Seminar Minutes of 9-19-74

I. Hela: Dinitrosoguanidine treated B. subtilis on minimal media having Galactose instead of Glucose, shows a very slow utilization of the Galactose.

1. This may be due to contamination by trace amounts of Glucose in the Galactose. Therefore, to the minimal media having galactose add trace amounts of glucose and observe the difference in growth.
2. Does this represent a lack of metabolic steps or inhibition? Gal^- may be self inhibiting and also for SB19 on Galactose medium. Therefore, cross streak the two; plate mixtures of Gal^- and SB19 in different proportions.
3. Various percentages of lactose were tested in selecting for the Lac^+ marker. The wild type grows only on 5% , and not with other concentrations of lactose. It may have the enzyme B galactosidase (which can be determined by colorimetric assay), but be missing the enzyme permease (the B. subtilis membrane may not be able to accomodate permease). Grow cells on glucose and a low level of lactose. Try to get maltose mutants and cellobiose mutants.
4. Get a collection of other Bacillus strains in order to look for crossing with the whole group of Bacillus. Transformation with high M.W. DNA may accomplish the same purpose in studying cross transformability (to get in + alleles by transformations). Two sets of reliable mutants are needed (there are problems if either one reverts). Build up multiple marker strains for recombination. Streptomycin Resistance is a good reliable auxotroph to cross with Streptomycin Sensitive prototroph; then look for Streptomycin Resistant prototroph. Order a dozen type species strains that are different from one another from ATTC.

II. Hela: Salmonella Rough cells + P22 DNA result in Transfection; complete phage are released providing plaques. The cells must first be made competent. The rough Salmonella have lost the surface polysaccharide and therefore cannot absorb free phage but can be transfected with the DNA.

1. Observation of the cells under the microscope after UV radiation following Transfection, showed clumps. Clumping occurs in $CaCl_2$; resuspension after one hour of shaking (which separates the clumped cells) could explain the later increase in the number of cell infectious centers (but the sharp increase of plaques after after one hour argues against this). When put in broth with growth for 21/2 hours a greater number of elongated cells were seen. It is desired that the radiation should have no effect other than to prevent cell division; the problem with UV is that it has a number of other effects. Therefore, look for other inhibitors to prevent cell division (e.g., crystal violet) and that operate at the level of the cell wall and not the DNA replication.
2. There is delayed bursting in about an hour; eventually the phage goes through the vegetative cycle. After Transfection perhaps there are lysogenic complexes that are not fully stabilized. There may be more lysogenic cells than cells with lytic phage. After lysogeny has occurred, irradiate the culture to induce lysis. Try induction with Mitomycin or other efficient inducers. Prepare lawns with 10^4- 10^5 bacterial colonies on each plate; these should be the bacterial survivers of the competency treatment and the exposure to DNA. Grow the cells for 8 hours and wash them well to eliminate excess free phage. Then test for lysogenics in the bacterial population. Look for clones that can still produce phage and release them upon induction. Use pour plates to see plaques in the vicinity of rough clones that produce phage; it is necessary to look for a later stage of phage release. Partially stabilized lysogenic complexes may not produce the normal amount of repressor that stable lysogenics do.

3. In assaying for infectious centers, observe the cells under the microscope for clumping at the various steps in the competency treatment. See if there is any correlation between the efficiency of Transfection and the qualitative level of clumping.

III. Hela: There are indications that the DNA alone may be toxic to the cells.
1. Results of the experiments to test this were not reproducible.
2. The sensitivity of the method is the crucial point.
3. Always do viable counts before and after adding the DNA.

IV. There was a discussion of terminal splicing of DNA,
1. The argument for end to end joining, that P22 is subject to random permutations of the duplicated ends, is not a strong argument since it is not a random ending.
2. There is an inability to get T7-P22 dimers. Also, Dusheo reports that T7 is not joinable to itself.
3. J.L. questions whether the difference in behavior of E.coli and T4 ligases (as reported by Sgaramella - T4 ligase with P22 DNA provides terminal joining of fully base paired terminii, whereas T4 ligase or E. Coli ligase with SV40 DNA provides cohesive joining of complementary single-stranded sticky ends) is truly the phenomenon that was reported. Even a minimal amount of erosion should destroy the flush endedness, and then there should be no more end to end linking. The behavior of the two ligases should then be alike, with sticky end joining predominating. It is questionable whether end to end joining does occur, the evidence being very weak.

V. Betty: Superbug Search
1. Samples were obtained from a variety of sources; creeks along Campus Drive, Stanford Ave., and Galvez and Arboretum; Baylands swamp and duck pond; and soils from garden, Medical School courtyard and Baylands swamp area.
2. Different type colonies were isolated and described both on pour plates (with 4mm soft agar depth) and streaked plates. From a number of the samples, large abundantly growing colonies were obtained with overnight incubation.
3. Growth on various media was compared: that on SOminimal media + 0.5% glucose gave the best growth, Glycerol + Vitamin Mix gave good growth with smaller colonies than on SO, Ethanol + Vitamin Mix gave good growth with colonies smaller than in Glycerol, and Ammonium Acetate + Vitamin Mix gave either very slight growth of tiny colonies or no growth at all.
4. Cell morphology of the isolated colonies are being described by gram stains.
5. Generation Time studies in Spizizen Minimal Media +0.5% glucose and in TB Complete Media are in progress for all the isolated colonies. One of the colonies isolated from the creek along Galvez gave a doubling time of 23 minutes in minimal media and a doubling time of 21 minutes in complete media (as compared to 28-30 minutes for E. coli, which was tested simultaneously). Its colonies are larger and grow more amply than those of E. coli on the same media incubated for the same period of time.
6. Suggestions made include: identify the above bacteria taxonomically, do viable counts from the minimal flask, try growth in a mineral mix recommended by J.L. , try Spizizen Minimal without the glucose (the citrate in the Spizizen may be serving as the carbon source), try growth in minimal media of different pHs, test the colonies that are difficult to separate and purify for symbiosis, follow daily viability counts of newly gathered samples.