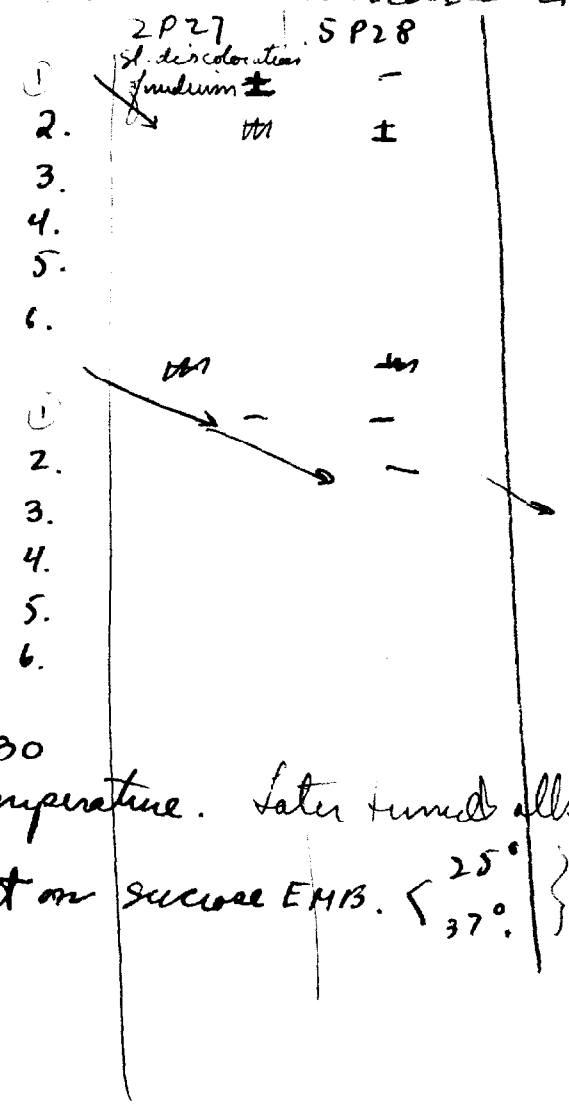
Selection for Sucrose + mutants.

Oct. 26, 1947.

Prepare 10ml. 2% sucrose 1% peptone, ~~0.1% y.e.~~ with Bromcresol Purple Indicator. Incr. as indicated, in series.



A. Y53. 10/26/47. (1). SP.

B. 58-161. (1) 10/26/47. SP.

(-)
IA3 was incr. into IA4 P30 and left overnight at room temperature. Later turned alkali. Not Sucr +
P4. (IA4) was +. Streaked out on sucrose EMB. $\left.\begin{matrix}25^\circ \\ 37^\circ\end{matrix}\right\}$ both Sucr -

Y105 —

Y106.

Abandoned A6.

Selection for α-methyl glucoside mutants.

Oct. 26, 1947.

1% α-methyl glucoside (EK) broth, autoclaved together. BC Pinds.

	2P27	5P28.
A. Y53. ① 5P	— ↓	– – ↓
B. 58-161 ①. 5P.	— ↓	– – ↓

growth only fair at 24hrs

abandoned A 6.

Oct. 26, 1942.

Glucose + 2% peptone. Vary glucose in methyl-red indicator to establish the critical level. K-12. 5P26. In duplicate.

Glucose: 2% 1% .5% .2% .1%.
Methyl red destroyed.

3P27. Growth + fermentation ++. No color changes. Test pH in 1 series.

pH:

add to MR	++	++	++	±	-
" " BCP.	++	++	++	±	-

Use glucose .3%; Bran Cresol Purple. Prepare series of tubes ē 2% peptone. Inoc. each with K-12. P28.

11A29. pH B. = ca. 6.8. pH. A. < 5.2.

(B) (-glucose).

Add ca 8ml B to A1. pH ca. 6.0.

(A.) (2) 1-10.

(1)

Retain sample in refrigerator for comparison to detect alkali production.

(A1). A30. Alkali produced. Transmit to A2.

(A2) A3. Alkali produced Add to (A3) i.e. mixcultures.

(A3). A6 Alkali produced.... Start A4

(4) A10 Alkali produced.

end. Expt.

10/25/47.

