

Streptomyces and streptothrinis

926.

March 27, 1952.

Test (cross-brush) S_m^R and S_m^S strains vs. off streptothrinis
 $10^9/\text{ml}$. W1922, W1607, W1177, 58-161, W677 all S_{TH}^S
trace showed a more ~~gradual~~ abrupt cutoff in streaks. Cf. previous
observations of S_m - S_{TH} cross-resistance! \Rightarrow These concern cross-
resistance at low levels only! ($5\mu\text{m} = 10\mu\text{g} S_{TH}$).

3/29. Selections for S_{TH}^R :

W1678, W1177 ca. $10^9/\text{ea}$ + 1,000 u S_{TH} . No survivors

4/2 Off. Add 10 ml growth culture 58-161 to 100 ml Pinassay + 50 u/ml
 S_{TH} . After 4 days streak and test survivors.
all tested were $S_{TH}^R S_m^S$. Pick 1 as W1969. When
cross-brushed against loopful of 1000 u/ml, shows slight
inhibition, whereas 58-161 is completely inhibited. S_{TH}^R may
differ from S^R in step-resistance.

Final run: T adsorption assay
with multiple filters

8/13 ± 152

λ (1439 Iwoffate) diluted to nominal $2 \times 10^6 / \text{ml}$.

W1655 young aer culture in NSB, nominally $2 \times 10^9 / \text{ml}$.

Mix in equal volumes $4^{50} - 5^{15}$ $37^\circ \text{ Incub. (no aer.)}$

(A) Assay initial phage ~~dilute 1:100 and filter~~ (uncorrected for 1:1 dilution)
with W1655. (132, 181) = 1.6×10^6

(B) Assay filtrate $\therefore \frac{543}{42} \cdot 5^{53} \times 10^4$

(C) Assay residue λ and bacteria $\frac{\text{Bacteria}}{(173, 1)} \frac{143 \times 10^7}{(25, 01)} = \frac{14}{96} = \frac{14}{96} \%$

(D) Assay diluted mixture.

λ 165, 162 1.64×10^4 Expt completed 5:38
Bacteria $.96 \times 10^7$

$$\text{Initial } \lambda \doteq 1.6 \times 10^6 \times \frac{1}{2} \times \frac{1}{100} = .8 \times 10^4$$

$$\lambda \text{ in mixture} = 1.6 \times 10^4$$

$$\text{Residue } \lambda = .17 \times 10^4 \times \frac{96}{14} = 1.16 \times 10^4$$

$$\text{Filtrate } \lambda = .54 \times 10^4$$

low e.o.p for λ , measured by incubation in cells?

March 28, 1952.

~~Hfr~~ Test of Mal-elimination in Hfr crosses.

Strain out cross-mixture as E17S lac. dosing difficult - ? ca 10% Mal +
30% lac or
Picks small lac+, looks for lac+

3/31/52. ^{E17B} Medium rather poor: characterization doubtful. 5? / 24. Best strain
A. Reheats from E17S lac. #1-5
Repeat. 40 addnl. test from crossplate 1? #6

	Lac	^{Hfr} lac
1	V	-
2	V	-
3	V	-
4	V?	-
5	-	-
6	X	+ +

Save + → T.O. 6/53.

Note 6/52. Unless these are homozygous, which is unlikely, elimination likewise occurs in Hfr x Het. The important point is not readily tested here, namely whether markers such as Ylo or Mal = in W1177 would be heterozygous. Selection for prototrophy invalidates search for TL, M heterozygosity which would be nearly as useful! Similar cross should be conducted on E17B lac son.

March 30, 1952

Lact^{S^R} had been noted separately in mixtures of W1895 and W1607, etc.
These might represent recombinants.

Cow (W1895, W1177), W1607 overnight in broth. Mix 1 ml
each + 10 ml Tnassay. Incubate 11³⁰ AM - 4 PM. Streak out on
EMB lac sm.

1895	no colonies
1607	all -
1177	

are semi-inactivated Hfr cells
participating?

1895 + W1177	ca 1-2% Lact ^{S^R}
1895 + W1607	" " R, but Lact ^{S^R} very weak (Col-?)
	↳ Lact ^{S^R} Mal Xgl - S ^R Aux.

3/31. 1:5 each 12 N.

A. 2 PM EMB lac: clumping not obvious. EMB lac sm 2+ 37-
well isol. streak out.

B. 5 PM. EMB Mal sm. 1 tab ca 500 - (streak)
EMB lac (clumping?) streak R → EMB lac sm
some rather small Lact^{S^R} or Lact⁻ + - (ca 2-3%)
Mal sm

#1

4/3/52 Redo as 928A

(dilution saline)

4/1 C. As above. 2⁴⁵ PM - 4 PM

D. Cow separately, then plate together

D: 4x 200 EMB lac sm → No Lact^{S^R} EMB lac: ca = mixture
numerous segregant colonies

C. 1 EMB lac - about like D, but ca 1% small scattered colonies.

2 EMB lac + sm:

	Lact ⁺	Lact ^{1/2}	Lac ⁻
1	5		78
2	3		92
2	3		63
3	6		116
1	5		82
2?	8		113

1895 tends to self agglutinate

ca 2% Lact⁺
6% Lact^{1/2}

11 30 546 7587

- E. Washed cello - to Penasay +
 F. Mixed in saline (dense) ca 10% ml each 37° -
 G. " " " 40° -
 H. as C. 2:30 PM - 4:15 ++
 I. " aerated (to stri!) ++
 J. 1177 cello + Hfr supernatant. -
 K. See 929. Y/Y. struck out EMB lac son.

Pool data of 929-1 and K:

929-1.	Lact+, +1-	-	K:	+	+1-	-
#1-11	7	84	EMB lac son	0	1	51
	"	"		1	3	65
			#12-25	1	12+1	64
				2	5	48

see over.

	EMB lac T1	0	0	60
	0	0	3	45

3: Lact, lac- RR. ← #26-28.

EMB lac. 67 56

KK. EMB lac see also 931B. 15 Total.

1-12 streaked 4/plate. 13-15 1/plate. a) pectenomis 1-4. b) Rylee all original plates.

Lact	lac-	Lact	lac-	Diagnose is V ₁ ; S
1 SR	RR	9 SS; RR	RR	no seen
2 RR	RR	10 RR	RR	(SR) → a few
3 SR	RR	11 SS; RR	RR	
4 SR	RR	12 pectenomis	SS	No recant.
5 SR	RR	14 SR	RR	
6 RR	RR	15 RR	RR	
7 RR	RR	12 SS	RR	Recant?
8 RR	RR	■		

inf. True segregations by adding in broth, 1/6 tube of deagglutination of
lac- vs lac+.

See 928cc

K: lac+ and - .. all are Mal-, Xyl-
(Bare V₁R selective)

Among lac+, 10 V₁^S / 17 V₁^R

Lac- , V₁^S / 19 V₁^R (coupled with
lac+ V₁^S).

a) Key this pair for test (#32)

b) Test all for prototrophy (B, agar).

3/8 lac+ { grows B, agar. Both are B, -.
2-10 lac+

∴ 2 B, - + + of 47 recombinants (ca 1/2 lac+ S^R).

3.2 lac+ V₁^S S^R

lac- V₁^S S^R

sci

both are T-B, -L+

In most tests, T- and L- are not distinguished (on replica plates), but
only on reductase in tubes. L+ V₁^S may be associated.

