(lab notes)

XII/16/74 **HELA:**

Repeated crossing of B.S.K. auxotroph SB1109 and Marburg auxotroph SB863, but this time used conditions from Kohiyama paper, namely grew cells only to late log. phase and then plated.
Media used was the same as the previous experiment, selecting for 1) his of SB1109 and aro group of 863 or 2) Meth of SB1109 and aro group of 863. Used also 1)+Sm (100γ/ml) and 2) + Sm (100γ/ml). Also plated on Minimal. To all plates added 0.01ml N.B.
After 4 days incubation at 37°C, all controls were perfectly clear, no growth. In plates with mixture of SB1109 + SB863, at 10^0 dilution saw tiny colonies, individually growing, between 60 to 100 in each plate. Colonies growing on - meth plates are bigger than the ones growing on - His. There was no growth in the Minimal media or in + Sm plates, or in the 10^{-1}, 10^{-3} dilutions.

Suggestions:
Pick colonies and test genotypes.

Using same Kohiyama growth method, repeated crossing of SB 863 + SD8, This time selecting also in media + Sm (300γ) to get rid of feeding effect.
Plated on 1) Minimal, 2) Minim + leu + his + cys 3) Minim + leu + his + cys + Sm (300γ)
Added to all plates 0.01 ml N.B.
Did 10^0, 10^{-1}, 10^{-3} dilutions.
After 2 days, there was no growth on either plate.
After 6 days, all controls were still negative, but there was heavy background growth on 2) and 3) at 10^0, less heavy at 10^{-1} dilution, and about 100, 92 isolated tiny colonies at 10^{-3} dilution on 3) and 10, 12 isolated tiny colonies at 10^{-3} dilution on 2).

Suggestions:

Add 1 ml of Spiz. to plate, scrape off, get cells in suspension, plate on N.A. and same selective media, at diff. dilutions to get single clones. Pick single colonies and test genotype.

Use also standard strains for crossing experiments and non competent cells.

Betty: 18-22 and 1-6

I. Salt-Preferring Bacteria

A. Loss of Viable Bacteria from four of the NA+0.2M NaCl plates

$16\text{-}G\text{-}2\text{-}I_1^4$, which had a doubling time of 24' in Spiz.+0.2M NaCl and of 22' in NB+0.2M NaCl

$16\text{-}G\text{-}2\text{-}I^4$ extra

$17\text{-}G\text{-}1\text{-}I^4$ the doubling time of which had not eyt been tested

$18\text{-}G\text{-}2\text{-}I_1^4$, which had a doubleing time of 24' in Spiz+0.2M NaCl and of 25' in NB+0.2M NaCl

1. There was no growth in Pen-Assay+0.2M NaCl
2. " " " " on SO + 0.2M NaCl plates
3. " " " " " YEA plates
4. " " " " " NA+0.2M NaCl slants, with or without NB added
5. " " " " " Pen-Assay +0.2M NaCl slants
6. Retrieval of $18\text{-}G\text{-}2\text{-}I_1^4$ was obtained by growing it in a YEA stab. From the YEA stab heavy growth was obtained on: NA+NaCl slant, SO + NaCl plate, Pen-Assay+NaCl slant. Attempts are being made to retrieve bac. from the other 3 plates in YEA stabs.

B. Measures taken to save the bacteria from the other 14 plates:

1. Lyophilization of 11, in triplicate, in Pen-Assay+0.2M NaCl, inoculated from the NA+0.2M NaCl plates. The remaining 3 grown in the Pen-Assay+salt as above, had the cells spun down, washed in 1% peptone, & were resuspended in 1% peptone.
2. Transfer to SO +0.2M NaCl plates
3. Freezing in liquid nitrogen (in Pen-Assay+salt)
4, Transfer to Pen-Assay+salt slants - refrigerated
5. Transfer to YEA stabs - kept at room temperature.
   After a few days aeveral stabs were observed to develop a dark grey pigmented area near the surface of the agar, spreading downward with time.
   (Suggestion - Perhaps the pigment is melanin. Grow the bac. in Spiz. Add tyrosin as substrate to reproduce this phenomenon.)

C. Doubling Time Data - showing marked change as the bac. remain on the NA+0.2M NaCl plates (inoculated 11-11-74)

1. 12-31 data:
   a. From the $18\text{-}G\text{-}1\text{-}I_1^4$ NA+0.2M NaCl plate, Spiz. + various molarities of NaCl were inoculated - no growth at all, tubes remaining perfectly clear.
   b. From the comparable SO+.5% glucose +0.2M NaCl plate, Spiz + various molarities of NaCl were inoculated:

O.D. of overnight inoculated tube	doubling time	Molarity of NaCl
176	no growth ?	0.05
120	36'	0.1
132	27	0.15
136	27	0.2

2. 12-13 (using the NA+0.2M NaCl plates, where the bac. were still viable)
   For $18\text{-}G\text{-}1\text{-}I_1^4$ as above, Spiz + various molarities of NaCl gave:

13'	0.05 M
(levelled off at a very low O.D.)	
18'	0.2M
20'	0.3 M
18'	0.4 M

3.11-21, The same 18-G-1-I_1^4 from the same NA+salt plate gave
25' in Spiz+0.2 M NaCl and 24' in NB + 0.2M NaCl

