1951-1952

Application for research support to

theRESEARCH COMMITTEE OF THE UNIVERSITY OF WISCONSIN

submitted by Joshua Lederberg, Associate Professor of Genetics

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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. Previous reports refer to the discovery of exceptional diploid clones of E. coli, as indicated by their genetic behavior. These cultures are being compared cytologically with the standard haploid forms, and a critical series of photomicrographs 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 chromosomes, 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 nuclei

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. W. R. Zelle of the Atomic Energy Commission, single cell pedigrees have been used to verify the diploid character of the exceptional clones.

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

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

C. Bactericidal mechanisms. 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 succeeding stage in the series diploid-haploid-death", i.e., that the nucleus or chromosome is the unit of inactivation, has been discarded. The analysis is continuing; a research grant from the Atomic Energy Commission for X-ray aspects is pending.

D. Gene enzyme 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 K-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 enzyme in short term or manometric experiments during which growth is precluded. Fortunately, one of the new fertile strains mentioned above produces the enzyme under simpler conditions, and should thus provide more workable material for the analysis of lactase-negative mutants. Preliminary experiments are now in progress to characterize the lactases of different crossable strains by the technique of electrophoresis on paper (cooperation of Dr. A. Dussard, 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. Hardy and Mr. S. Ruby at the Enzyme Institute.

4. FINANCIAL SUPPORT requested:

Personnel. (1)	Three (3) Assistantships, 12-mo. @ 1200	$ 3600
Equipment (2)	Repurchase of research microscope with phase contrast accessories	750
Hourly help	(for dishwashing and technical assistance by undergraduate students)	300
	Total	$ 4650

Explanation-

(1) Two of these assistantships are for renewal:

Miss Ethelyn Lively and Miss Phyllis Fried.

These assistants have carried out their duties very satisfactorily 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 $1200, is in use 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 of equipment is already difficult to obtain, and ought to be retained.