4/2/52

C The pure lac^S are especially significant as they exclude the possibility that residual Hfr cells fertilize with mucoidomies on the agar (after agglutination and clumping).

c2 - pure lac+ Restreak: 6 ✓ lac+ pure. Pick for Mal, V, test.

c3 - ^{pure}
several lac+/- Restreak: 4 small colonies for ~~pure~~ lac/S seen.

4/4/52 c2-3. (lac+) all Mal-. c2: 3V,R 3V,S

c3: ^{7V,R}
⁺⁵ ^{5S}

^{15R} ^{13S}

C1 In each are isolated lac+ S^R identified, few or no lac+ S^S?
the small restreak colonies presumably are related to Hfr recombinations.

928 A) Repick 8 lacv? colonies:

1	-, v, +
2	+,-, v
3	+,- (v?)
4	-
5	+ - v
6	+ - v
7	+ - v
8	+ - v

Report lacv. Restreak EMB Lac, Mal, lac_{sun}, EMS, Xyl-Galv Auxotrophie

→ C3 Nutritional tests: aggs.

all these are Mal Xyl Gal- Tubes:

	V,	BB, TL	BB, M			
11	R	+	-			
12	S	+	-			
13	S	+	-			
14	S	-	-	MTL - ?		
15	R	+	-			✓ M-T-
16	R	+	-			
17	R	+	-			
18	S	-	-	MTL - ?		
19	S	+	-	± MTL+	I. Probably B- or B,-	✓ M-T-L- ✓ B,-
20	R	+	-			
21	R	+	-			
22	R	+	-			

Test associated lac- on V, !

Summary sheets

928cc

C3

EMBL strains

	Re-organize C3 tests			Lac+	Lac-	components	+ and -
	Lac+	V,	lac-	M Nat.	RepL	all Mal+	
1	-	S	R	-	+	-	
2	-	S	R	-	++	-	
3	-	S	R	-	++	-	
4	-	S	R	-	++	-	
5	-	S	R	-	++	-	
6	-	S	R	-	++	-	
7	-	S	R	-	++	-	
8	-	S	R	-	++	-	
9	-	S	R	-	++	-	
10	-	S	R	-	++	-	
11	-	S	X	-	-	-	
12	-	S	X	-	-	-	
13	-	S	X	-	-	-	
14	-	S	X	-	-	-	
15	-	S	X	-	-	-	
16	-	S	X	-	-	-	
17	-	S	X	-	-	-	
18	-	S	X	-	-	-	
19	-	S	X	-	-	-	
20	-	S	X	-	-	-	
21	-	S	X	-	-	-	
22	-	S	X	-	-	-	

KK

Lac+
Eff/Lac

	Lac	V,	lac	S	TLB, growth	Bm	P _r , no +
1	+	-	S	R	+	-	-
2	+	-	S	R	++	-	-
3	+	-	S	R	++	-	-
4	+	-	S	R	++	-	-
5	+	-	S	R	++	-	-
6	+	-	S	R	++	-	-
7	+	-	S	R	++	-	-
8	+	-	S	R	++	-	-
9	+	-	S	R	++	-	-
10	+	-	S	R	++	-	-
11	+	-	S	R	++	-	-
12	+	-	S	R	++	-	-
13	+	-	S	R	++	-	-
14	+	-	S	R	++	-	-
9a	+	-	S	R	++	-	-
10a	+	-	S	R	++	-	-
11a	+	-	S	R	++	-	-

Cross?

12 Cross?

#4, 5 are exc. Bm - lac+ V^S S^R (bumping error)

#9, 11, 12 were Hfr type, + usual pair

#10 had ① usual pair V^R; ② TL-lac-V^S S^R

See also 931E

∴ will + 2 Recombinants
 Lac+ V^R Lac-V^S = 2 zygotes?
 Lac-V^R Hfr missing

KK:

Most sectors are ~~Lac+~~ ~~(V, S^R)~~
~~Lac-~~

of 17 seg. colonies, 3 showed Lac+ S^s / Lac- S^R, and
may not have been recombinants.

The remaining 14, the Lac- component (1 part. exception)
was V^R S^R. The Lac+ was also S^R, 5 V^s,
6 V^R.

Sample type colonies from each sector for further
test.

4/3/52. 1ml each /5 Penassay:

1. W1895 - W1177 -
2. W1895 - W1607 -
3. W1895 - W1876 -
4. W1678 - W1876 -
5. ~~W1895 - W1876 -~~

6. W1922 - W677

7. W1678 - W1177 -

8. W1895 + W1177. Zone ca ^{each} 10^{10} /ml fresh Penassay 3:00 PM - 5:30 PM.
0/80 lac-. (Numerous + on $\frac{1}{2}$ M13 lac)

4/4 : W1177 + W1895. evening.

~~1.5 ml~~ 1ml ea + 5ml Penassay 3 PM. A
W1177 control. $\frac{1}{2}$ 4:50 PM B

Microscopic clumping ^{W1895} " C
mic and A.

SM Crosses and F₁ gradient.

93⑦

May 13, 1952.

W1922 = W1895 S^R. Hfr status?
 1903 = ~~1678~~ S^R

- A)
- | | | D(0) | D(su) |
|---|---------------|-----------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 | W1895 × W1903 | +++ | ++ |
| 2 | W1922 × W1678 | ca 50 | 12 |
| 3 | W1922 × W1876 | +++ Hfr? → ^R 1678 → 1678 Mal → Mal + | closer comparison with lower grades needed. Note that both A1 and A2 are reasonably futile. S ^R segregation here? Does it mean that W1678/Su can act as F+ to W1922 (Hfr?) Or is W1922 no longer high level F+? |

B)

		D(Su)	D(0)	→	Mal	Loc	D(Su)
1	W1895. 1678 1177	+++	+++	→	→	.	.
2	" 1876	++	+++	→	→	.	.
3	" W1903 1876	+	++				R > S
4	W1922 677	-	+++		.	.	.
5	1896	1 col. -	+++		.	.	.
6	1678	+	++				S? > R
7	Hfr test ✓ 1876		+++		→ +	-2+	.
8	1903 1896	1 col? -	-				.
9	" 677	-	++	→ ca 2+	→		.
10	" 1802	-	++				S > (ca 10% R)
11	1678 1177	+	+ ±		.	.	.
12	1876	2	13	→	→	.	.
13	1607	+++	+++		x	.	.
14	1875	+++	++, ++	→	.	R >	.

new
diploid
D(0)
D(Su)

All -/+ ratios agree with F₁ gradient. Note Hfr × 1678 less than maximal yield! Does 1678 transduce F+?

9306

Preliminary test maturing / diauxotrophs.

EMS Lac

cont.

3/31/52

W 1765

\times W 1177
Yield Lac⁺

W 1876
Yield Lac⁻

1688

+++ $\bar{20\%}$ +

++ $\bar{20\%}$ -

background only! λ ?

$F < 1876$.

1920

++ $\bar{5\%}$ +

\pm $\frac{1}{5}$ -

$F_{1920} \geq 1876$?

Test vs. 1607, etc.

1 hr crosses

931

W1895 - W1177

April 5, 1952.

5:10 PM

A. Mix directly from growth broth; no fresh broth

B. 1/10 each to " "

C. 1/10/ml each " "

(Thermotaxis?)

D. Grow together overnight.

B: 20 minute intervals: 0, 20, 40, 60. (ca 2x growth assumed)

Plate at 10^{-6} , 10^{-7} of original cells ($\frac{=10^{-5}}{=10^{-5}}$, " of susp.)

