

August 28, 1949.

a) large series of lac- and Mal- mutants obtained from
 W1069 and W1077 ca 8/25. (W1140 = $\text{Lac}^- \text{Trp}^+$ - Lac- ;
 W1141 = His^+ - D^+ Val- Mal-).

N28. Mix W1140, W1141 in Purnasoy tubes.

N30. Wash and plate on T(0); EMS Lac

P31. 1 colony / 15 plates.

spread out on T(0) agar. 4 s.c. picked and streaked on lac; Mal EMB.

Non-coliforma dominated, but contamination with a Lac+ Mal+ seen.

Purify and test mutations:

These derivatives of W1045 show no signs of recombination.

August 28, 1949

Mix heavily in Pennassay:

A) W1059 + W814

B) W1059 + W1084

A). Malac ~~75~~ 5 x 100. 13% Malac - 3/500 Malac v.

Test on other sugars, ~~is~~ T1.

Lac 25 x 100. >1% Lac v.

Mal+ "heteromlac" 33 L- (2 L+) (loc 2 indif.) were (B).
 Mal- 10 L- 50 L+

Mal+ Xyl+

B) Malac 10 x 100 = 1000 No Malac!

Mal 5 x 100 = 500. 6 Mal v. ?

Lac 25 x 150

Mal+: 56 Lac- (from Malac platings, all
 33 Lac- 2 Lac+ Mal- are Lac+).

Mal+: 89 Lac- 2 Lac+

September 2, 1949. xyl tests

	Lac + vac -	
1.	-	-
2.	-	-
3.	-	+
4.	-	+
5.	-	+
6.	-	+
7.	-	+
8.	-	+
9.	-	+
10.	-	+
11.	-	+
12.	-	+
13.	-	+
14.	-	+
15.	-	+
16.	+	-
17.	-	-
18.	-	-
19.	-	-
20.	-	+
21.	+, -	+
22.	-	+
23.	-	+
24.	-	-
25.	-	+
26.	-	-

of 25 lac v, 10 were Xyl - Lac -
 15 Xyl + Lac - // Xyl - Lac +

Among Lac	Seq. Col's. Rec.	10	15	35
	Whole pop.	24	25	
		34	40	

Sept 2, 1949.

602A = mixture of Lac-Mal recombinant colonies (5).

Grow overnight in ~~2~~ Penassay.

A) Assay cultures

	$\times 10^7 \text{ ml}^{-1}$	\bar{m}
602A	2,5	3
K-12	42,64	53
1033	20,27	23
58-161	40,56	48
Y10	35,33	34.

Sept. 2.

B) Inoculate .001 ml 602A + following: into Penassay tubes.

A	K12 1ml	46+/59-	31+/27-
B	W1033 .5ml + Y10 .5ml	213+/0-	136+/0-
C	58-161 .5ml + Y10 .5ml	71+/2-	156+/3-

Plate out ca 10^{-7}

Sept 5, 1949.

Lac Mal

B. 17 Lac y. Test +, - on Maltose. (5 each)

1-16 parentals only.

#17. 4 Lac+Mal-, 1 Lac+Mal+, 5 Lac-Mal+

September 7, 1949.

Irradiate W466 and W477 on EMB Mal 7 sec. UV.
25 plates each. ca 200/

W466 12 mutants purified. #3 is glucose -
All but #3 and #10 are maltose - slow. T.O.

#3 Mal - Glu -
#10 Mal - Glu +

1187?

W477 16 mutants purified.

#3, 7, 13 are glucose -. #2 is "this".

#s. 1, 2, 8, 11, 14 are maltose slow

#12 forms minute colonies.

#3 Glu -
4
5
6
7 Glu -
9
10
12
13 Glu -

1178-1188

check for 30° fermentation of Glu - - None were temp. sens.

September 15th 1949.

as 604. W466. 20 Mal EMB plates; 200/ = 4000.

1-8 are slow or nearly - fermenters of maltose. Test on 12/4

9, 10 are Mal - . Streak out. (Islet); W1208-1209

11 as streaked from 1st isolation had mostly Mal-(slow) colonies, but 3 colonies sectoring on maltose. [These may be suggested to be

Mal+	Let Mal
Mal-	Mal-

]. No Mal+ were seen. Streak out on Mal EMB.

