
9. Bear YB - YS3, Y40
10A10. Bear (15 min NSB) YS3, Y40 (A)
1P10 bear YB - Y40 (B)
4-5P10. Wash (A) cells.
1. Mix YS3-Y40 cells.

8P10. Searched YS3(A) cells in T-minimal, accumulate 3 shuttles 36.
    Sediment (C,D) and mix with washed Y40 (B). 2. plate 0.
3. Mix supernatant (C,D) mix with washed Y40 (B) 3. plate 0.

1. 5x10^2 prototrophs.
2. C > 10^2 " included for count.

3. Filtrate:
   C - 1 prototroph??; F supernatant was not entirely free of cells
   ?? by the centrifugation. Repeat 2 controls
   on influence of dilution of cell type on prototroph yield.
January 10, 1947.

Y40 + Y53 m1 T(0), T(81) again

Pick colonies to EMB media. 112/y = 1-15. 8, 13 +

Streak out densely on (A) BMTL-lactose (B) BMTL lactose + glucose.

Compare the B1- types separable from these plates.

Colonies:

Column 1: B1-m1.  A  B  C  D  E  F  G  H  I  J  K

Column 2:

Lys 1 - S(R)  - S  - S  - S  - S  - S

Lys 2 -

Lys 3 [R] + S  - S(R)  - S(R)

Lys 4 - S  + S  - S  + S  + S  + S

Lys 5 - S  - S  - S  + S

Lys 6 - (R)  - R  - R  - R  - R  - R

Lys 8 X  - S  - S  - S  - S  - S  - S  - S  + R

Lys 11 - R  - R  - R  - R  - R  - R  - R  - R  - R

Lys 12 - S  - S  - S  - S  - S  - S  - S  - S  - S  - S  - S  - R

Lys 13 + R  + R  + R  + R  + R  + R  + R

Lys 15 - R  - R  - S  - S  - S  - S  - S  - S  - S  - S  - S  - S  - S  - S

BMT + R

TLB - S
January 11, 1947.

Y64 x 58-161.

TLB: lac- T\textsuperscript{R} x BM lac+ T\textsuperscript{S}

good material.

a. prototrophs

\[ T\textsuperscript{R} \text{lac-} A + S - S + \]

42 1 53 23

R = 36\% 64\%

lac- = 80\%

b. B\textsubscript{1} plates. Much more numerous colonies (10 x)

(not well visible)

(colonies impure)

8 0 5 10
January 11, 1947

Y67 (Y53M) x 58-161 (Try x Y40.)

muc-het - lax - sm-het - muc-het - sm-het +

P3 17 1) 9 lac- = 0.66

Y57 (Y53/345, M) x 58-161 (Try x Y40.)

Y68 (58-161/M) x Y53. (Try x Y64.)

Segregation: M+ M+ + ml-

6 P 22 P 12 7

M68 Induced to lac-

Interaction of expression of lax- + Hift-
on EM plus medium?

Note variation in shrunken kinetic character?
January 11, 1946.

110 oz. 100 ml (1/4 fl.) YB - YS3.

III. Centrifuge 250 ml (step 25 - 1). Resuspend cells in 15 ml 9% NaCl. Add trypsin-entanate for autolysis, shake at 25° (1200 RPM - 3K RPM) Centrifuge "free cells" and mix 5 ml of 1 ml Y40 suspension + plate 3 x 2 ml samples into T(0) agar.

P74.

- 10 large 10^2.5 small 0 halos v. clear plates.

See 394

Plate Y55 (H...lac-) into lactose-minimal at
various dilutions: (Assor. = 10^4)

10^8 extremely crowded.

10^6 about 10^4 visible colonies

10^7 about 200 large colonies, with absence of small ones. Smaller colonies much smaller than below.

10^2 about 5x. 10^3 small colonies; 6 typical colonies (probably lac+). Affinitated

1) The reversion frequency, as estimated from EMB plates is very high, (ca 10^{-4} to 10^{-5} / generation ?)