A: EMB lac son

	-	+-	+
• (6)	297	7	
• (7)	38	0	

335 7

ca 2%

C: " " (6) 2x 757 30.4 ✓

ca 1/500. This applies
for non-random contact.

B: 0 M. 0+ / 30, >1000.

20 M 0, 1/34; all appear. +/ 12/ca 1000

40 M 1+/139; ~~18/139~~ mostly, +/- 18/ca 1000

60 M. 7: 0/36

sm. 1v 133

1v 139

2/108

7: 0/32

T16: 3/322

See 330
excepting
for other
plates
at 10^{-7}

6 10 7-
9 1000
13

32.

∴ ca 3%

Dried mixtures may do nearly as well as morula into fresh broth.

B: pink ~~red~~ lac_v colonies on EMB lac. Post with 928 KK.

Hfr crosses

931E

April 6, 1952

E. W1895 + w1177 ca. 50 minutes

18 plates ca. 60/plate EMB lac + son 7 lac + oxy/- (+ others
+/-)

16 plates EMB lac. Picks lac "v". Also

up to EMB son; T1 to check on frequency of lac^R, v.
+ R separately function of colonies, or at previously published loci.

13 lacs from EMB lac. On basis of 928K, pick only 1+, 1- from each and check through.

Also, plate at 10x, 100x D(0), D(B₁).

D(0) : 1 100x

D(B₁) : 25 100x (about half are v. small)

Lac	Mal	S	V ₁	Nutri require.
1 +	- -	- R	R A	R TLB, MTL ←
2 +	- -	- R	R R	R TLB, TLB,
3 +	- +	- S	R S	R BM TLB,
4 +	- -	- R	R R	R TLB, TLB,
5 +	- -	- R	R R	R TLB, TLB,
6 +	- -	- R	R S	R TLB, TLB,
7 +	- -	- R	R R	R TLB, TLB,
11 +	- +	- S	R S	R BM TLB,
12 +	- +	- S	R S	R BM "
13 +	- +	- S	R S	R BM "
14 +	- +	- R	R R	R TLB, "
15 +	- -	- R	R S	R TLB, "
16 +	- -	- R	R R	R TLB, "

Cross?

{ Cross?

Only 2 colonies tested per plate plating

See also 928CC

Hfr crosses. T2.

932

April 7, 1952

2:15 PM - 4:50

+ W1895 1 hour 1:10 each.

A. Cross W1177 overnight in T2 broth.

B. Control 1177/1895

C. W677/1922 Close T1 EMB Lac.

A) (To test crossability of W1177 T2).

EMB Lac sm:

exc!

Lac + / -	5	48
4	21	
4	32	
	13	104

Very high yield!

B

prob. underestimate

est. 40. 500

C) EMB Lac sm.

60. all Lac+

14 339

EMB Lac T1 Pickle and streak out Lac+ ~~all~~! (~~Flav~~ ~~white~~) (close)

20 Lac+ pairs: Lac+ and - : all V, R+ $\frac{5}{5}$ Xgl-

5. Lac+ " "

W1177 is clearly still Hfr.

Therefore Lac/T1 selection still leaves
only the Mal- suggestion

? Are Mal+ recombinants present in any form? Mal vs T1; sm; $\frac{BM}{TB}$ are possible.

See D.

D. 4/9/52. = A 5:20 - 8:30 PM. Plate on EMB Mal, EMB Mal sm ...

E " x 1611 EMB Lac sm

F " x 1590 EMB Lac+ sm

4/10: D- ca 40 Lac-... purple

plates	# exc.	
E MB Mal sm	3	0
" Mal T1	3	0
E MB Mal sm	3	0

E MB Lac sm	3	7
E MB Mal	2	0 (no Mal+)

E ca 600 col. No Lac+ & R

F ca 700 EMB Lac sm No " . Why?

EMB Lac: 8 Lac? / ca 1000 Lac, -
Restrains. None Lac+ ^{Giving a few} _{restrains in smalles}

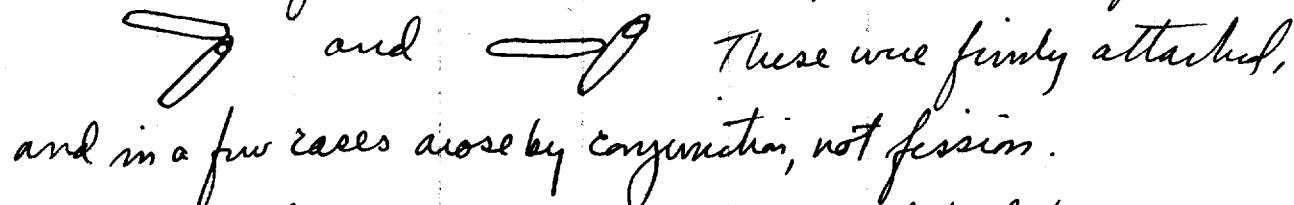
April 6-10, 1952.

- A. Use of tetracycline as tag for parent type: Cells grown in .005% Tz/
Penassoy broth. W1177 over 95% of cells have 1, usually polar,
Tz granule. This does not segregate (obserr. on agar-coagulase mounts)
PM 4/9. Several cell fissions are of the form:



This does not necessarily mean that the mitochondrion "itself is genetically discontinuous.

- B. In mixtures of W1895 + W1177 numerous pairs have been found



and These were firmly attached,

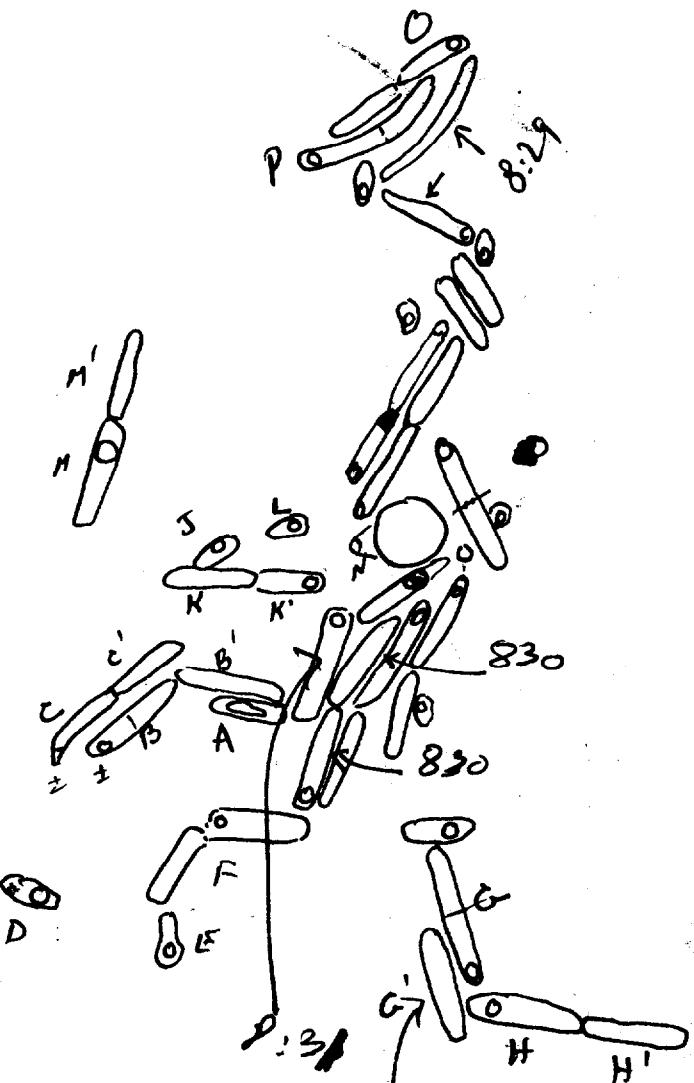
and in a few cases arose by conjugation, not fission.