Harvest Y53 from 2×25 ml/125/f. YP broth and from 3 × 10 ml YP agar slants 20 hrs. old. Suspend in 10 ml M/10 phosphate buffer at pH. 6.0. Add 20 mg HN2 nitrogen mustard [$(Cl\,Et)_2 NCH_3 \cdot HCl$] in 10 ml buffer. Let stand 30 minutes. Sediment cells and replace with peptone 10 ml. 1% Inoc 1 ml samples into 25 ml YB broth and incubate to prepare inoculum for mutant detection. Spread .1 ml ea check on survival → ca. 10^4. Estimate.

$pS = \log 10^{10}/10^5 = 5.$ Many mucoids noted!

	Plates	Colonies/plate.	Total colonies.	Suspected mutants
10/28/47.	40	250	10,100.	-1 not Mal- (mucoid). -2 -3

	T1	T4	T6	T7	Lac	Nutr.	
-1 (W1)	S'	R	S	S	—	T-L-B, ±	∴ mutant!
-2	R'	R	S	S	—		
-3 (W3)	S'	R	S	S	—	?	Mal- "papillae" on test streak. Reisolate.
Y105	R	R	R?	R	+		
Y106	R	R	R?	R 1 plaque	+		
N-12	S	R	R?	S	+		

10/29/47. Spread similar populations on galactose EMB plates. 11AM.

(colony dimorphism noted • S/R?)

Of ca. 40 × 250 = 10,000 colonies, no galactose - were noted. However, one colony which was unusually light purple was noted + picked for further study = 4-4.: Not Gal-.

Oct. 28, 1947. Test the cultures indicated on the following EMB:

	Y53	Y87	58-161	Y53	Y81	58-161	
Galactose	++	18 hrs. ++	++	→			✓ *** same pitted!
Rhamnose	−	−	−	−	−	−	+ + + ✓
Inulin	−	−	−	−	−	alk	
Melibiose	+	+	+	+	+	+	✓ *
a-methyl glucoside	−	−	−	−	−	−	
Cellobiose	−	−	−	alk	alk	alk	✓
Dulcitol	−	−	−	−	−	− ppt.	− ± ± **
Sorbitol	++	±	+	++	+	+	✓
Sucrose	−	−	−	alk	alk	alk	
Ethyl Butyrate	inhibited			→			produce alk
Lactose	−	−	+	−	−	++	

on $CaCO_3$ (.1%) peptone agar:

Lactose Minor clearing around lac+ colonies. Not definite enough!
Ethyl Butyrate (add "sterile" to hot medium.)

Cellobiose α-methyl glucoside Sucrose Melibiose

W-1
W-3.

* Streaks not uniformly dark: lighter in centres. However, this is rather a thin plate.

** Streak out the papilla.

P 31. Y53 is Dulcitol negative; Y87 + 58-161 Dulcitol +.

[dulcitol is related to galactose!)

~~W-1~~ W-1. Melibiose slow +
10/31/47. α-methyl glucoside −
Sucrose −
Cellobiose −. Papilla (?) on 58-161 and Y87?

Streak out again

Carbon source utilization.

T(m) + Indicated Carbohydrate (½%) + BM for 58-161
+ TLB for Y53...

G = glucose ½%
g = glucose .05%

16 hrs. 37°.

	O	G	g	Maltose,	+d.m.g.	d.m.g.	+g	+G.	Cellobiose	+g	+G
W-1	−	++	++	+	+	±	++	++	±	++	++
Y53.	−	++	++	+±	++	±	++	++	+	++	++

	Sucrose	+G	+g.
W-1	±	++	++
Y53.	±	++	++

	−BM O	+BM O	G	Lac	Suc
58-161	−	−	+++	++	±
Y87.	−	−	++	±	−

48 hours:

	O	b	g	M	M+d.m.g	d.m.g	+g	+G	C+g	C+G	Suc	+G	+g
W-1	−	++	++	++	+	±	++	++	+	++	++	±	++	++
Y53	−	++	++	++	++	±	++	++	+	++	++	±	++	++

	BM	Gl	Lac	Suc
58-161	−	++	++	±
Y87	−	++	+	±

Use .05% in testing sugar utilization in future growth experiments.

Mutants in S8 161. MUSTARD. 7.

P 29. Inoc S8-161 into YP broth. 2P30 Harvest and suspend in 1 ml
M/10 Phosphate pH 6.0. Add 10 mg. to each of two tubes -
HN2.

a. 2:28 PM - 3:02 + 8 min centr. 34 min.
~~b. 2:48 PM.~~ b: 3:02 - 3:17 + min centr 15 min.

at 3:02 PM Centrifuge dilutic peptone 10 mins. Resuspend in peptone
Inoc 1 ml each into 25 ml YP. Spread .1 ml samples to
assay killing.