2) At least on this medium, lac- is capable of developing to some extent. Since they develop bialaphos, it is likely that there is a territorial factor in the agar which favors reverse growth.

Test large colonies on EMB:
January 11-12, 1947.


P 12. Wash + plate.

\[(Y43 + Y44) \quad 0, 0 \quad \text{red turbid} \quad (exc.)\]

\[(Y43+Y44) \quad 0\]

\[(Y43+Y53) \quad 0\]

\[(Y43+Y53) \quad 0\]
January 12, 1947

Plate Y40, Y53 (cultures as in 391) in T(0) as initial controls.

9-10 PM. .5 ml. 25°

T0 = ca 2-300.

a. Keep Y40, Y53 in water (.9% NaCl) 25°. Plate YP13

b. Keep (Y40, Y53) in water. Plate YP13

c. Keep Y40, Y53 in T(0). [Add 1 ml to 10 ml T(0)] Plate YP13

d. Plate Y40, Y53 in superficial layer of agar -- 4 colonies.

e. Fresh Y40, Y53.

\[ \begin{array}{cccc}
10^2 & 2.5 \\
10^2 & 10^2 \\
10^2 & 10^4 \\
10^2 & 10^2 \\
\end{array} \] cleaner plate

cells will react if kept in water for 24 hours and then mixed. but not many more found if they are kept together. recombination takes place in the agar.
Differential centrifugation of *E. coli* - Preliminary Expts.

0. Density - in sucrose buffer, centrifuge 10 min eq. at 10,000 rpm.

<table>
<thead>
<tr>
<th>Density (g/ml)</th>
<th>1.0</th>
<th>1.04</th>
<th>1.08</th>
<th>1.12</th>
<th>1.16</th>
<th>1.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>20%</td>
</tr>
<tr>
<td>Time (min)</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.q.: ++ ++ ++ ++ ++ ++

1.16 = 20g sucrose / 100cc water

1:4 bacterial susp. in H2O.

6.9 g for density.

Repeat, using 20g sucrose / 20g H2O as d = 1.25. (actually 1.23)

\[ \left( \frac{1 + 0.01d}{4 \text{H}_2\text{O}} \right) \]

<table>
<thead>
<tr>
<th>Density (g/ml)</th>
<th>1.0</th>
<th>1.05</th>
<th>1.10</th>
<th>1.15</th>
<th>1.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>±?</td>
<td>-</td>
</tr>
<tr>
<td>Time (min)</td>
<td>20m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.15 1.20

20 +

Use heavier susp. cells.

This might achieve some separation.
1/13/47.

1/2 ml of various dilutions.
1 ml + 1 ml

<table>
<thead>
<tr>
<th>No.</th>
<th>NY3</th>
<th>NY40</th>
<th>ca. 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10^{-2}</td>
<td>10^{10}</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>10^{-4}</td>
<td>10^{10}</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>10^{10}</td>
<td>10^{-2}</td>
<td>8</td>
</tr>
<tr>
<td>4.</td>
<td>10^{10}</td>
<td>10^{-4}</td>
<td>1</td>
</tr>
<tr>
<td>5.</td>
<td>10^{-2}</td>
<td>10^{-2}</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>10^{-4}</td>
<td>10^{-2}</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td>10^{-2}</td>
<td>10^{-4}</td>
<td>0</td>
</tr>
</tbody>
</table>

Dissolve Y400 by dissolving 1 ml/10 Y400. Incubate 18 hours at 37°C. Dilute 1:100 and plate on EMB media. 20,000 colonies examined.

3 colonies, but rather small colonies were found. Pick and test further.

1 lact + mucoid colonies found. Pick + streak out to isolate.

all lact + lact muc = Y69
January 17, 1947

See 383 (1-6)

P21. Colonies have taken a blue tinge. Make streaks on plate to Y53.
All show coloration in leuc. zone to T1 virio.
5, particularly, shows few or no papillae. Y70.