- C. Intercutting cells with small bud frequently noted.

108.9
51.8

W1177 T^t
5:20





$P \quad 8:10$
 $F \quad 8:05$
 $G \quad 8:15$
 $G \quad 8:15$

Hfr X

934

April 11, 1952

EML irradiated W1895 UV 8 sec. EMB lac. Obtained ~5 lac-
12 plates $\frac{4 \cdot 8 \text{ sec}}{8 \cdot 7 \text{ sec}}$. 200. 400/plate. stock out EMB lac in

A	1?	<input checked="" type="checkbox"/>	= W1940	most suitable
B	2?	<input checked="" type="checkbox"/>	1941	
C	1?	<input checked="" type="checkbox"/>	1942	
D	No-noted	<input type="checkbox"/>	1943	slow + at R.T.
X	1922	<input type="checkbox"/>	1944	" " "
E	No lac-noted	<input type="checkbox"/>		

Box A-E + W1922 in Penicay. 10 AM. Brief stock-out tests.

A	-✓
B	-✓
C	none noted
D	-✓
E	?

Use W1941 for further experiments on self-crosses. lac+ ones?

See 937.

In allelism tests x W677, EMU recorded lac+ recombinants in 1945, 6, 7; 1951. 1940-44 not yet tested.

Rechecks ~~1948~~ 1941. Use 1948 in further test.

6/25/52. W1941 x W1956 gave few or no lac+ recombinants. W1940, 42, 43, 44 gave lac+.

Rechecks 1941, 1948. Use others to search for lac+ balanced diploid vs. lac-, esp. W1940

Unfortunately, W1941 was used in some subsequent Hfr Q nupts., but its lac+ allelism should be further verified.

April 13, 1952.

- A. W1895 + W1177 (overnight no lysis). Agar plate on EMB Mal
- B. " " EMB Mal T1 $B' = 10 \times$ $B'' = 100 \times [= 10^{-5}]$.
- C. " " EMS Mal TB, 4 plates all -.
- D. " " EMB Lac sm. (for "control").
- E. " together 1 hour. EMB lac sm ^{and EMB lac = +} (for double cross test).
- F. " " " D(B₁) for mapping: 100x
- G. " " overnight ... EMS Mal SM (BM).

→ G! 1 G" 3+2. stuck out EMB lac + lac - lac+

A. 5 plates ca 100 colonies. Pick all colonies that might conceivably be Mal⁻ or Mal⁺.

- | | | | |
|----|---------------------------|-------------|--------------|
| 1 | + small +? | EMB(0) Agar | all + |
| 2 | ① prob conic. | | + - |
| 3 | tiny ± | | all + |
| 4 | like 1 | | n.g. |
| 5 | black near +. | | Mal+, Mal- |
| 6 | like 1 | | + |
| 7 | ① darker + lighter; not - | | dark + light |
| 8 | mid-size, badge, mottled? | | 3 + |
| 9 | " " | | 4 + |
| 10 | prob conic. | | near -. |
| 11 | like 1 | + | |
| 12 | " | + | |
| 13 | like 8 or 5. | + | |

Mal	Lac
1 -	+ - +
2 -	+ - +
3 +	+ + +
4 +	+ + +
5 - + -	+ +

no evidence of Mal, Lac Recomb.
Test on S, V₁, Natr.

B. ca 30 per plate 5 plates.

$B' = 200 \cdot 300$? 1 Mal+ ② → all Mal- (uninfected col.?)
 B'' crowded 1000? None observed.

D.	Lac-	+	+/-
31	1	0	
42	1	3	
332	6	12	

E. 9 plates. Count 1:
 109 4 3.

Total 10
plates:

See over.

100 19 42 1 ~~12~~ 61

Pick "pure +" 17 total.
 But only 8 got only +
 colonies in EMB. Very rare -
 others. 1 lacv? #10
 +, - and mostly v
 Mal-Xyl-Aer.

H. Somewhat crowded & separation of lac S.
 (over)

935 E. (lac^+ from EMS Lac ser) all are Mal -.
Repl to E17B17al T1.

"Pure+":

1. S
2. Pure R
3. Pure R
4. "
5. R
6. "
7. "
8. "
9. "
10. R

Note preponderance of
 $\text{lac}^+ V_i^R$ in these colonies.

No evidence of $\text{lac}^+ V_i^R$
 $\text{lac}^+ V_i^S$

Apparent excess of $\text{lac}^+ V_i^R$
may be due to selective advantage
of certain types.

#11 Pure lac- V_i^R $\text{lac}^+ V_i^S$.

F. Picks to EMS Lac B:

Lac-	+	+ and -
9	8	1
5	10	2
7	11	2
11	8	1
8	10	1
5	10	2
<hr/>		
45	57	9

H		EMB	Lac	Lac	Hal	V.
1	(1)		+,-	+ - +	-s	R -
2	large	++	+ rare-macrescible	+ +	s	/
3	tiny	o	+ -	+ - -	-R	R *
4	"	o?	+ -	+ - -	-s	R x
5	(1)		+ only (rare, macc -)	+ + *	s	/
6	"		+ -	+ - +	-s	R -
7	(1)		+ rare.	+ +	s	/
8	(1)		+ -	+ - +	-s	R -
9	(1)		all+	+ +	s	/
10	"		all+ 1-	+ # +	s	/
11	(1)		+ - (scme)	+ - -	-R	R *
12	(1)		+ -	+ -	-s	/
13	(1)		+ -	+ -	-s	/
14	(1)		+ -	+ -	-s	/
15	(1)		+ -	+ -	-s	/
16	(1)		+ -	-	-R	*
17	(1)		+ -	-	-R	*
18	(1)		all+	+ -	-s	/
19	tiny	o	+ -	-	-R	*
20	(1)		+ inf. -	+ -	-s	/
21	(1)		+ -	+ - +	-s	R -
22	(1)		+ #	+ (or +)	s	/
23	(1) small		+ -	+ - -	-R	R *
24	(1)		+ rare -	+ (or +)	s	/
25	(1)?		+ - (infreq)	+ - +	-s	R -

18 complete pairs (Lact, -).

Recom { 6 are Lact + Mal - V₁^R / Lac - Mal - V₁^R
 { 1 Lact + Mal - V₁^S / Lac - Mal - V₁^R
 Recomb { 11 Lact + V₁^S Mal + / Lac - Hal V₁^R Mal -.

∴ only small colonies
are likely.

Incomplete pairs: 7 - parental.

Recomb other markers?

April 14, 1952.

Mixed 2³⁰ - 6⁰⁰ PM

- J. W1941 x W1922. Plate on EM^{lac}B; EM^{lac}Bacus for lac_s; lac-S^R.
- K. W1922 x W677. Plate on EM^{lac}B T1 Plate 10X or ('') 100X
- L. " " Plate on EMS Lac SM TLB.
- M. W1895 x W1876. Plate EM^{lac}B; sm; T1 FOX
- N.