Killing a) >> 10^4

b) > 10^4 survived. pS < 5. Spread out +

Retreat survivors also!
Spread on Lac, Gal. and Mal.

Galactose. 83 plates. Sharp division into large + small colonies
noted. ca. 300 large + 900. small noted. Noted
noted on Lac or Malt plates Lac + Malt plates are v. crowded ē
"uniform" colonies - ca. 1000/plate ∴ are some cells inhibited by
galactose?? Only large could be scored: 25,000 colonies. Ca 7
most likely possibilities. Also reisolate G^R and G^S colonies +
test as contaminations? 7:1-7.

Lactose: 37 plates. Ca 800 on each plate scoreable = 30,000.
Almost 1 lac - per plate noted. 12 selected for further study. 7:11-30
(1/1000)
Maltose 63 plates ca 55,000 colonies scoreable.

8 apparent Mal - noted. 7:41-50.

Total tests: 110,000

Reversion of Lac + Mal in W-1 and W-3.

Streaks out papillae of W-1, W-3 and YS 30 cultures on Lac and on

Nov. 2, 47 Malt. EMB agar. Note that on original plate, W-3 had some papillated, some non-papillated colonies.

W-1. Lac. All Lac -. Papillae in streak?
Mal. alkali. All Mal -.

W-3. Lac All -.
Mal All -!

} Hold for evaluating

Restreak A6.

W-1. Recorded for Lac found. Purify and number W-33.
W-3-(Malt) all - ; papillae are not Mal+!
W-3-(Lac) + and -. Purify. ✓ W-34

Mustard killing : 58-161.

Add 10 mg mustard to 10 ml 58-161, assay = 3×10^9, in phosph. M/10 pH 6.0. Dilute 1:100 at intervals and spread (on sucrose EMB which is available). .1 ml. Colony count = survivors/ml $\times 10^{-3}$

0 time assay = 3×10^9

60 mins assay = 2×10^6. pS = 3+. for 60 mins.

Assume 10% survive for each 20 mins. at .1% in phosphate buffer. Use .2% and 30 mins. treatment.

Nov. 2, 1947. G^S G^R

P1 - Inoc small and large colonies of 58-161 on galactose EMB plates of Expt. 7 into YP 25 ml. Inc. overnight.

P2. Treat c̄ .2% HN2 in phosphate buffer 40 mins. Add 1 ml susp. to 25 ml YP for further incubation.

Incubate G^S in left (sinistral) side of incubator.

G^R: Lac. 70 pl. x 20 col. → 1400 tests → 12 cols. from unts. inoc all Lac +.

ca 50% of colonies are Lac −. (In inoculum?).

Pick colonies from a Gal plate inoc. with the untreated suspension. Pick 3 colonies to slants W30, W31, W32.

Pick to sl. 10-1.
Test nutrition: BM ✓✓

Malt. 16 x 40 = 640 tests.

1:1 dimorphism of very dark and less dark colonies. Both are + and have a sheen. (Corresponds to above??)

Gal. 23 x 30 = 700. No mutants.
some contaminated

G^S. Lac. 65 x 100 = 6500. No mutants.

Malt. 17 x 80 = 1360 No muts.

Gal. 23 x 30 = 700 No muts.

Nov. 3, 1947. Characterization of Mutants of Exp 7.

A. Galactose mutants – 1st. streak on galactose EMB.

7-1. Majority of light purple colonies ⊙ with a few typical Galactose +. Pick to glucose slants. BM W-2.

7-2. Gal ± as in 7-1. No Gal+ BM W-4

7-3 do. BM W-5

7-4. Majority of alkaligenic, v. small colonies. Streaks of heavy streak, a few Gal+, larger colonies. Test as Lac: Lac- alkaligenic. throw out.

7-5 as 7-1. BM W-6

7-6. Do. BM W-7.

7-7. As 7-2. BM. W-8.

B. Lactose mutants.

7-11. Typical Lac –. n.g. in 16h. not B-M-! W-9

7-12. Do. Papillogenic W-10

7-13 Do. Pap. W-11

7-14. Do. + two Lac + colonies. W-12

7-15 Do. W-13

7-16. Do. W-14

7-17 Do. W-15

7-18 Do. Colonies smallish. Lac + in heavy streak W-16

7-19 Do. Weak utilization? W-17

7-20 Do. W-18.