N18

When streaked out, Mal_v colonies above gave mixtures of pure + and - and no apparent Mal_v. One possible Mal_v (unusually conglomerated) was noted. Streak out: These colonies are very difficult to interpret, mainly because there were no pure + colonies on the original plate. Conceivably, there had been induced an unstable intermediate allele which usually shifted to Mal - but rarely (i.e., within 3 colonies) reverted to Mal +.

September 13, 1949

(cont.)										
W1178-1183	x	W1014	}	Mal EMS(B ₂).						
W1187	x	W814								
(W1186)										
	Mal+total proct.									
1187	20% +									
1178	0/100				MAL1					
1179	30%									
1180	1/3 (ca 20% pro.)									
1181	80% +									
1182	30% +									
1183	0/100				MAL1					

677 SR x^w478
= W1177

Cultures purified

612
M. Dambroff

culture	from	Malt	Streptomycin	Lactose		lac+	lac-	Total	
1	malt synth	+	-	-	Strep resistant	malt+ 0	2	2	
2	"	+	-	+		malt- 4	5	9	
3	"	+	-	-					
4	"	+	-	-		Strep sensitive	malt+ 13	6	19
5	"	+	-	-			malt- 0	0	0
6	"	+	+	-					
7	"	+	-	+					
8	"	+	-	+					
9	"	+	-	+					
10	"	+	-	+					
11	"	+	-	+					
12	"	+	-	+					
13	"	+	-	-					
14	"	+	-	+					
15	"	+	-	+	Strep sensitive	malt+ 4	4	8	
16	from Malt synth + B'	+	-	+		malt- 6	13	36 (44)	
17	"	+	+	-					
18	"	+	-	+					
19	"	+	-	+					
20	"	+	-	+					
21	"	+	-	-					
22	"	-	+	+					
23	"	-	+	+					
24	"	-	+	-					
25	"	-	+	-					
26	"	-	+	-					
27	"	-	+	-					
28	malt. Synth	-	+	+					
29	"	-	+	+					
30	"	-	+	-					

Total organisms tested

Tested for lactose

lac+ lac-

Strep sensitive.	Malt+	30	2	16	10
	Malt-	0	0	0	0
Strep. resist.	Malt+	8	5	1	4
	Malt-	44	36	6	23
		<u>38</u>	<u>44</u>	<u>17</u>	<u>23</u>
				<u>6</u>	<u>14</u>
				<u>17</u>	<u>23</u>

Cross: 677 SR (1177) X 478.
4 pertinent diploids selected, Segregated

Original diploid	Segregant	Lactose	Synth	Resisting Streptomycin
1	1	-	+	+
	2	-	-	+
	3	-	-	+
	4	-	-	+
	5	-	+	+
	6	+	+	+
	7	+	-	+
	8	+	-	+
	9	+	-	+
2	10	-	+	+
	11	-	+	+
	12	-	+	+
	13	-	+	+
	14	-	+	+
	15	+	-	+
	16	+	-	+
	17	+	-	+
4	18	+	-	+
	19	-	-	+
	20	-	-	+
	21	-	-	+
	22	+	-	+
	23	+	-	+
	24	+	-	+
	25	+	-	+
	26	+	-	+
	27	+	-	+
	28	+	-	+
6	29	-	-	+
	30	-	-	+
	31	-	-	+
	32	-	-	+
	33	+	-	+
	34	+	-	+
	35	+	-	+
	36	+	-	+

Ma. 1

Diploids grew on streptomycin EMB, showed segregation into lac+ and lac-, all growing on streptomycin. Diploids could be reisolated from streptomycin plates.

Some colonies grew

??

Hence: Streptomycin resistance is dominant, ~~not~~ recovered in all segregants for lactose fermentation & nutrient deficiencies. sensitivity is lost in cross. Hemizygous?

W 108 mutations occurring spontaneously on nutrient agar.