1 very few papillae
2 papillae
3 papillae
4 few, but some papillae
5 none no papillae
6 few, but some.

coli Hs offi - papillae very varied. Than 11-12, but some papillae
are formed. Comparison should be
made of some protot photons saguaries.
This slide may now to Y53-Lab.
January 18, 1947.

P17 - P18. Yehom cultures Y53 autolyze 300 ml autolysin = 300 ml washed cells in 2L under 3 hours shaken at 28°C.


Emptied - use washed cells of above 5 autolyzis x Y40.

See also 399.

<1/2
Turbidity of autolyzate was more than that of the 1:100 dilution m 399
Keep 30ml overnight at heavy layer of benzene and repeat later.

Hold autolyzates overnight on cold.

6:19 - remove benzene from sample by evaporation.

A. Y40 + 0.2 autolyzate 0, also m 393 0
B. Y40 + autolyzate 0,0
C. autolyzate 5 + Y40. 0,0.

autolyzate is stead; no protozoa.
January 20, 1947.

A: Y40, Y10, Y64.
B: Y41, Y46, Y53

- S not added. + R  - R  + S  - S

<table>
<thead>
<tr>
<th>#</th>
<th>#</th>
<th>1</th>
<th>1</th>
<th>#</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>9</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

B: S-161, Y46, Y53
B: Y46, Y53

- R unadded. + R  - S  + S  - R

<table>
<thead>
<tr>
<th>#</th>
<th>#</th>
<th>#</th>
<th>1</th>
<th>1</th>
<th>#</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>#</td>
<td>#</td>
<td>1</td>
<td>1</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>#</td>
<td>#</td>
<td>#</td>
<td>1</td>
<td>1</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>#</td>
<td>#</td>
<td>#</td>
<td>1</td>
<td>1</td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

20 34 5 40

Some mistake?? See 911 for repeat.
3-way cross.

Y10

BM lac+V1^R

Y40

TLB, lac+V1

Y64

TLB, lac+V1^R

Y46

TLB, lac+V1

58-161

BM lac+V1^S

TLB, lac+V1

YS3.

TLB, lac+V1^R

TLB, lac+V1^S

TLB, lac- V1^R

TLB, lac- V1^S

BM lac+V1^R x TLB, lac+V1^S -> all types

LM lac+V1^S x TLB, lac- V1 -> all types.

BM lac+V1^R x TLB, lac+V1^S -> all types.

BM lac+V1^S x TLB, lac+V1^R -> all types.

BM lac+V1^R x lac+V1.

already done!
**January 18, 1947.**

Half needles:

<table>
<thead>
<tr>
<th></th>
<th>$Y53$</th>
<th>$Y40$</th>
<th>$Y53$</th>
<th>$Y40$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$10^0$</td>
<td>$10^0$</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>2</td>
<td>$10^{-1}$</td>
<td>$10^0$</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>3</td>
<td>$10^{-2}$</td>
<td>$10^0$</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>$10^0$</td>
<td>$10^{-1}$</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>$10^0$</td>
<td>$10^{-2}$</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>$10^{-1}$</td>
<td>$10^{-1}$</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>$10^{-1}$</td>
<td>$10^{-2}$</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>$10^{-2}$</td>
<td>$10^{-1}$</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>$10^{-2}$</td>
<td>$10^{-2}$</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

$\rightarrow f(Y40)$ $\rightarrow f(Y53)$

$Y53$: $10^0$ 120 $Y40$: $10^0$ 120
$-1$ 60 $10^0$ 120
$-2$ 8 $10^0$ 120

$Y53$: $10^{-1}$ 13 $Y40$: $10^{-1}$ 23
$-1$ 23 $10^0$ 23
$-2$ 16 $10^0$ 16

$Y53$: $10^{-2}$ 8 $Y40$: $10^{-2}$ 8
$-1$ 8 $10^{-2}$ 8
$-2$ 1 $10^{-2}$ 1

$Y53 + Y40$: $10^0$ 120 $10^{-1}$ 23
$10^{-2}$ 8 $10^{-2}$ 1
January 17, 1947.

