New heterozygotes

December 4, 1948.

A. W65 × W595

B. W48 × W595 No yield

C. W182 × W595

12/5/48.

~~By~~ W45 × W595 on Lac EMS.

12/8. No yield! (3+ colonies in 15 plates!)
2 not cli 3'd +4.

Note. Hfr. A12 Streaks out W-1 to W595 series to establish mutability.

		on lac
Y53	Lac −	M (irregularly; many colo. stable).
W1	" Gal −	M consistently.
W566	" Gal −	S
W582	" Xyl −	S
W583	" Mtr −	S
W595	" Mtl −	S

The mutation to Gal- seems to have been accompanied by stability of Lac, −, possibly fortuitous.

12/28/48. Test other Gal- mutants of this series on Lac EMB for mutability:

W 565	Stable, thin colonies	575 mostly small stable colonies; some large mutable.
W 566	" heaped-up centres.	
567	" (very occasional papillae).	576 small colonies uniformly.
568	Stable.	
569	v. sm colonies; some revertants	
570.	typical mutable.	
571	like 565	
572	like 567	
573	stable, large colonies	
574	typical mutable	

12/6/68

A. W126B x Y87B see 373.

B. W495 x W45

C. " W48

D. " W65

E. " W182

Yields low:

A. 5 plates 100/plate. 3+ colonies. S.O. on Lee EMB + EMS. ++

B. 10 plates ± 2/plate 2+ colonies. ++

C. " ca 1/plate No +

D. " ca 1/plate No +

E. 9 " " No +

12/5/58.

Pick 1 – colony from each of four mosaics of H119 - H122 & test as indicated.

		Lac	V_1	Bu Gal	Nutr.
119	A	– S	S	–	TM
	B	– M	R	+	TM
	C	– S	S	–	TL
	D	– M?	S	± M	T * ✓ –
120	A	– M	R	+	M
	B	– M	R	+	MT
	C	– M	R	+	M
	D	– M	R	+	T
121	A	– M	R	+	MT * ✓ –
	B	– S	S	–	(MTL ✓
	C	– M	R	+	~~(MTL)~~ M * ✓ –
	D	– S	S	–	MTL
122	A	– S	R	–	MT ✓
	B	– S	S	–	TL ✓
	C	– M	R	+	MT ✓
	D	– S	R.	+	MT ✓
temp new data	Y87	– M	R	+	BM
	W126	– S	S	–	TLB,

6S:10R

M = mutable
S = stable

Summary:

Bug	V
8	+ R
1	+ S
2	– R
5	– S

suggesting linkage of Lac, and R.

Note preponderance of T- and M- S streaks out indefinite Bu Gal tests. *

There is a general correlation between mutability and Bu Gal – but it is not perfect here

Maintenance of heterozygotes.

12/14/48.	H1	v?	vv	
Lac	22	All+	All+	Returns to previous EMS plates.
Lac	52	v	vv	
Lac	62	v	v	
Lac	72	vv	v?	
X-gl	85	v	v	
X-gl	93	vv	vv	
Lac	118	v	v	

+ colonies from previous EMS plates restreaked on EMS. These restreaked, 2/type, on EMB and streaked on EMS; also on Nutrient agar slant (subculture 1).

A6. Streak out NA slant from H1 and H118 to determine feasibility of recovery at this stage.

4 tests each.

H1. 1- 3 Var. 4++ or Var? H118. All 4 are Var.

This may be a suitable method

Dec. 13-14, 1918.

A. W45 x W595
B. W45 x W583
C. 58-161 x W595.

} EMS Lac

(1). 15 plates each P13. A16: all but blank.

A: 7 colonies altogether. 5 possibly +. All --
B: 17 " " . 10? " +. All++ or --
C. - + 4 tested: 3++ 1-

} No Var.

Pick all possibly + colonies and streak out on EMBlac, ~~sucrose~~ + EMS.

(2). 15 plates A + B A14. Low yields, but quite up apparent +'s. (58)
Mostly — mostly scored as +. Not variegated.
Some weak(?) + noted. Type by on Lac EMB

#19, 2, 6, 14, 16 Keep as W-460
#16 is Gal++ Lac++ others are Gal- Lac very weak

Papillae seen on lac + gal plates. Streak out on both media.

Gal + pop:	Lac	Gal
6	++	++
14	++	++
Lac+ from 6		
0	++	++
1	++	++

Note: on lactose, residual Gal- colonies show near + reaction when they are situated in vicinity of + colonies.

Conclusion: The Gal- in these stocks is also an inhibitor of lactose fermentation, in distinction to W-255. H93, therefore, may now be Lac, ± and Gal-. ∴ It is not proven that Lac- can be homozygous!

12/23/48.

Cf. W460 on 1% and 3% Lac EMB.

At 48 hrs. W460 is nearly +++ on 3% lactose
still slow on 1% ".

Streak out W695 on EMB galactose for reversion.

Test revrts on lactose for mutability.
(W660.) #4. Allac Lac mutable like Y53.

Dec. 18, 1948.

Cross W45 × W595 on lac synthetic media.
A) "EMA" .5% asparagine as C source.
(B) EMS, fresh batch. Na succ " .
(C) EMA+B. Asparagine + Succinate .5% each.
(D) Lilac B. Bact standard.

