UV - Killing - Liquid suspensions.

Jan. 9, 1947.

Irradiate 5 ml standard suspension in water of 1/10.

Plate out .05 ml samples.

T. (secs)	S.
10	3
20	46
30	1
45	0
60	0
90	0
120	0
180	0.

Irradiate flasks at aperture of lamp with shielding by sand.

1/9/47 PM. Repeat.

~~10 S.~~

20 S.

~~30 S.~~

Jan 8, 1947.

as 75. Irradiate Y10.

Maltose: 36 plates x 200 = 7,000 cols.

1. ● +and- Restreak to purify. −102 +103
2. ○ faint+. All−. Pick as W95.
3. ◉ +and−. + W97 − W96.
4. ◉ +and− − W98 + W99.
5. ◉ +and− Restreak to purify. 104 − 105 + 106 − 107 +
6. ◉ +and− "
7. ◉ +and− − W100 + W101

Rae: 36 plates. x = 7,000 cols.

1. ◉ +and− W108− W109a+
2. ⊙ Restreak
3. ◉ + and ± (⊙). W110± W111+ See 197.
4. ◉ All +.
5. ◉ + and− W112− W113+

[Cross-test these].

Jan 9, 1948.

Lactose analogues 1% EMB

		b-Me-galact	b-N-butyl gala.	O-Cresyl-b-galac ·
Y10	Lac+	++	++	± v. slow pegillate similar to
Y53	Lac$_1$-	± - + slow	++	± o-phenyl.
Y35	Lac$_2$-	—	—	— strangers inhibitors
Y45	Lac$_2$-	—	—	—

The β-N-Butyl galactoside gives the most straightforward differentiation so far noted.

Sucrose & Melibiose & Raffinose.

	Raf 3%	Melibiose st. fil.	Sucrose.
" Raf ± "	±	slow ++	—
" Raf - "	∓	slow +	—
Y40	∓	slow +	—

Melibiose activity should be enhanced before attempting to test on raffinose!

Fructose sterile filtered.

Y40	+++
W-1	+++.

Cross-test Lactose mutants

January 4, 1948.

Inoculate YP broths with following:

Y53 (Lac_1-) and:

Cross each on three plates.

A8. (proportion +)

1	W-30		
2	W-35	++	1/3.
3	W-40	+	Like 65. ca 1:100.
4	W-42	-	All - [1/>200 + noted J!
5	W-43	1/100 +	Like 65. ca or < 1:100.
~~6~~	~~W-44~~		
7	W-45		++ 1/2 - 1/3.
~~8~~	~~W-47~~		
9	W-48	1/100 +	
10	W-65	-	All - [1 + colony!] 1:100.

no growth.

Harvest and mix cells. Plate dilute on EMB-Lac(B_1).

∴ None seem to be allelic with Y53. Lac_1 -.

a) W35, W45 1/2 - 1/3 Lac+ recombinants

b) W40, W42, W43, W48, W65. ca 1% Lac+ Recombinants.

c) Y53. (Y87?). Original data on Y87 were more limited than these.

Streakout all Lac- and Mal- mutants for recheck!

Maltose segregation: Effect of preadaptation on maltose. 82

January 8, 1948

Prepare inocula overnight in YP broth.

Y40 10 AM add 2-3 ml to YP-maltose (A,B) and YP-glucose (C,D) broths.

Incubate W-1 similarly in YP for five hours to 2 PM Cultures of Y-40 are actively producing gas at this time. Was and cross samples of A,B,C,D, with W-1. Plate on synthetic EM-Maltose(B_1).

Count Sectors as +.

A: (M2)

M+	M-	% +	
4	130		0
3	78		0
3	88		0
6	113		1
3	156		0
9	248		0
3	177		0
12	398		1
2	64		1
			2.

3.099%

B:

0	68		0
1	179		0
12	435		2
7	236		2
9	384		0
12	284		2
1	70		1
10	237		2
4	135		
46	2028	2.74	

2.218%

91	3480	3571	

2.548%

Conclusion: No effect of preadaptation.