D. Beneckea

1. The lyophilized strain received from the ATTA was labelled SB1092 (X/22) &put on a YEA plate & NA slant by Hela, who isolated one colony in a YEA plate & labelled it SB1097 (X?23); a YEA stabe was also made of 1097, as well as a NA+2%NaCl plate.
2. From the 3 plates (1092 YEA, 1097 YEA, 1097 NA+2% NaCl) the following media were inoculated for growth curves & incubated 37C with aeration:
   Spizizen + 0.25% glucose + 2% NaCl - no growth
   TB + 2% NaCl - no growth
   NB + 2% NaCl - no growth
3. Fresh YEA plates were made. Three were dividedinto 8 segments each, & 8 colonies from each of the 3 Beneckea plates ere used for inoculation onto a segment. After 37C inc. - all segments were negative for growth.
4. YEA plate was inoculated from the 1092 NA slant - no growth
5. Both the 1097 YEA stab and the 1092 NA slant were used to inoculate:
   BM + .25% glucose
   BM + .5% glucose
   YEB
   in duplicate, 1 set being incubated at 37C & the 2nd at 25C, both aerated.
   a. No growth obtained in any media at either temp. from NA slant
   b. Growth occurred in all the media inoculated from the YEA stab.
   c. Best growth was in YEB at 37C.
   d. There was better growth in BM + .25% glucose than in BM + .5% glucose, but far less than in YEB
6. A new YEA stab was made from the 1097 stab & a fresh YEA plate. Both had heavy growth overnight. Also, the turbid YEBof 1097 was lyophilized in 9 tubes.

II. Saccharin Study using 14 samples from nature

A. Saccharin as a S Source, using Spiz minus S (NH_4Cl & MgCl instead of $(NH_4)_2SO_4$ &$MgSO_4$) + Glucose + citrate + 0.1% saccharin
The same + 0.5% saccharin.
The control was Spiz - S + G +C and no saccharin
There was comparable growth in all 3 sets of tubes (either all - or ±? or ± or 1+?); also, the E. coli control was 1+
Therefore, addition of saccharin as a S source made no difference.
Fresh Spiz. minus S medium must be made.

B. Saccharin as a C Source
Using, Spiz -G -C + 0.1% saccharin and the same + 0.5% saccharin .
There should have been a set of control tubes without any carbon source, i.e., -G -C - Saccharin (S)

1. Several tubes became ± after 9 days and 1+ by the 12th day. These were used for serial transfers of 0.1ml into 5ml fresh medium & for plating & then were stored in cold room. A few of the 1st transfers became 1+ & were used for 2nd serial transfers & plates. None of the " " " ever became +.
2. The plates streaked with the + tube contents were S0 -G -C +0.1% or 0.5% S (depending upon which%S was used in the tube). These plates were made 11-1-74 & stored in cold room & were probably of Pfizer agar. The plates became + with mold or bac or both.
3. Transfers were made to:

a. Difco agar plates SO-G-C+S & became +

b. Another set of Pfizer agar plates (for comparison with the Difco plate results, transfers being made concurrently) & were +, with more growth

c. NA plates which gave very heavy growth

4. Transfers were made from the NA plates to another set of Difco agar SO plates -G-C+S, to see if the orgs. are capable of using S as a carbon source after being on NA & these were +

5. Control: Transfers were made from the first set of positive Difco SO-G-C+S to another set of Difco Agar SO plates -G-C-S, i.e., without a carbon source supplied.
These are being observed & will be compared with the plates having saccharin to see if the saccharin plates have growth where controls have none or if colonies on " " arelarger than on control plates

III. Suggestions:

A. For Beneckea and other salt-preferring bac. there are 2 main problems:
Survivability of cultures over a long period of time
Rescue of cultures losing viability

1. Try Bayland water as the medium. Sterilize by filtration of several liters. Buffer the filtered bay water with TRIS, TES or 5mMPO_4 buffer. Begin with minimal amount of buffer & then add more.
2. To retrieve the 3 lost plates: Try overnight inc. If not successful, try scraping into YEB & then plating. Try deep pour plates as well.
3. Oxygen supply may be the issue. These bac. may be poisoned by too much oxygen. Does pour plate give better viability than surface agar plate?
4. pH control must be better; there may be pH change over a long period of time on the plate which may be very detrimental. Must heavily buffer the medium. Check the pH of Bayland water.
5. Check viability of lyophilized samples; check viability before & after lyophilization. Wash & resuspend cells in 1% peptone before ".

B. Use Galvez Stream bac. CG1 for genetic studies. Is it mutatable? Use dinitroso guanidine. Does it lend itself to the penicillin method? Make 2 auxotrophs. Does one quickly get evidence of genetic recombination or not? Recheck the doubling times of the plates first.

C. Saccharin Study

1. Scan Chem. Abstracts through 1940s and 1950s to check sulfanilamide to see if any bac. grow on it.
2. Under which conditions (pH, etc.) does the imide group of saccharin get hydrolyzed?
3. Try the soil sample with buried saccharin again since there has been good rainfall.
4. Phone Mike Doudoroff, Microbiology Dept., Berkely to ask if he knows of any work on microbial attak on benzene sulfonic acids & their derivatives or if he knows of anyone with such information.
5. Check the lit.,looking up his name, for aromatic metabilism - the breaking of aromatic rings by bac. Then check the various co-authors names for work on this topic.
6. Ifcolonies are present on saccharin plates that are not present on control plates or if colonies are larger than those on the controls, isolate these as pure colonies on plates.