Strain: Isolated 60+ on: Gluc Galact malt. Lact. Xylose Mannitol Treh

Strain	Isolated	Gluc	Galact	malt.	Lact.	Xylose	Mannitol	Treh
1	+	S	-	S				
2	+	S	-	S				
3	+	S	-	S				
4	+	S	-	S				
5	+	S	-	S		S	-	+
6	-	+	+	+	+	+	+	-
7	- (S-)	+	+	+	+	+	+	-
8	-	+	+	+	+	+	+	-
9	-	+	+	+	+	+	+	-
10	-	+	+	+	+	+	+	-
11	-	+	+	+	+	+	+	-
12	- S-	+	+	+	+	+	+	- (S-)
13	- "	+	+	+	+	+	+	- "
14	- "	+	+	+	+	+	+	- "

5
6
7
8
9
10
11
12
13
14

Glucose
" maltose.
Galact
Lactose

(not very strong)

+

+

- (S-)

- "

- "

D. D.

9-15-49 613

Fermentation tests on Shapiro's cultures.

	Sucrose	Rhamnose	Arab	Inositol	Xyl	Mil	Gal	Mel	Alu	Sorbitol
W1115 (1)	+		+	-	+	+	+	+	+	
W1116 (2)	-							+		
W1113 (3)	+							+		
W1114 (4)	+							+		
W1045 (5)	+							+		
W1176 (6)	+							+		
Sh 7	+							+		
8	+							+		
9	+							+		
10	+							+		
11	+							+		
12	+							+		
13	+							+		
14	+							+		
15	+							+		
16	-							+		
17	+							+		
18	+							+		
19	+							+		
20	+	+						+		
21	+									
22	-									
23	-									
24	+									
25	+									
26	+									
27	+									
28	+									
29	+									
30	+									
31	-									
32	+									
33	+									
34	+									
35	-									
36	+									
37	+									
38	-									
39	-									
40	-									
W1222										

All slow +

-+ are unstable

Sept. 16, 1949.

A.	58-161 x W1178	*6 tests ; all Lac ⁺⁺ ; 96 tests.	1 Lac ^v	2?
B.	58-161 x W1183	52 tests.	2? Lac ^v ; 60 tests	6 Lac ^v 2?
C.	^{Mal⁺} W478 x ^{Mal⁻} W1178	100 tests.	4 Lac ^v	
D.	W478 x W1183	52 tests.	1 Lac ^v	

A. 2, 3 (?)

B. 1 het \leftarrow Lac^v Mal⁺⁺

614-B1

C. 1-4 Lac^v Mal⁺⁺

614-C: 1-4

[#1 and 2 throw off frequent lac-prototrophs I.]

D. 1 Lac^v Mal⁺⁺

614 D1

B. 1-6 Lac^v 7, 8 Lac^v?

5, 6, 7 are Mal⁺, - 8 is Mal⁺ Lac⁺⁺.
1-4 are Mal⁻

M.O.
* Miscalculated.

See 618

September 17, 1949.

	on EMS Lac;	EMS Mal.
A. W1178	x W828	52 tests 1?? Lac _v
B W1178	x W836	100 tests. 45 Lac _v 6-7?
C. W1178	x W760 very infertile	52 tests 122 Lac_v 6 tests. 2 Lac _v .

In 615 B, both on Mal EMS and Lac EMS, - colonies seem to grow better than + ! streak out from Mal EMS:

A Mal -

B 1-4 Mal - -
All Lac_v.

C 1-2 Mal -

5 Mal+ (v??); 6 Mal+, - 7 Mal -
(Residual on resolution)
Isolated Lac_v of 5, 6 were
pure Mal+, Mal - resp

See 618

September 17, 1949

- D. W1189 x W1195 [Val^{A.C}Mal¹- x Leu²Tryp²Lac-]
- E. W1189 x W1205 [Val^{AA}Mal¹- x Thr³Hist³Lac-]
- F. W1195 x W1191 [Leu²Tryp²Lac- x Thr⁴Hist⁴Mal-]

Wash cultures from 12. Conc. 5x. Use 1ml/plate

Controls:

1 (W1189)	4 colonies / 4 plates	Lact Mal-
2 (W1195)	0 " / "	
3 (W1205)	0 " / "	Lac-Mal+
4 (W1191)	8, 12, 16, 11 / 4 plates	ca. 12/plate!

- D. 2 colonies / 9 T(0) plates 2 Lact / 4 EMS Lac plates
- E. 7 colonies / 9 T(0) plates 1 Lact / 2 "
- F. 10, 6, 2 / T(0) plates

W1189 and W1191 appear to be exceptionally unstable. Their nutrition should be carefully checked.