} Very heavy (4× conc.) inocula.

A) 8+ /11 plates. A few lac-. Pick+ test +
1-8.

B) 7+? /13 plates. Surval -.
9-15

C) 12 plates. Poorly scored, but yields much higher.
16-48 25+.

D) 4 plates. 1? +.
42

Very few scored + on EMB. Some were lac mutable. (W45?)
6++ altogether. Numerous slow + à la 389.

Test media for crosses.

December 22, 1948.

Cross W478 × W595 on various media using constant inoculum. (1 drop ½ dil. parents)
Also conc. inoculum on Lac EMS.

+B₁ : ⟶ 1–6.
5 plates each.

+ +, — 100cc	
Na Succ.	.5g
$(NH_4)_2SO_4$	.5g
NaCl	.1g
4 $MgSO_4$	0.01g
agar	1.5g
K_2HPO_4	.2
Eosin Y	0.04
Meth. blue	0.0065
Lac	1g
8,	

eosin Y, blue, Meth Blue

12/24:

T(B₁).	51,	43	49	46	43	m = 46.4
T(0)	4	3	5	10	1	m = 6.6
(1) EMB.	1	0	0	0	0	.2
(2) MB	0	0	0	0	0	0
(3) E	0	0	4	1	0	1.0
(4) No dye.	10	8	14	4	10	9.2

The dyes are certainly inhibitory, but the minimal medium base is certainly not very satisfactory, possibly due to use of lactose as main carbon source.

From 20 plates mainly inoculated [illegible] lac EMS, about [illegible] [illegible] [illegible] papillae [illegible] + [illegible] [illegible]. [illegible] [illegible] [illegible] [illegible] lac +.

D478 x D57.

3934.

12/29 - /48.

1. 26 ✓
2. 46
3. 171 ✓
4. 188 ✓

not heterozygous.

: H139, 140, 141.

	Lac	Xyl	Mannitol	Gal	Malt
139:	±	±	−	++	
140	±	±	±	++	
141	±	+	−	++	

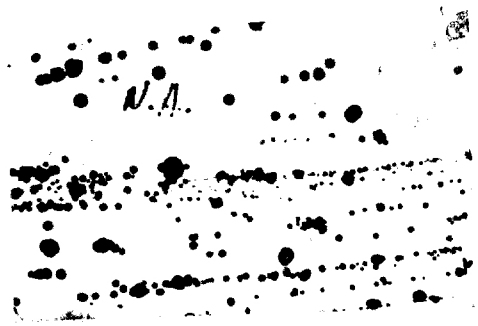
12/23/48.

Recover H93 from nutrient agar slant and from Xyl. S plates from NA to Xyl EMB. Pred Xyl-. Ca 2% mosaic colonies.

nutrient agar probably remains a preferred means of maintaining heterozygotes.

similarly on EMS Xyl. Pick a few to Xyl EMB to test recovery of H-93.

from EMS plate 7½ are still mosaic. Recover heterozygotes from EMB; EMS Xyl.



Where a heterozygous colony is streaked on EMB:

Gal negative

Xyl almost all mosaic colonies

Lac slow + (1 or two colonies finally ++).

Lac 3% full +; no sign of variegation.

H93 is therefore probably Xyl +/- Lac +/+ Gal -/-

December 24, 1948.

S(lac) 7 carboys ca 30 hours

1/20 mL .251 .340.

i. Each ml of culture will provide ca equivalent of [illegible] of 319A.

104 g. collected from two carboys (70 Liters). i.e. equivalent to 20 ml 319A.

58 g. suspended in a very heavy cream in 1/80 for grinding but ~~no~~ pump did not draw properly. Return cream & remaining paste.

12/25/48. Re carbeties mill & grind remainder of cells. Use entire basis. (ca 40-50 g. paste probable).

Ca. 10 ml of extract. Assays 3970 u/ml.

Galactose mutations
Xylose.

12/24/48.

Y87 7 secs. etc. 80 plates ca 200/plate
Galactose 16,000

W870 7 sec. etc 33 plates. ca 300/plate
10,000

→ W641.
642 :
643 very thin.

Xylose.

Galactose :

W			Lac
644	1	—	M
45	2	– thins	S
46	3	slow +	M
47	4	slow ++	M
48	5	– small col.	S
49	6	slow ++	M
50	7	slow +	M
51	8	—	S
52	9	slow +	M
53	10	slow +	M
54	11	slow ++	S
55	12	slow ++	M
56	13	slow ±	M
57	14	– thins	pap. to thin colony, prob. sat +
58	15	– thins	S
59	16.	– thins	S.

use 644 for 2 pathways.

Inhibition by various batches of MB; Eosin. – Crosses.

12/9/5

Weighed out 40 mg Eosin Y and 6.5 mg MB of batches indicated. (Certification numbers ...)

	Eosin	MB	Colonies −	Colonies +	Average.
1.	23	27	58	18	
2.	23	28 ✓	113	38	
3.	23.	29	93	25	
4	24/24	11	56	8	
5	14/24	28 ✓	103	23	
6.	24/24	29.	49	8.	
7.	22±	28	236	23	
8.					
~~12~~					
8.	7(0).		146		

all batches gave results comparable to 7(0).