J. ca 150 purple EM^{lac}Bacus: 3 plates
No lac-S^R.

EM^{lac}Bac 10 plates. ca 100%. 4? lac_v → lac+ only on EM^{lac}Bac.

#5: + and - lac-S^R col. noted. #5 #6? all +. This ① n.g.
mostly lac+ S^R and lac-S^S
2 lac+S^S; 1? lac-S^R found. owing to proximity these might
K. 5 plates. No recomb.

M. EM^{lac}Bac 5x ca 150/plate. No lac_s

N. T1. 5 plates → 3? lac_S. ✓ → lac+, -. Repl → EM^{lac}Bacus

L. 1 tiny colony: 5 plates

all 3 give lac+ { lac-S^R
lac-

~~Hf~~ ~~F+~~ Mal / T1

957
936'

April 23, 1952

W1957 x W1702
Hfr V^R Lac-Tal-SK

(Overnight growth).

① EMB Lac son factors Lac-
7 200 replica to EMB Lac- T1.
after plating Lac+, test G.

② " + T1 100X

3 plates + lac+ (est.)

Phenotypic delay?

6 testing. - all V₁^s

Replica → no intact V^R

3 isolated new colonies, probably mutants

Rechecks W1957 / T1. — as recorded this is V_{1a}^{ER}, unlike V.R.!

Hfr ⊗

937

April 17, 1952

A. ~~W1895~~ W1955 × W1922 EMB Lac

Lac- V_1^R Lac+ V_1^S S^R

B. " " EMB Lac T1

B. 7 plates ca 250/plate Not lac+ V_1^R !

A. 3 plates. 6 ?? lacs. Mostly ① types.

Lac- V_1^R Lacs. ~~EMB Lac + son.~~

1	S	R
2	S	R
3	S	R
4	S	R
5	S, R	R
6	S	R

Only possible lac/s Recomb. ✓ V_1 .

937AS.

1	-	S	V_1^R
2	+	R	S PAR.
3	-	S	S

other parent? Should have
killed more colonies!

Check these for Hfr.

all 3 were Hfr (× W1958!) Save ① for further use in crosses. W1970.

C 4/28. W1970 × W1895 plate EMB Lac + son. (Appropriate lac-?)

2 sm. 4x150 = 600. 3 Lac V_1 .

1 sm. 2x300 2 Lacs

$Hfr \times F+$

938

April 22, 1952

E. W1922 x W1896 (3PM - 8PM). Plate on EMB lac + sm
 $lac_+ s^R$ and D(8) → no colonies!

low density plates. EMB lac: ca 60/plate

6 EMB lac sm. No lac- s^R

6 EMB lac 1 ?? lac_S. Desaid.

Repeat 4/25. (overnight)

4x 150 plates. No lac- s^R

These crosses apparently much less fertile than $Hfr \times F-$.
Return to unselected crosses W1895 x ~~W1876~~ W1876 for further study.

April 17, 1952

C W1895 x W1876

$10\frac{30}{5}$ - 6⁰⁰
EMB lac sur 4 plates ca 250 lac-/plate
2 lac s.

D " "

EMB lac 7 plates 500/plate
but $\frac{1}{3}$ demand!

Rate of Recombination much less than W1177 experience.
D

4/18/52.

$135 - 5\frac{35}{5}$
EMB lac sur

E W1895 x 1177

F. " 1876.

E: EMB lac sur

+ 10	+ 16	- 44
5	7	3
4	3	-

F: + 1 1000.

0	2	-
0	1	-

Abandon.

Hfr x F+ is much less fertile than x F-!

F: only 2 likely lac s on EMB lac. ~~Abandon~~
Reinfection EMB lac. add to D.

c. B² and 936N. 3. all Xyl - Mal - (S^R). abandon.

D _p	P _p	Tac	Mal	Xyl	Mtl	S	T ₁	R	BMB,	TLB,	
+	+	-	+	-	-	-	S	R	+	-	f. every recomb
+	+	-	+	-	-	-	S	R	+	-	has to Mal+
+	+	-	+	-	-	-	S	R	+	-	
+	+	-	+	-	-	-	R	R	+	-	
+	+	-	+	-	-	-	S	R	+	-	
+	+	-	+	-	-	-	S	R	+	-	
Par	+	-	+	-	-	-	R	S	+	-	
Par	+	-	+	-	-	-	S	R	+	-	
											N G
											see 945

sic! house of 1895 x 1177? Try W1922 x W1896!

Note crossovers of Mal/S!! Mal-S-Hillock!

2 types:

① Par₁ / Par₂ Mal+② Par₁ lac+ Mal+ / Par₂ - Mal+!all showed a Mal+ S^R camp., but might be S^s. been screened!

Further content
of these colonies
should have

5/3/52

See 945

E W 1970 x W 1918 EM Blact + dm

F " Y10. " "

E. 4x200. lac sun. No lac +.

5x350 lac crowded No lac sun.

F. 4x200 1 lac s^R!

5x350 3? lac s.

April 21, 1952

A 58-161 B W1957.
 $10^6/\text{ml}$ $10^7/\text{ml}$

ca 25 minutes in 1mg/ml HN2 in D(m)

37° dilute 1:10 in Penassay.

streak out. Plate and dilute by spreading serial plates.

A. sterile B. ca 200 on plate 2. (10^7). $\rho S = \text{ca } 5$.

Replyia to D(0) + W1177 for replica-plate test of F⁺
 $or + 58-161$.

C #. 4/22/52. As above. 20 minutes exposure.

Ca 50 on plate 2 (i.e. .1ml from 1/10 dil to Penassay)

1 Lac- \rightarrow addt. lac-? Pick + naturals. W165

rene Hfr.
occ. phototrophs Replyia to D(0) + W1177. (spot W1895 control)

(D) 4/23. As above Plate 2 only.

only 2-3 coli/plate. B. subtilis does contaminant.

E 4/25 $10^6/\text{ml}$ 10ml 5mg HN2 in D(m) 20m. 37° .

at 20m, dilute 1:10 in Penassay express dilutions from this as
 10^0 . $2 > 400$.

3 60.

~~1 Lac-~~?

This establishes suitable dose level

No Hfr noted this run.

In preliminary HV run, 1 colony 58-161/ca 60 plate was noticeably nibbled. This proved to be λ^+ , similar to W1655. Discard.

Summary.

940

- | | | |
|---------------------------|---|-------------------------------------------------------------------------------------------------------|
| A) Lac + / - | { | Almost all <u>TL- M+ S^R Mal- Xyl- Htc-</u> . Segregating |
| B) Lac + S ^R / | | Lac- V ₁ - V ₆ in more or less linked pattern. |
| C) Prototrophs! | | (V ₆ - Lac - TL-). <u>Also upheld in C.</u>
B ₁ -> B ₊ . ca 10:1. |

In Hfr x F- lysis appears to be a constraint favoring TL- ... as well as S^R Mal- M+ [Rothfels crossed S^R M- x S^S M+ ... Almost all recombinants in his experiments was also T-L-Tac- V₁^R just as me! This was concluded to be based on the M+ - lac - ... linkage. It can be reinterpreted as a lac - ... TL - ... Y linkage, with Y a hidden selector. But, the order V₆ lac V₁ TL Y would (if applicable to Rothfels) would give a different sets of auxotroph single crossovers! The conditions of mating do not preclude a limited degree of F⁺ - transduction.

It may be conclude that selection for TL+ essentially discounts the effect of Y over the lac - T1 region, but leaves this influence at the left end, so that all of this set of prototrophs are Mal-.