Tests on "cross" prototrophs

	Mal	Lac
D: 1-4	+	+
E 1, 6	+	-
2-5, 7	+	+
F 1-16	+	+

These prototrophs are clearly either contaminants or recombinants, probably former. Parents had been checked on EMS and found pure ✓.

Tests on "reversion" prototrophs

1	Mal+Lact
4	Mal+Lact

} must be contaminated!!

P19

A20

1	0, 2, 2	1113:	True Hist. x	Val Arg.
2	1, 2, 2	1113 x 1114	"	Val-200, Arg.
3	2, 1, 4	1113 x 1115	"	Leuc Tryp.
4	0, 0, 0	1113 x 1114	Val Arg	Hist Leuc
5	0, 2, 1 ^{sm sm}	1113 x 1115	"	True Hist
6	1, 2, 0	1114 x 1114	Val 200 Arg	Hist Leuc
7	2, 0, 0	1114 x 1115	"	True Hist
8	7, 4, 8	"	"	Leuc Tryp.
9	0, 0, 0	1115 x 1115	True Hist	"
10	>15.	1113 1115	Val Arg	True Hist

Prototrophs occur amidst rather heavy syntrophism!

Pick colonies from #8, #10. + streak on T(0).

Each of 12 tested from #8 and #10 grew out as single colonies on T(0), and were further picked to EMB lac, Mal, Xyl in which they agreed with their parent in being + + +.

September 25, 1949.

Collect following heterozygotes: 4 = 614 5 = 615.

5A = 1178 x 828

4A = 58-161 x W1178

4B = 58-161 x W1183

5B = 1178 x 836

4C = W478 x W1178

4D = W478 "

5C = 1178 x 760

mENSkc

614 A. 1 Lact+ Mal+ } Result!
 2 Lact+ Mal+ }
 3 Lact+ Mal+ }

0
0
0

614 B 0 1, 2 Lac^v Mal+ or v?? Mal+.
 1 Lac slow? Mal-
 2 Sac slow? Mal-
 3 Lact+ Mal-
 4 Lac^v "
 5 Lact "
 6 Lact "

C 1 Lac^v Mal^v?
 2 " Mal+
 3 " Mal^v?
 4 " Mal+

D Lac^v Mal+



615 B 5 (4 tests) #1, 4 Lac^v; All Mal+ but #1 shows nothing on Mal. but Mal+

6 (4 tests) All Lac^v Mal- (segregating blue + white) Use for Rev
 60 (1 test - broad streak) Many Lac^v. Almost completely Mal-.

615 B5 = Lac^v Mal+
 615 B6 = Lac^v Mal-

See 615 for data on other Lac^v.

Sept. 23, 1949.

W466 Mal_x- x W677.

A) W1208 x W677.
2 Mal+ / 600 Mal -.

B) W1209 x W677
5 Mal+ / 600 Mal -

steals out + photostops:

A) Pure Mal+

B) 3 Mal_v; 4 ~~Mal~~ Mal++.

4 plates Mal EMS

In view of rarity of Mal+
in W466 x W677, these low
frequencies do not necessarily
speak for close linkage.

Reconstitute [to use for lac reversion
studies] 3.

September 28, 1949.

Mixtures grown together 48 hours.

Plate ca. 5ml \approx /plate T(0).
Inc. 48 hours.

A	W1189	0	0	0	
B	W1191	0	0	0	0
C	W1195	0	①	0	①
D	W1205	⑤	①	⑤	0
E	W1189-1195	2	3	5	4
F	W1189-1205	3	1	2	0
G	W1195-1191	0	0	0	0

Lac+M - Valkey Sh 3

Lac+M - Th Hist Sh 1

Cont? L-M+ Leu Try Sh 1

<sup>L-M+
Th Hist</sup> Prototrophs grew more poorly on T(0) agar than those below.

Picks, dilute and test on Lac; Mal:

E	2	Lac - Mal+	
F	10	All Lac - Mal+	
G	12	All Mal+; 8 Lac - 4 Lac+	
H	6	Lac+ Mal+	
		Lac - Mal+	

These results strongly suggest recombination between W1189 and either W1195 or W1205. However, there is a curious instability of the individual parents. The Lac+ Mal+ prototrophs are, however, unique.

Repurify and retest parents!