7-21. Do. — what is the nutrition of these creatures? — W-19
7-22 do. — W-27
~~3~~-MALTOSE.

7-41. Majority are light purple. Some Mal+. BM W-20
7-42. Mal+.
7-43. As 41. BM W-21
7-44. All Mal+. BM W-22
7-45 do. BM. W-23
7-46. do. BM W-24
7-47 do. BM W-25
7-48 do. BM. W-26.

Test nutr. by comparison of T(B+M) c̄ T(0).

58-161. G^R — W-28
G^S — W-29

Galactose Inhibition

Nov. 3. Dimorphism on galactose was noted in 7.

G^S + G^R were streaked out on EMB.

	G^S	G^R
Lac	++	++
Gal	++ only sl. smaller than.	++
Mal	++	++
Sule.	± (slow)	± (slow).

The galactose effect was not reproduced here, nor in the plates streaked from cultivated G^S, G^R, in expt. 10. These were, however, a different batch. Tested on:

galactose 1%	peptone 1%
galactose 1%	NH₄ amine 1%
galactose 1%	N-Z Tone 1%.

Dulcitol

Nov. 3 '17.

K-12 ferments dulcitol only weakly. Inoc into broth + compare. By 16 hours, dulcitol broth is vigorously fermented by 58-161.

A. from broth
B. from plate (a slow colony). } Both form only "weak" colonies.

Streak A. again. - slow + as before!

?? Reduction of Methylene Blue??

Galactose media.

Nov. 4, 1947.

EMB - Gal 1% - agar 1½%

A. Peptone 1%

B. " 0.3%

C. N-2 - Annie "B" 1%

D. N-2 - Tara 1%.

Streak out G^R + G^S colonies. P4.
A5.

A No dimorphism

B large + small colonies. Not as marked as C.

C large + small colonies.

D. no dimorphism.

Mutant Rem

4
Nov. 19, 1947.

Mutant Rem. Treat a single colony culture of 58-161. E HN2 .2% for A) 5 mins. B) for 30 mins. Lac mutant plating.
Lo. W-1 for galactose mutant plating.
Incubate 24 hours before plating per recent technique

A. 58-161 (treated) - 50 pl x 70 cols. on Lac = 3500 tests

No mutants

B. W-1. 35 pl x 100 cols on Gal = 3500 tests

No mutants.

Induced sugar utilization.

Nov. ~~15~~ 4, 1947.

See 14A. for Treatments.

Add ~~Plate~~ ca 10^{9} cells (survivors of mustard) to:

			A5	A6	A7	A9
58+161 :	1.	100 ml T(m) + (BM.)	±			
	2.	100 ml T(m) + glucose .05%	+++			
	3.	do. cellobiose	±			
	4.	do sucrose	±			
	5.	do sucrose 200 ml.	±			
	6.	do. sucrose 200 ml.	±			
	7.	do. α-methylglucoside.	±			
W-1.	8.	do. sucrose TLB, 300 ml.	±			

- No change

Ditto

Ditto.

abandon experiment on Nov. 9.

Nov. 5, 1947. U.-V. Killing Rate.

Hanovia Lamp. 6" from plates. Spread .1 ml (10^8 cells) of young cultures of 58-161 on EMB lactose plates. Irradiate as indicated.

t.	colonies
0	$>10^5$
5 sec.	$>10^3$
10 sec.	112
15 sec.	ca 100.
20 sec	50
30 sec	28
1 M.	20
2 M	7
6 M	3.

There is certainly a break in the killing curve between 5 and 10 seconds, or else, the survivors of higher doses represent large clumps.

10 secs. is a convenient irradiation time which has a $pS = \log 10^8/10^2 = 6$.

One "weak" lac colony noted on 10 second plate.

Streak + compare with subs.

β-methyl glucoside

Nov. 4, 1947.

Sample from H A Lardy. (mp 106-108°)

1) 1% in EMB.

W-1	—	A6. dark spot - Restreak. →
Y53	—	
Y10	—	all —.
58-161	—	
W-22	—	

P5

2) .05% as C-source in T(m) + BM. 58-161 mor. P5.

	A6.	A7	A10.
1) β-methyl gl.	—		+
2) α-methyl..	—		—
3) α + β	—		+
4) glucose	++		++
5) β + glucose	++		+++

The Lac- W-30.

Nov. 6, 1947.