Y57 x Y68 (TLB, +le - \( V_{13,5}^R \) x BM - Muc)

No prototypes!

See 404

of 387 for recording.

Y53M

Y67 x S8-161 work.

Y68 x Y53 work.

S8-161M
January 19, 1947.

TH19. Lay on 1/2 ml benzene on 1 ml Y40 in water. Keep on dark.

dc. in H2O.

2. Remove water layer; evacuate to remove benzene.

1. Plate to determine killing of Y40. — 0.

2. Add 1ml fresh Y40 to aqueous layer + let sit for 24 hr. Plate.
January 20, 1947.

P14. Grow Y40, Y53 into YB+ Tween; A20 + Tween likewise; plate in T(0) agar + 1% Tween.

A  10%  no growth
B  0.1%  no effect
C  0.05%  no effect

P19. Grow Y40, Y53 into YB. etc.
Plate into T(0) agar +

A  0.1%  Tween
B  10%  Tween

all ra 10^2

no particular effect of Tween could be established.
January 20, 1947.

5 1 ml samples 58-161 grown 18h. in YS. Wash +
incubate 2hrs. add 1:100 in nft. sol. 1/2
1 1/5
3 4 5
Survivors

58-161 is evidently more sensitive
than YS3. (which has had further
X-ray + u.v. exposure).
January 22, 1947.

1. Y65 × 58-161 (Y10/17) (in 1:100 def.)
2. Y57 × Y68 (Y10 Y53/1 × BM Huc)

1. Shows no recombination, prototrophs. (Do Y65 unable to recombine?)

See 379, 390

2. Plate e on 1% (no survivors). (Try at 9%)

Tag Y64 × Y68
January 21, 1947.

250 ml of blood from cell of YS3 harvested from YB-9 cell stock. Autotype Wb. under Wayne at room temp.

P22. Add 440 cells per plate.

P24 - no colonies.
January 22, 1947.

Plate $\beta^-$ colonies into TLA agar + BMTL. Use plates which relatively few, isolated prototrophic colonies.

0 Y65 x 58-161
1 Y40 x Y53.

Test original colonies for $U, R, lac$:

$\begin{pmatrix}
1 & 2 \\
3 & 4 \\
5 & 6 \\
7 & 8 \\
9 & 10 \\
\end{pmatrix}
\begin{pmatrix}
+ S & - R \\
- S & + R \\
- S & - R \\
+ S & - R \\
+ S & - R \\
\end{pmatrix}
\begin{pmatrix}
10 S \\
3 R + 3 S \\
7 S \\
3 R + 3 S \\
10 S \\
\end{pmatrix}$

Plate colonies into BMTL. Pick + test samples of colonies which arise.

$\begin{pmatrix}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 \\
10 & + R & - S & - S & - S & + R & 7 S + S & 8 + S \\
6 & 5 & 2 & 1000 & 1000 & 300 & 300 & 1000 \\
2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \\
200 & 200 & 200 & 200 & 200 & 200 & 200 & 200 \\
8 & 9 & 10 & 11 & 12 & 13 & 14 & 15 \\
10 + R & + R & + R & + R & + R & + R & + R & + R \\
9 & 8 & 7 & 6 & 5 & 4 & 3 & 2 \\
10 + R & 7 + R & 6 + R & 5 + R & 4 + R & 3 + R & 2 + R & 1 + R \\
16 & 17 & 18 & 19 & 20 \\
10 + R & 9 + R & 8 + R & 7 + R & 6 + R \\
\end{pmatrix}$

How explain "10" - Recession of $\beta^-$?

R test. 405-2. in plate. Dil to ca. 100% mcl + pneumo plate 5

1. BN  346
2. BMT  60
3. MBL  60
4. BMTL  60
5. BHTL  365