	R	S	T	U
Na I:	8	5	5	0
	7	2	7	2
	15	7	12	2
Na_2S –	8	3	5	0
	4	3	11	1
	8	2	9	1
	8	5	6	0
	6	3	9	0
	9	5	2	0
	11	2	6	0
	54	23	48	2
	71	30	60	4

C: (G1)

	M−	M+.	S	
1.	3	207		
2.	2	47		
3.	2	109	2	
4.	3	135		
5.	8	263		267
6.	2	85	1	
7.	2	98	1	
8.	0	71	0	
9.				
	22	1019.	4	total: 1041

D: (G2).

11	269	3	
8	213	3	
3	108	1	
14	357	3	
5	165		
			1153.

41	1112	1153.
63	2131	2194.

~~2.727 % Malt.~~
2.871 % Malt.

Comparison.

Glucose.	58.6 59.	2135	
	63	2131	2194.
	91	3480	3571
	154	5611	5765

3806

$\chi^2 = 16\left(\frac{1}{63} + \frac{1}{91} + \frac{1}{\;\;} \cdots\right)$

$= .5$

Mean: Malt = $\frac{154}{5765} = .027$ %

Y105 Mutation run: Lac attempts

Jan 12, 1948

Irradiate .1 ml per plate (LacEMB) 9 secs. under Hanovia.

71 plates x ca. 30 colonies or 2000 colonies.

3 suspicious colonies streaked out:

1:

2: No mutants

3:

Jan. 13, 1948.

Plate mixtures on Lactose-$EMSB_1$:

	$Y87(Lac_1-)$	$W-45(Lac_2-)$
Y53	(>1000) ✓✓✓	+++
W108	++✓✓✓✓	++✓✓✓
W-112	>1000 − ✓✓✓	++± ✓✓
	a	b

see 81 ✓ are replicates. Lac_3-.

On Maltose $EMSB_1$:

	a $W56(Mal_1-)$	b $W-60(Mal_2-)$
W-1	(>1000) − ✓	+ ✓
W95	? ∸	++
W-96	∸, +	++, ✓
W98	∸ (1:1000)	+±
W100	∸, −	+ ✓
W102 (circled)	±	+±
W104	∸	+±
W106	∸	+± many sectored.

Mal_1-: W-1, W-56.
Mal_2-: W-60
Mal_x - all others.

+ = 1:100

++ = 1:10

+++ = majority or same order magn.

Parentals checked. ρ = papillation in heavy streak.

Y53 − ρ
W45 − ρ.
W108 − ρ
Y87 − ρ
W112 − no or few ρ.
W102 − ρ
W56 − No ρ.
W98 − ρ.
W96 − ρ.
W95 do.
W100 ∓ ρ

W78. Slow but ++ utilization
W60 − No ρ.
W20 slower ++ utilization.
106 slow + ρ
104 slow + ρ++
W71 ± ρ.

Polysaccharide Utilization

Jan 10 ff 1948

Test strains indicated on T(m) plus .05% substrate.

A. Inulin	W-55	Ap✓ (39)
P12 (48h)	—	—
	—	—
B. "Bacterial Dextran" Lot L-10 from K.P.Link	—	—
	—	—
Inoc. P12		
C. "Soluble Starch" as above,		
A14	±	++ → dodine color red-violet
A17	±	+++
A25		

A25 —

W55 Hm+ seems to accumulate a red-staining "erythro" from Amylopectin and soluble starch, but utilizes amylose completely. "Saccharifying amylase ??"

Cross available B-M- Lac - mutants with TLB, Lac, and Lac3 testers, ~~Y53~~ W112 and W-108

	A ① W-112	B ③. W-108.
Y87.①		
W31	n.c. N.C.± 'col.	no.col. ✓✓
W35		+ ✓ ++
W40		+ ✓ ++ ✓
W42		✓✓ ++
W43		++ ✓
W45①		n.c. ✓
W 48		++ ✓
W55		+ ✓ also intermediates ??
W67	? + ✓ + and intermediates?	n.c. ✓ + ++ sm. cols (poor plate).
W72	n.c. + should be underlined.	n.c. ✓ n.c. ✓
W74	+ * ✓ ✓	++ ✓ ✓
W76	+ ✓ ✓✓	++ ✓ ✓ ✓
W83	+ ✓ +	n.c. ++ ✓
W87	+ ✓ ? + ✓	++ ✓✓

Jan. 16, 1948.