W-30 represents a series of Lac- colonies which constituted 50% of the colonies found upon spreading mustard-treated 58-161 on Lactose EMB. 12 colonies derived from the untreated inoculum were all Lac+. The inoculum was derived from a single large colony (3 R) of a type constituting about 1/4 the colonies formed on a galactose-N2 dennis B-EMB plate (see. Expt. 7) In Expt. 10, the ratio of Lac+ : Lac- was:

	+	-	
plate 1.	18	10	
plate 2.	10	4	
plate 3.	8	6	
plate 4	12	16	
	48	36	/ 84.

A large fraction of both these types was found on each of the 70 plates of the experiment. (10)

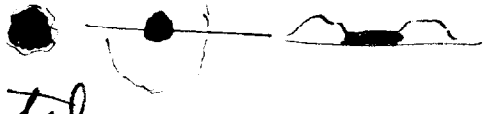
A). Pick 5 colonies from plate Expt. 7 in attempt to reproduce the expt.

B) Pick + streak out 5 Lac+ colonies from Expt. 10 to determine stability of these Lac+ : all plates show all Lac+ ~~-~~ !

c) Set up selection Expts. between these Lac+ and Lac- in broth.

On the Lac plates, W-30 forms broad flat colonies. These seem to engulf Lac+ colonies which they may contact.

On maltose two colony types are seen. Initially, they differ only in that some are less intensely colored on EMB than the others. Later they develop large beads of gummy material which projects from the surface.



On galactose no peculiarities had been noted.

C. Selection Expt.

A. Broc Lac+ colonies 2P6 into YP broth 25 ml.

B. Do. + 1 loopful of Lac- (W30) suspension.

Streak out duplicate Lac plates for initial assay.

A: all colonies Lac+ (Lac- < 1/100)
B: ditto.

		P7.	
C. Streak out	A	A 6: all+	all+
D.	B	A 6.: all+	all+.
		A10	
A2	Streak out P7.	all+	all+
B2	" " .	all+	all+

The combination: W-30 and the population of Lac+ from on these plates does not seem to satisfy selective hypotheses. The Lac+ may be resistant to the (hypothetical) inhibition. 58-161 (18-A) should be used instead.

D. Selection Expt. 11/10/47. Mix 1 ml broth culture of 18A1 with .05 ml similar suspension of W-30 from s Lamb in YP broth. Streak out initially, etc.

			A 11
(A).	18D-A1	Streak 18A1 on EMB-Lac	all+ all+
	-B1	18A1 + W-30 on EMB-Lac.	ca 100:1 +:-
			A12
N12	A2 ✓		all+ all+
	B2. -		ca 20:1, +:1
A12	B3		ca 20:1 +:1

This selection expt. does not explain original findings.

Nov. 6, 1947.

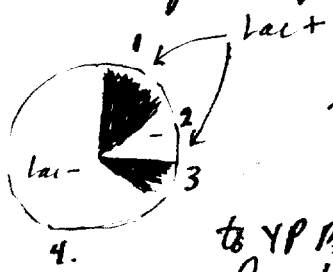
Spread .1 ml 58-161 culture on EMB-Malt and Lac plates.
Irradiate each for 10 secs. c̄ Dr. Smith's Hanovia lamp.
ca. 120 / plate

Lac (10) Nothing noted on 9 plates. See below for 10th. *

Malt (10) No mutants or sectors noted.

~~No mutants or sectors noted.~~

* A colony was found of the following appearance.



This suggests further a delayed effect of the mutagenic agent.

Pick from each of the 4 distinguishable zones to YP Broth + incubate. When grown spread on ~~and streak out.~~

1	W-35	[illegible]	38
2	W-36	W-35	35
3	W-37	[illegible]	37
4	W-38.	W-36	36.

Lac EMB.

(A10).

1. Ca ~~100~~ 50:1 Lac+ : Lac-. No evidence of sectoring of the +.

2. Ca 50:1 Lac- : Lac+. No sectoring

3. Ca 100:1 Lac+ : Lac. No sectoring.

4. All Lac-.

Streak out and transfer to slants.

Reversion of Lac - 1953.

Nov. 6, 1947.

P3. Streak out Y53 on EMB-lactose - 3/10% peptone.

A6. Papillae well developed. Pick papillae to small H_2O and streak out.

#P8. All of 20 plates pure Lac+ exc. for 1 which was streaked from a mixed colony. ∴ Lac+ is a stable reversion in most cases.

Nov. 8, 1947.