- ① Further Project: Compare Hfr x F+ crosses.
- ② Study T-L-V, more closely

April 22, 1952.

EMB Lac

Shows. Plate on EMB Lac ± sim and D(B₁).

3-4% lac+^s 16 lac^s streaked out. Pick +, - to EMB Lac.

B EMB Lac sim.

B' (+ recognizable only).

C D(B₁) 100x. Ca 50 purple Pick 40 to EMS Lac B₁.

940 A: (Check for possible lac^v) none.

B 20 lac^s 3 were pure + (test for V, R/S)

20 factors. Uncertain relationship of lac-, do not consider these.
Isolate lac+ (and -) and test.

1 pure S V_6^S check reaction V_6

1 pure R V_1^S

R/S. Both V_6^R Check mutation of V_1^S . at bottom of test plate

- B " Many of the isolates were mixed +/- on Lac EMSB, despite care to obtain single colonies and despinning of the colonies.
- | | | | |
|----|------------|-----|---------------------------------------------------------------------------|
| a | + (supuf.) | 16 | Upon restreaking on EMB Lac, almost |
| b | - | 8 | all showed a lac- component. |
| c. | +,- | 18. | Picks + and - whenever observed.
(for later confirmation of mutation). |

Again, 9 unselected Lac segregates were all $Mal - S^R$.

7 Lacs also Mal^S were parental for every marker
and must be assumed to be trivial clumps.

	Lac	Mal	S	Gal	Xyl	Mtl	T1	T6	BMB, agar	TLB, agar
C	-	+	R	-	-	-	R	R	-	+
D	-	+	R	-	-	-	R	R	-	-
A	x	+	R	-	-	-	R	R	-	+
A	-	+	R	-	-	-	R	R	-	-
D	x	+	R	-	-	-	R	R	-	+
Par	-	+	R	-	-	-	R	R	-	-
A	-	+	R	-	-	-	R	R	-	+
E	-	+	R	-	-	-	R	R	-	+
F	-	+	R	-	-	-	R	R	-	+

1-10 show 5/10 segregations. Mal-Xyl-Mtl-S, linked Lac-V₁-V₆-TL-BMB

9 total.

4	A	-	+-	-R	R	-	+-	--	-R	R	R	S	-	-	+	+	
0	B									R	S	R	S	-	-	+	+
1	C									R	R	S	S	-	-	+	+
2	D									R	S	R	S	-	+	+	-
1	E									R	S	S	R	-	-	+	+
1	F									R	R	R	R	-	-	+	+

clerk

Exc. ? 12. None fail to show Lac + S^r!
... recombination. But note rarity of recombs. in Lac- selection
1:9

Note rarity of crossovers between
M-TL despite Lac, V, syn.

12 may be
a recomb. (of Gal)

typ Gal
wild type?
but see 12

- ① Verify lac- V_6 linkage
- ② Probably V_1 -TL linkage (all 7 TL^+ are V_1^S)
- ③ Probable lac- V_1 linkage (?) $\frac{V_1^S}{V_1^R} > \text{in lac+ then -}$

These data suggest a constraint favouring TL^- as well as $Mal^- M^+$. # II (unless coincidence) may point to $Xyl - M$.

?? Are we missing lac-zygotes? Try looking for rare Mal/S recombinants, or $S/V_1 [1895 V_1^R \times \dots V_1^S]$

B1

V⁺
T-L
galB(B)

	lac	Mal-	S ^R	Gal	Xyl	M ^R	T1	T6	BMB, agar	TLB, agar
1	-	-	-	-	-	-	SD	S	(S)	(+)
2	+	-	-	-	-	-	RA	SS	R	-
3	+	-	-	-	-	-	RA	SS	R	-
4	+	-	-	-	-	-	RA	SS	R	-
5	*	-	-	-	-	-	RA	SS	R	-
6	*	-	-	-	-	-	S.B.	SS	R	-
7	*	-	-	-	-	-	R-A	SS	R	-
8	*	-	-	-	-	-	R-F	SS	R	-
9	*	-	-	-	-	-	S.C.	SS	R	-
10	*	-	-	-	-	-	RA	S	R	-
11	"	-	-	-	-	-	S.G.	R	S	-
12	"	-	-	-	-	-	S.B.	SS	S	-
13	"	-	-	-	-	-	S.E.	SS	S	-
14	"	-	-	-	-	-	R.A.	SS	R	-
15	"	-	-	-	-	-	R.F.	SS	R	-
16	"	-	-	-	-	-	S.B.	SS	R	-
17	"	-	-	-	-	-	S.B.	SS	R	-
18	"	-	-	-	-	-	R.A.	SS	R	-
19	"	-	-	-	-	-	R.A.	SS	R	-
20	"	-	-	-	-	-	R.A.	SI	R	-
21-31	+	-	-	-	-	-	S.G.	R	S	+
2	+	-	-	-	-	-	R.A.	SS	-	-
3	+	-	-	-	-	-	R.A.	SS	-	-
4	+	-	-	-	-	-	S.R.	SS	-	-
5	+	-	-	-	-	-	R.A.	SS	-	-
6	+	-	-	-	-	-	S.R.	SS	-	-
7	+	-	-	-	-	-	R.A.	SS	-	-
8	+	-	-	-	-	-	R.A.	SS	-	-
9	+	-	-	-	-	-	R.A.	SS	-	-
30-40	+	-	-	-	-	-	R.A.	SS	-	-

Among 16 lac-S^R selection, only 1 recombinant. [complement to lac+ V_b?]

40 lac+ ... no parental (re mutation... S^R- mut linkage?).

Type of lac+ (Mal-S^R Gal+ or ++).

1-20 are parental +
21-40 are recombinant +

A. 22 - Xyl M^R T1 T6 BMB, TLB, standard

B. 5+1 - - S S S + V_b

C. V - - S + R ← - + V_bV_b

D. V - - S ← R ← + ← + V_bV_bBM

E. V - - S ← S + + + V_bBM (TL)

F. V - + + + R S S S - Xyl TL
G. V - + + + R S S S - Xyl T1 BM T1
H. V - + + + R S S S - Xyl T1 BM T1
J-E Xyl T1 BM T1

Note preponderance of Mal+

Prototrophs

940C. Single factor ratios:

	V_6	Lac	V_1
R, -	35	32	28
S, +	29	32	36

About equal lac+ :-
valid unless single
prototrophs are not segregating
units.

Linkage:

$$V_6 \begin{array}{c} \diagdown \text{Lac} + \\ R | \frac{7}{S} \frac{28}{\underline{25}} \end{array}$$

$$V_1 \begin{array}{c} \diagdown \text{Lac} + \\ R | \frac{4}{S} \frac{14}{\underline{28}} \end{array}$$

$$V_6 \begin{array}{c} \diagdown V_1 \quad R \quad S \\ R | \frac{14}{S} \frac{21}{4} \end{array}$$

consistent with

$$V_6 - \text{Lac} \longrightarrow V_1$$

$B_1 - M$ $\delta B_1 + / 58 B_1 - \} \text{ independent}$

$\text{Xyl} - M$ $2 \cdot 3 + / - \} \text{ independent}$

No Mal + !!