(Y10)
Suspend cells from slants. Spread on Lac EMB (ca. 100,000, 100/pl) and irradiate ~~[illegible]~~ 15 secs. under Stauffer's lamp. as supra.
× . = colonies.

Run n.g. Evidently, wrong cells (mixture Lac+ / Lac-) were used for irradiation.

TP from W94. 89

Jan. 17, 1948.

Grow 12 l. W94 in N2 Case 1%, Glucose ½% (ster. sep.) and $K_2HPO_4 + KH_2PO_4$ (3:1) .4%. 15 gallon Pyrex carboy 24h. at 37° with aeration.

Collect 53g. paste in Sharples. Resuspend in .9% NaCl 2 litres and recover 39g. washed paste.

Mix paste with 2 parts pepsin and q.s. citrate saline and crush in portions in a Pyrex cone mill, ~~c~~ assistance RH Barnes. Resuspend in 200 cc citrate saline (.1m each). Sediment glass & debris and collect supernatant juice. add 2 vols alcohol and store in refrigerator. To 100 cc portion. (A).

To remainder, (40 ml.) add 1/30. chloroform & ~~1/30~~. 1/10 vol. 6m NaOH (B) Mix and store.

P18 (A) Decant and reject supernatant from A. Sediment and redissolve in 50 ml .1M NaCl. Add 2 vols 95% alcohol in a sterile flask. Repeat. → 3.9 gms. alc.-wash. paste.

(B). Reject gelled $CHCl_3$ - 6m NaOH - protein. Sediment and decant supernatant. Retreat with $CHCl_3$ overnight. Repeat twice.

Store bulk of extract A. in 95% alcohol.

Suspend 1 gm. paste A in 20 ml NaCl. Add 5 ml aliquots to sterile test tubes and add 10 ml alcohol to each (use acetone for B4). allow to stand for stratification, sediment and replace alcohol with sterile saline, 10 ml. These will contain 1 gm paste / 40 ml saline.

Sol. "A" 90A

B. Third "Swagging" → almost clear, opalescent. y.g. liquid. Remove from residual $CHCl_3$ and ppt. with alcohol 2:1 as above. Sediment and wash with 95% alc. to remove exc. $CHCl_3$. Resuspend sediment in 10 ml H_2O, add 5 x alcohol. Ppt. fibrous. Lift out with glass rod and resuspend in .1M NaCl → clear but str. opalescent solution. Repeat with remainder of sediment. Have very little fibrous sediment, considerable granular which is thrown out. Final suspension presumably polymeric NA. in 10 ml NaCl. ~~"Sol B"~~. Sediment with 5 vols. alcohol in sterile tubes, and resuspend in sterile NaCl, 40 ml. "Sol B." 90B.

Note N24, 1 tube of (B) ppt'd with 2 vols. alcohol. No fibrous ppt. formed suggesting depolymerization.

TP Activity.

January 19, 1948.

(Add 1 ml. 90H +B. resp to 10 ml YB broth tubes (5 ea.).
Use 2 for sterility tests. Inoculate each of the other three with 48hr. culture Y138. Also 3 tubes of C smears for no-treatment controls.

Read A 20.

1	A1			all Mal +	
2	A2			all Mal +.	(A phage plaque ?)
3	A3			all Mal +	
4	A St	turbid		Some everything	Not coli
5	A St	turbid.		~~No colonies~~ Some everything.	Not coli.
6	B1			All Mal +.	
7	B2			all "	
8	B3			all "	
9	B St	Turbid!	str. out	Cont. Not coli	
10	B St.	Clear	str. out.	= No colonies.	
11	C1			All Mal +	
12	C2			"	
13	C3.			"	

Streak out all tubes on Mal ~~and~~ EMB.

↳ ~~Streak out~~ Test on ~~Mal~~ Y138

	O	A	L
1-1	0, 0, 0	0	11, 7
1-2			
1-3			
2-1	0 0 0	0 0	5, 1
3-1	0 0 0	0 0	15, 5
4-1	heavily loaded with septicomycete contaminant		
5-1	heavily contaminated.		
6-1	0	0	34
6-2	0	0	3
6-3	0	0	0
7-1	0	3	30
8-1	0	0	45
9-1	Loaded with "mucoid" contaminant.		
9-2			
9-3			
11-1	0	0 1	16 1
11-2	0	0	1
11-3	0	0	0
12-1	0	1	32 26
13-1	0	0	46

There is no evidence from this experiment of transformation of the H- or L-loci either by the crude extracts or by the fibrous material of "B".