Spread 10^8 cells of a single cell culture of 58-161 from YP on maltose + lactose EMB plates. Irradiate each plate ca 10 secs. c̄ Hanovia u-v lamp at 6". Score at 36 h.

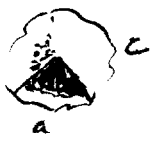
Maltose. 24 pl x 60 cols. = 1440 colonies tested. All colonies +.

Lactose 59 pl. x ca. 150 scoreable colonies = 9000 tested.

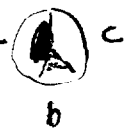
12 plates showed 14 likely colonies. Some of these are clearly simply mucoid.

4 clearly sectored
3 not sectored
5 mucoid
2 ??

(1), (2) Mucoid.

3. 

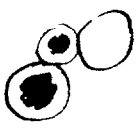
4. Small white colony ✓

5. 

Streak out 3 comps.

6 M

7. ✓

8.  ✓

9. M

10

✓

11.

✓

12. → no Lac. on streaking

13.

all + c̄ mucoids.

14. O mucoid.

3a 1:1 +:− (+) w50
3b almost pure(−) w48
3c 1:1 ± and − (−) w49

4. pure −. w39.

5. a. 20:1 Lac+:Lac− Puffy+ : w41.
   b. 2:1 + : − →
   c. pure − . w40.

7. pure − w42. (slight utilization?) ✓

8. 1:1 +:− The lac− is w43.

10. a. 10:1 +:− +, w44
    b pure − −. w45

11. a. 2:1 +:− (+)/w46

    b. pure − w47.

12. all +. Not mutant.

β-~~pha~~ methyl glucoside

Nov. 10, 1947.

Compare 58-161 and 17-1.

	glucose	β-Me Gluc.	cellobiose	—
(on glucose) 17-4.	± +++	− −	+ ±	− −
(on β-methyl) 17-1.	++ +++	− ±	± ±	− −
(Maltose Neg.) W-20.	± +++	− −	− −	− −

def. diffusion
maltose contam?

24hrs., 36h.

A15. do. only glucose is +++. others from − to ±.

∴ 17-1 is only slow utilization.

5 A16:, P17

	β-Me Glu.	Cellobiose.
17-~~4~~1	+± ✓	±. ✓
17-4	± ✓	± ✓
W-20.	± ✓	± ✓
17-1A.	~~+~~ +.	±.

Transfer 17-~~1~~ to similar series → much slower on β-methyl than on glucose.

U-V. rem.

Nov. 14, 1947.

Irradiate 50 ml of 24 hr. ~~W-37~~ W-37 conc. to 10 ml. with washing. 3 ml / 25 ml quartz flask. 40 secs. at 6 in. from Hanovia Lamp., with manual twirling. Spread .1 ml on EMB.

30 Lac, 20 Gal plates. All too dense (ca. 10^5 - 10^6 / plate).
(Autoabsorption rather marked!)

1 ml sampler, irradiated 5 secs moc into synthetic minimal.

- glucose
  sucrose
  α-methyl glucoside
  cellobiose

all neg. after 4 days.

Attempts at induced utilization of sucrose + other sugars.

Dec. 10, 1947.

Prepare suspensions, ca 10^{10}/ml, of Y10. Irradiate 3 ml at a time 5 secs. in a quartz flask rotated at hood of Hanovia uv lamp. ½ ml inocula into 50 ml / 125 ml flasks containing T(m) + sugars at .05% (except. affinose .15%) ± glucose .005% (g.)

or. 4P10.

	9A11.	2P11.	P611	9A12	P13	P16.
1. Sucrose		±				
2. "		±				✓
3. " g.		++	✓ + No +		* No +	✓ (*)
4. " g.		++	✓			✓
5. Ref.		±				
6. Ref.		±			* No +	
7. " g.		++		++		
8. " g.		++±	* No +	+++		✓ (*)
9. Cellobiose		±				
10. " g.		++	✓ * No +		* No +	do. W55.) (*)
11. d-Me gluc		—	+ No +			✓
12. " g.		+				✓
13. Trehalose	+++	—				
14. "	+++	—				
15. Glucose	+++	+++				✓
16. g.	+	+				✓
17. —	±	— baseline.				

Experiment terminated 12/24, without the recovery of any plus-variations in this series. Y55 (Salicin-plus mutant) should be tried on the beta-glucosides.

* Streaks out on corresponding medium.

[.005% glucose is apparently an excessive "boost". Use .001%.]. Also these sugars were evidently somewhat broken up by thin prolonged autostaving.