T-L-V_i linkage

940B3

Compare mutation of (TL) - V_i^R vs. V_i^S. (lac+generally)

Collect occurrences in following array:

	V _i ^R	V _i ^S	D()	TLB _i	TL	TB _i	LB _i
A	1	14	+	+	-	--	--
B	2	4	-3P	-	+	±	-
	3	2	6	+	-	↓	↓
	4	3	9	↓	↓	++	↓
	5	4	12	↓	↓	++	↓
	6	5	17	↓	↓	-	↓
	7	7	18	↓	↓	++	↓
	8	10	23	↓	↓	±	++
	9	14	25	↓	↓	! +	++
	10	19	56	1			

$\therefore 16 = B_1 - \checkmark$

Same ~~L-T~~. Presumably this is correct order: ~~L-T~~ T-L-T

T-L+

lac-V_i-T-T
$$\begin{array}{c} \uparrow \\ V_i^S \\ \uparrow \\ V_i^S T-L+ \end{array}$$

D₁, ± Prototroph recombinants

9/4/52

Second 5/1/52.

	Lac	Mal	S	Gal	Xyl	Mtl	T1	T6	defects D(1a), D(1b)
1	+	-	R	R	I	-	-	S	R
2								SSS	RRR
3								SSS	RRR
4								SSS	RRR
5								SSS	RRR
6								SSS	RRR
7								SSS	RRR
8								SSS	RRR
9								SSS	RRR
10								SSS	RRR
Pure and lact									
2									
PAIRED									
1	+	-					-	S	R
2								SS	RR
3								SS	RR
4								SS	RR
5								SS	RR
6								SS	RR
7								SS	RR
8								SS	RR
9								SS	RR
10								SS	RR
R									
1	+	-					-	R	R
2							+	S	S
3							-	SS	RR
4							-	SS	RR
5							-	SS	RR
6							-	SS	RR
7							-	SS	RR
8							-	SS	RR
9							-	SS	RR
10							-	SS	RR
11							-	SS	RR
12							-	SS	RR
R									

- a). Note high frequency of mixed pairs (22 pairs). Some of these might be lac → lac+ recombinants (especially if concordant for V₆). 7 may well fall in this category, and should be checked further. However, remaining 15 are discordant for V₆ also. Remaining pairs are, for V₁: SR8 RSO SS7 RR1
but no pair was concordant for Gal!
~~Dropped lac + of the 7 concordant pairs~~ all pairs: 8 0 12 2

42 colonies streaked out. 20 were substantially pure, 10+, 10-. Remaineder were mixed, pick 1 lac+, 1 lac-.

← over

May 1, 1952

AU 943. W1895 x W1956 on D(B₁) and EMS lac B₁. Incubate 3 days.

EMS lac B₁: Superficial appearance. An unusual proportion of + - -(+) + (-) sectored colonies is indicated.

34 2 5 7 Owing to size differential, the

figures do not show their proportion adequately. Sectoring texture is also notable on D(B₁). Picks well-separated colonies from D(B₁) and streaks out on EMB lac.

Yields, as usual experience, about 10^{-4} g of medium.

	Pickle 32, random streaks on EMB lac			
	Proportion lac+	(est.)	Weighted average: 110/32	ca. 3 lac+
1	.5			
2	.1			
3	.5			
4	<.1			
5	0			
6	<.1			
7	0			
8	.1			
9	0			
10	.5			
11	1.0			
12	.9			
13	.4			
14	.8			
15	0			
16	1<			
17	<.1			
18	.5			
19	0			
20	.1			
21	.9			
22	0			
23	<.1			
24	.1			
25	<.1			
26	1<			
27	0			
28	.3			
29	0			
30	1			
31	0			
32	.8			

" EMS lac B₁, colonies picked to EMS lac B₁: ca 30% +

~~Effect of EMS medium??~~

various conditions

April 23, 1952.

B. Unseeded. 5½ hr cultures. Mix 2 hours ca 5%?

A. Aerate " " (separation).

A1 -

A2 heat 60° 5 minutes. Plate 10x. No kill!

A3 UV 0, 10, 20, 30 sec. on plate

A4 Partially sediment (ca 90%) mixture. Sediment dil. 20+ / 288

A5 ↓ Supernatant dil. 6+ / 264.

It appears superficially somewhat less. Should be repeated.

A3): 0 incomplete counts. ~~22~~ / 350 21 / 363
 10 " " 14 / 280.
 20 " " 5 / 184
 30 ca 30% surv.

slight effect?

4/24/52.

C. Aerate overnight W1895, W1956 T2. Reincub (in air) 10 AM.

Mix and re-grow 2 PM.

		(Pore est. of counts)	Total counts
		EMBLac min.	
1.	1 ml ea parent / 10 ml 10 essay. Air	3, 6, +	40, 34
2.	W1956 control "		
3.	.1 ml ea par. No air	0	40
4.	.01 " " "	6	129

Confirms very high relative rate of recombination in diluted mixtures.

Start:
 1 C1 3¹⁸
 2 " plate mixed to 50% before fix & heavily overgrown.
 3 C2 " "

9/25/52

D. 1895, 1956 Tz grown overn. aer. 10AM - 2PM Region.

Mix ca 5ml each + 5ml Penassay 2PM — 3PM EMBLac sen.

1 (+ 90 min room temp.)

< 1% bact

2 sediment after strong centrif.

"

3 Resuspend in saline. Re-sediment: supernat.

"

*cultures may have
detergent or moderate
addit. content*

E ~~mix~~ Dilute 1:100 3PM. Mix in 10ml: (assume 10^{10} /ml initial)

1 1ml ea (ca 10^8 /ml) → ca 1/2% bact SR.

2 .1ml each (ca 10^6 /ml) "

" "

3. .01 ml " (ca 10^5 /ml) 0

Review of D these results are minimal. However, the development of zygotes at extremely low dilutions is confirmed. Competitor sys?

Flagellar phage: Salmonella

944
942

April 24, 1952

Received this date from Boulgakov

1 "Stain" 372 = ~~H901~~ Sutie - Boulgakov Rough
 2 377 H901
 3 383 = Felix 6.396 V/S

A ~~VIII~~ - 113 1936 } had been propagated on H901.
 B " "
 C " Passage 372
 D " "

3/21. Open 1, 2, 3, A. Test by cross-blush on EM/Bac

	A
1	S
2	S
3	S (late secondary R)
stanley	R
O-901	S!
LT-2	R
LT-22	R

3/25. H901 ~~A~~
~~O-901~~ A B C D
 S++ S++ S+ S+
 S++ S++ R R

Apparently C and D fit description of flagellotropic phage. Should be single-plated to verify effect of propagation on H901, supposedly the sole distinction of A+B.

Test various Salmonella types on EM/Bac vs. C.

1+C cleared after ca 3 hours in Penassay. streak out for 0% survivors

Reinfect 1+C, 2+C. Pick single colonies: motile; test motility.

1: 3 ~~1/2~~ most promising

← like #1 →. Re-test

↓
 Reinfect by single colony isolates.

2: 3 motile, 1, 2, 4 re-tested: show limited motility overnight. Detect single colony isolates 1PM - 10PM (from #1)
 (returning spreading)

→ H901 controls! — clump of large bodies seen on soft agar!

Motility of H901, & non-motility of O-901 verified microscopically. (over)

O-form from S. typhimurium.