Replate cells in series 1 on A + L agar.

[illegible] later.

Preparation of I.P.

Jan 23, 1918.

Grow W-94 "anaerobically" in 12 l. N2 case medium, 2 d. 37°. Yield: 17 g. Sharples paste (~~1~~ 1/3 aerobic yield). Suspend in 170 ml NaCl (physiological) + blend ē. 2 ml toluene. Let stand 4 hours, sediment & ppt. supernatant ē 2½ vols 95% alc. V. little sediment formed. Separate + store in 70% alcohol. (C)

Specific reversions of W108.

Jan 27, 1948

Streaks out the following "reversions" of W108 on the homologous medium, as indicated, to purify.

From glucose. EMB plates of 93. — to lactose & maltose.

	L	M
	Y10	Y10.
1.		
2.		
3.	Test 31 "reversions" on glucose plates on	
4.		
5.	lactose and on maltose.	
6.		
7.	All 31 glucose-reversions are also lactose & maltose +.	
8.		
9.		

plates M1, M2, L1, L2

From Lac + Mal EMB. Streak out to Lac + Mal + Man.

+ Man.

10 Mal + are Lac +

6 Lac + are Mal +.

L, M, Man = ½ Man + also Mal + and Lac +.

3 Man + are "weak", fourth is "strong".

Purify + compare ē Y10.

From 93 Broth. 108 M / M & L resp.

From 93 T(m). Maltose 108 M (Tm) / M + L resp.

→

All reversions are non-specific for glucose, maltose + lactose

Nos. tested:

Glucose	31
Lactose	6
Maltose	13
Mannitol	4

54 tested altogether.

Select. area as W-108^R = W116.

Characterization of W-108.

January 28, 1948.

T(m) T+B, + : .05% = W108 (autoclaved together). Y10.

glucose.	−	+++
α-ketogluconic	−	+
hexose ~~di~~ diphosphate.	++	++.
" + glucose		+++.

The HDP was prepared from the Schwarz Ca salt product by adding excess oxalate and neutralizing with NaOH. The solution contains exc. oxalate, which is evidently not inhibitory considering the control. In autoclaving, the HDP solutions turn quite yellow so that breakdown must be suspected. Repeat expts. using filter sterilized HDP.

Test Proteus X-19 on HDP. Add to T(m) + nce :

	A29.	A2
glucose	−	++
fructose	−	−
HDP.	−	++

Salmonella cross.

Jan 29, 1948.

S. dublin I IV g,p ; — Ara+ B_1 −

X

S. paratyphi A. I II XII a ; − Ar+ B_1+ Meth− Tryp−.

on arabinose minimal medium.

Inoc sep. + together into YP broth. (1) S1 (2) S37 (3) S1+S37 X

(A). Plate .1ml washed samples of 16 hr. cultures on arabinose T(m) minimal.

1. S1 12 cols. 4. S1+S37. 2. S1 reverts on ~~agar~~ arab. minimal.
2. S37 —
3. X ca 10-20 cols.

(B). Do.

1. S1 0 2 large 1 sm. many small,
2. S37 0, 0
3. X 0, 3 sm. cols., 10 cols., 0, 0
4. S1 + S37 3 c., 10-20 c., 100 c., 100 c. 100 c. many small.

Read 2/4/48.

(4) may represent a cross. Addn'l differentiating characters needed to eliminate S1 reversion.

Jan. 29, 1948.

Test 93. W108: glu+ and tre+ on glucose & trehalose EMB.

1. Glu+ On EMB, all white colonies with no glucose + trehalose
2. Tre+ In T(m). Both grow rapidly on glucose, fairly quickly on ~~glucose~~ trehalose, T+ both.

Streak from Glucose plates to EMB glucose.

(1) —
(2) — in 24 hours.

Take 99-1, impure, as W-117

W-117 is either an aerobic oxidizer of glucose or else a slow fermenter.

Compare on glucose and on K gluconate:

W117:	EMB:	
glucose	+ weak +.	Use these colonies for pure W-117
Maltose	± - +	
lactose	—	
K-glucon.	+++	