Application for research support to theRESEARCH COMMITTEE OF THE UNIVERSITY OF WISCONSIN submitted by Joshua Lederberg, Associate Professor of Genetics January 12, 1951

TITLE AND REASONS FOR STUDY

Bacterial Genetics. The broad objective of this continuing project is an informed insight into the most fundamental aspects of microbial biology- the hereditary mechanisms. The most incisive tool of genetic investigation, recombination analysis, is being applied to problems of formal, physiological, and cyto-genetics in as many bacteria as can be shown to be technically feasible.

RESULTS OBTAINED TO DATE and PROCEDURE for projected study.

A. Cytogenetic comparisons of haploid and diploid Escherichia coli. Provious reports refer to the discovery of exceptional diploid clones of S. coli, as indicated by their genetic behavior. These cultures are being compared cytologically with the standard haploid forms, and a critical series of photometrographs is in preparation. Haploid and diploid cells can be distinguished by their nuclear structure, although there is considerable variation in the appearance of each type through the culture cycle. The diploid nuclei have a more open, dispersed structure than the haploid, but it has not yet been possible to resolve countable chronoscres, or to outline the presumed mitotic cycle in detail. The work so far has used primarily killed and stained preparations. However, there are published reports, which we are attempting to verify, that bacterial muclei can be resolved in living cells by phase contrast microscopy. It is hoped to use this technique together with the isolation of single cells to correlate cytological and genetic events of segregation. In collaboration with Dr. V. R. Zelle of the Atomic Energy Corruission, single cell podigrees have been used to verify the diploid character of the exceptional clones.

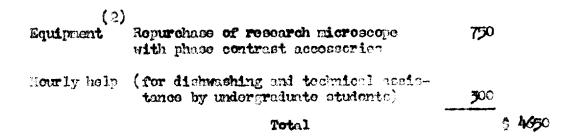
B. <u>Outcrossing bacteria</u>. To date, work on genetic recombination in bacteria has concerned mutant derivatives of a cingle strain of E. coli: "K-12". Attempts to find other bacteria which could be crossed, either introgressively, or with other strains, had been unsuccessful, ewing to the limited number of tests which could be made. Nethodological improvements have made it possible, however, to screen large numbers of strains for "crossability" with K-12. About 5-10% of isolates of S. coli from human urine cultures have proved to be inter- and intra-fertile. Among the many research possibilities thus opened up is improvements analysis, on which we are unbacking in a preliminary way.

A related project, sponsored by the National Institutes of Realth, involves recombination in Salmonella spp. This project is being pushed in parallel with E. coli studies, and has benefitted similarly from improved methods.

C. <u>Bactericidal mechanisms</u>. In this project, the effects of bactericidal agents (ultraviolet light; X-rays; chemical mutagens and other chemicals) on diploid cells have been examined to try to determine how bacteria are killed. There has been no indication that lethal mutations play a role, although other more complex genetic effects have been found. The results so far have not supported a hit or target theory, even for radiations, in contradiction to most speculations (including my own) based upon kinetic data only. The hypothesis presented in a previous report, that "killing is possibly the succoording stage in the series diploid-haploid-death", i.e., that the nucleus or diremesome is the unit of inactivation, has been discarded. The analysis is continuing; a research grant from the Atomic Energy Cormission for X-ray aspects is pending.

D. Gene engune relationships. Further study of the nature of genetic control of lactase in E. coli has been impeded, in part, by the fact that the enzyme is formed in strain 2-12 only in cells which have grown in contact with lactose or analogous substrates. It was therefore not feasible to study the conditions of formation of this ensure in chort term or manometric experiments during which growth is procluded. Fortubately, one of the new fortile strains mentioned above produces the enzyme under simpler conditions, and should thus provide more workable material for the analysic of lactasenegative matants. Freliminary experiments are now in progress to characterize the lactages of different crossable strains by the technique of electrophoresis on paper (cooperation of)r. A. Bussard, research fellow, Zoology Dept.). It is anticipated that inter-strain crosses will give information on the genetic control of enzyme quality, as well as on the formation of the "standard" enzyme. Large-scale preparations and purification of E. coli lactase are being explored by Dr. H. A. Lardy and Mr. S. Huby at the Enzyme Institute.

4. FINAMIAL SUPPORT requested: (1) Personnel. Three (3) Assistantships, 12-500. § 3600 © 1200



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(1) Two of these assistantships are for renoval:

Hiss Ethelyn Lively and Miss Phyllis Priod. These assistants have carried out their duties very satiofactorily to date, and would be difficult to replace. The third assistantship is to be filled by a candidate to be selected from current applications. Unless urgently required by the best qualified candidate, this assistantship will be revised to a 10-month appointment.

(2) This microscope, originally costing 31200, is in uso in connection with a contract with the Chemical Corps on related projects. This contract will expire during 1951-52, at which time title to the microscope will revert. The repurchase price will be subject to negotiation, and may be more or less than the figure given, although the value suggested is probably a fair one. This type if equipment is already difficult to obtain, and ought to be retained.