942-2-1 4 colonies tested. #1 did not migrate overnight

#4 ++

↓
should be
suitable

942-1-4 " " #2 +

#4 +++

Compare motile + non-motile "mutants" for sensitivity to EC

clearer phages 942-1 942-2
alike ↓

H901	S (measured)	[culture old, from liquid]
O901	K	
3 motile mutants of H901/c	{ S+++	[from motility agar]
1 NM-H901 R.		<u>Save</u>

		A	B	C
1		S	P	
2		S	P	
3		S	P..	
4	(Edw.)	0901	S	R
5	(Kauf.)	0901	S	R
6	stanley	R	S	
7	entiret	R	R	
8	para B.	S	3	
9	gallin.	P	R	
10	Ty 2V	S	R	
11	T4T2	R	R	
12	LF2	R	SR	
13	LT22	R	R	
14	K.	R	R	
15	K.	223	R	
16	K.	2Y8	S	
17	[+C]	R	R	
18	SW 579			
19	SW 570			
20	SY 79			

Phase C.

LT2 meshed
SW 519 S±
SW 570 S+
SY 79 meshed S.

Lg growth with C → motile

Inoculate from lytic area to Pinesay 10+ AM.

3PM: paraB + φA Motile → 3/3 brimotile for 8 hours
 paraB + φC NonMotile → " " "#1
 stanley + φC NonMotile → 1/3 " " "#1
 LT2 + φC Motile! *Reinje LT2 + φC*
also remained until phase?

4/28/ Grow 1 plaque of φC on #1. = φ 942-1 } (Pinesay 50 ml)
 " #2 = φ 942-2 } behave alike H, 0901
 contra A, C as rec'd!

Responses of SW579, SY79... phase variation? WV variation?

Replicates of A to EMB lac +; EMB Mtl.

1. of 31 lac+, 5 were S^R

2. No Mtl+ lac were seen in 5 plates (ca. 1000 lac-Mtl-)
31 lac+ Mtl+

Owing to desirous ratio of lac+:lac-

and overall ^{low} number (5) of recombinants

This experiment is not conclusive

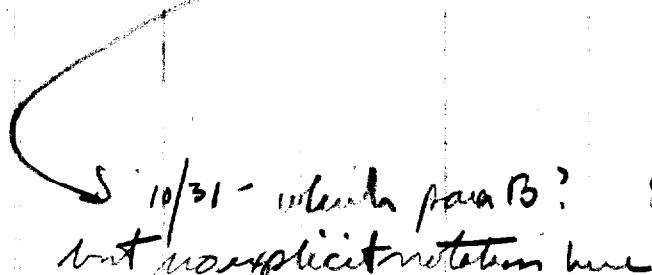
Bordet-Galvoor flagellophage phage

942

Summary

4/19/52.

1. Two phages (A-B) (C-D) received from Bordy above.
2. AB is essentially a typhi (does paratyphi 1^b) phage, but independent of sonata (842-1 rough) or Vi antigen.
3. C-D Accords to description of flagellophage phage. High (?) titer obtained from single plaques either on H901 (942-2+) or Sutie Rough (942-1+) S.typhi. This acts on H~~9~~. Typhi, probably inactive on Vi+ (Ty2V, Sy78) It has given non-motile secondary growth and L-forms of variable stability with S.typhi (Sutie R unstable; H901 "stable"), para B and Stanley. Although masked lysis is seen with typhi murinus LT2 and SW519 (Typhi; i -), secondary growth remained motile (Vi, O antigenic ??)
4. Motile "virus" from S.typhi became again sensitive to OC.
5. Seed for further work: NM typhi H901, para B; Stanley. Should be reduced. Also lytes ~~from~~ single plaques.
6. L-forms not often motility agar. See 944



10/31 - which para B? Stockbook records SW533 (703)
but no explicit notation here

Kinetic studies Hfr x F-.

943

4/28/52.

	W1895	+ W1958 T2.	Overs.	Acute 930 to 245.	Hfr to 445
A	"	"	in 10 ml	^{Lac +} 21+, 379-	^{Lac} ca = +, -
B	1	.01		4-	
*	C	.01	1	3+..;	28 Lac +, > -
D	.01	.01		H 22-	68+, 17-, 33

plate on EMB lac + sss.

Again note relatively high efficiency of diluted crosses
(esp Hfr x F-).

4/29/52.

1895 T2 + W1958. Acute overnight. No aer 34 (T2). 1:10...
(no second incubation) minute

A	1	+	1	^{Lac +} 0	! in 10 ml.	2 PH...
B	.1		"	0		
C	.01		"	0		
D						
D	.001		"	0		

5/1/52. ~~No aer~~ overnight. 1:10 Pen acute 10 AM - 2 PM. Suspend air ca 30 minutes to reduce T2

1895 T2 1 or .01 ml / 10. No air. 2-430
1958 EMB Lac

A	1	1	ca 20- : 1 + !! (lac + fails to grow in mixed broth?)
B	1 .1	1	{ lac + V. infected
C	.01	1	{ V. infected meat
D	.01	.01) only lac - seen.

rate → 100% of Hfr cells Replica A to EMB Mtl EMB min.
← (see over)

S. typhi H901 large bodies

992C.
944

4/27

Many colonies of ~~large~~ L-type growth noticed in course of motility tests in H901 controls.

H901 was inoculated from EMB plate (?) to semi-solid agar incubated ca 24 hours. Room temperature 8 hours. Inoculate to semi solid agar (5 ml ±) + 500 units penicillin.

- 4/28. - penicillin showed same interplay of bacilli and sphaerules
+ " no macroscopic growth; sphaerules were prominent. These are very transparent, practically invisible except to phase microscopy, possibly accounting for infrequency of reports on them.

Similar admixtures of sphaerules noted to varying extent in semi-solid agar smears of S. stanley, 842-1, as well as isolates of H901! (Test LT2, K12...).

- 4/29. - Similar observations without & with penicillin. However the L-colonies are much less prevalent than in H901.

W1895 and W1956 inconspicuous in motility agar. Their transfer to motility agar + penicillin 500 u/ml. Occasional L-type, usually not colonial, seen with and without bacteria respectively. Small & very large sphaerules seen

- 5/1 Similar stunts with B. subtilis and staph. aureus.

Further course of examinations showed similar sphaerules singularly in uninoculated plates, also in freshly poised medium! Doubtful connection with bacteria!

May 3, 1952.

see 938

W1895 + W1876. Grow overnight Penassay. Mix 12N 1ml 10ml each.
Incubate to 130.

A. EMB Lac 9 plates ca. 100/plate. 2 ?? lac+

B. EMIB Lac son (10 and 100x). B- 0+; B': 2 0+ B" 3: 3+?

C. EMIB Mal son (1 and 10x)

D. D(B.)

C: ~~78- 7+~~ ~~75- 8+!~~ ~~43- 9+~~ Contrast very low frequency of Lac+ S^R. These Mal+S^R appear to be unselected. Possibility of contamination in parents?
~~196- 24+ → all lac-~~

W1895/1958 "control" - Lac EMIB son. 94-: 6+. Conditions are suitable.

5/4/52. Repeat C and also plate on EMIB Mal. Replica A to EMIB Mal.
As above. Reincubate ca 5 hours. Mix 3⁴⁰ - 6⁴⁰.

E EMB Mal "W1876" streaked out gives 10% Mal+
F EMIB Mal son on EMIB Mal son.

G EMIB Mal These expts n.g. except rare Lac+ S^R

H 1895 + 1958. Spotted on D(B.) 3⁴⁵. Incubate to 7PM. Examine under

phase microscope. Numerous nucleocystes. About 1/100 is partly or fully lysed with many granules of various sizes (such as mentioned by Post?) and control!