-	-		
N(Urea).	6 K-12	-	÷.		
Urea + NH_4Cl	7. K-12	-	+++	+++	Urea not inhibitory.
NH_4Cl.	8. K-12	-	+++.		

In following, omit glucose; add NH_4Cl for C-utilization: ~~K-12~~. + TLB, (Y10).

				P8	
11.	—	-	-		-
12.	.1% glucose	-	+++	++++÷	✓
13.	glycine	-	-	-	-
14.	asparagine.	-	+±.	+++	✓

Compare the N-utilization of glycine! (Acid acetic; glycollic acid!)

Fermentation Tests.

March 6, 1948.

EMB:

		Lac	Megal.	Mal	Gal	Sna	Glu.	
1	319	—	—	—	±?	-?	-	Growth limited.
~~2~~	~~320~~							
3	321	±.	+	±?	±	+	-	(109)
4	322	—	—	—	±	+	—	(108) Megal -!
5	323	—	+	+	+	+	+	
6	324	—	+	+	+	+	+	
7	325	+	+	+	+	+	+	
8	326	-	—	—	±	+	-	Glu- Megal -! of. 108
9	331	—	+	+	+	+	+	
10	332	—	+	+	+	+	+	
11	333	—	+	+	+	+	+	
12	334	—	+	+	+	+	+	
13	335.	—	+	+	+	+	+	

Mg: ~~W. 329~~ ~~W-330~~ ~~W-335.~~

			TRE 24. →	TRE 36h.	48.	
Tre.	W-1		+++	+++	✓	
	W-60		- .. ±	±	—	
	W-102		+++	+++	✓	
1st. rdg.	W-108		—	—		
	W-145		+++	+++	✓	
24h.	W-306		—	—	—	with +++ pop.
	W-327	108 Mal+	—	—	—	
	W-328	108 Mal+	—	—	—	
	W-117		—	—	—	

[Repeat tests on purported negatives. / Select for specific reversions.]

W-60

March 5, 1948.

Heavily inoculated: P7

W-243. Lac +++ * 99%+. Test on Glu, Mal. 60: Mal+. 34 Glu+ No S.R.
Mal −
Glu −

W-145 Me-gal. ++ * Mostly weak+. Test on Lac. 16+. Test on Suc, Mal.

W-125 Me-gal. +++ * All+ Test on Lac. 10+.

W-120 Me-gal. ± ±+

W-45 Me-gal. ± +++ * 41+/Lac all+.

P8. P9.
W-117 Tre. +++ * 85%+. Test on maltose 15 all+. Test Tre on ≠ glu+lac. All +, +.

W-60 Tre +++ * 60% weak+. Test on maltose. (6−).
Retest on trehalose: +±. S.O. ① on trehalose.

≠ W-117 controls easily distinguished from +'s., and between glu (±) and lac (−).

Papillae from 327, 108 on trehalose tested on glucose.
327: 4+, 2− } Retest on trehalose. 149-1-6
108: All−. } 149-7-10 (11, 12 S.O.)

When retested, no distinctive Tre+, unless Glu+, noted.

Test Recombination of C2 mutants.

March 6, 1918.

Prepare washed suspensions & plate .1 ml each on Lac EMS '16.

	A 8.
1. W93	—
2. W138	—
3. W139	—
4. Y87 × W93	—
5. Y87 × W135	—
6. Y87 × W139	—
7. W93 × W138	—
8. W93 × W139	—
9. Y87.	—

No evidence of recombination. Mixed culture must be tried.