Interim Report to the Natural Sciences Division

Rockefeller Foundation

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Project: Genetics of Bacteria,

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1. Gene-Enzyme Relationships in E. coli.

In order to elucidate gene-enzyme relationships, attention has been focussed on a single enzyme, many mutants obtained in which the enzyme is deficient or modified, and genetic and physiological differences between the mutants studied. The enzyme chosen for study is beta-d-galacto-sidase, or lactase, in Escherichia coli K-12. This system was selected because (a) lactase works on a readily obtainable substrate, lactose; (b) it proved to be a simple matter to isolate any number of mutants deficient in lactase, by the use of indicator medium which told whether a given colony on a plate was producing acid or not, according to whether it could split and ferment lactose; (c) a very precise spectrophotometric method for the determination of the enzyme was developed using the analogous substrate, nitrophenyl galactoside. The compound is colorless, but when nitrophenol is split off under enzymatic action, it gives a yellow color which can be quantitatively measured in the spectrophotometer; and (d) it proved to be relatively easy to extract the enzyme from dried cells and to study its properties in vitro.

Several hundred independent lactose-negative mutants have been isolated from nearly a million colonies examined on indicator medium, after ultra-violet irradiation. Most of these have been crossed among

themselves in order to define their allelic relationships. At least seven well-defined groups have been found, such that a lactose-negative from one group will give some lactose-positive recombinants when crossed with a lactose-negative from another. Within the same group, lactose-negative parents give only lactose-negative progeny. The conclusion drawn is that there are at least seven distinct loci interdependently controlling lactose-fermentation, so that mutation of any one of them interferes with or modifies the formation of the necessary enzymes.

Studies on extracted galactosidase support the contention that it is a single enzyme critical in the pathway of lactose metabolism. It is completely or nearly absent in extracts and intact cells of each of the mutants.

In addition to the multigenic control of galactosidase, some of the mutants show alterations of several enzymes. For example, the mutant class "Lac$_3$-" fails to ferment glucose, maltose or lactose. These effects are probably due to distinct enzymes, because (1) the enzymes occur independently of one another in wild type cells adapted to different substrates, (2) different suppressor mutations have been found which will reverse the different components of the effects of Lac$_3$-, and (3) a temperature-sensitive allele at the Lac$_3$ locus has been found, which is like the wild type at 30°, like typical Lac$_3$- at 40°, but within the temperature interval has a different threshold for the different effects. Temperature affects enzyme formation rather than action; cells adapted to lactose at 30° retain their activity when tested at 40°; cells fail to adapt to lactose at 40° and later show no activity when tested at either temperature.

Several findings have suggested that enzymatic adaptation rather

than the specificity of the final enzyme produced must be scrutinized. Lactase is fairly strictly adaptive, i.e., it is present in very small amount in cells which have not recently been exposed to lactose. It seems likely that the site of action of most of the mutations may be at early stages in adaptation. For example, the "Lac_1-" mutant type which produces practically no lactase in response to lactose, adapts to a very appreciable extent (about a third as well as wild type) on other galactoside substrates such as butyl galactoside. Thus, Lac_1- cells grown in the presence of lactose ferment lactose very weakly, while cells grown on butyl galactoside ferment lactose nearly as well as wild type. The same probably holds for Lac_3-. On the other hand, Lac_2-, Lac_4-, and Lac_7- seem to be completely devoid of lactase under all conditions tested. Lac_5- has a pleiotropic effect similar to that of Lac3-, except that glucose is fermented, and it is gluconate which is not. Lac_6- ferments lactose, but only very slowly.

These observations suggest that the genetic control of galactosidase is quite complex, but they do not yet afford more than leads for further research in elucidating the mechanism of this control.

This material has not yet been published, except in abstracts, and in passing in a review article "Bacterial Variation" which will appear in Volume 3, Annual Reviews of Microbiology, 1949-50. A description of the wild type enzyme and the assay methods is in manuscript.

In collaboration with a group of biochemists at the University of California, Berkeley, the mechanisms of maltose fermentation in the suppressor-mutant combination of Lac_3^- which ferments maltose but not glucose has been investigated. It was found that maltose is polymerised to a starch-like polysaccharide under the influence of an enzyme named "amylomaltase". At a later stage, the polysaccharide is phosphorolysed to yield glucose-1-phosphate which is then further metabolized. Thus the splitting of maltose circumvents glucose, but goes instead via starch to glucose-1-phosphate. However, this explanation is incomplete. Amylomaltase in, e.g., dried cells, converts maltose to glucose and starch in equimolar proportions, and one mol of glucose accumulates for each mol of maltose utilized.

(1) $n\ (G\text{-}G) \xrightarrow{\text{amylo-maltase}} n\ G\ \#\ (G)_n$

n maltose — n glucose plus polysaccharide of nth degree.

(2) $(G)_n\ \#\ n\ H_3PO_4 \underset{\text{phospho-rylase}}{\rightleftharpoons} n\ G\text{-}H_2PO_4 \longrightarrow$ further glycolysis

starch plus phosphate — glucose-1-phosphate

But intact cells do not accumulate glucose during maltose fermentation, although they will not metabolize exogenous glucose supplied to them, even during maltose utilization. In some way, the cell must be able to distinguish between the glucose which is released from the amylomaltase reaction (1), and that supplied from without. It is not yet clear whether there might be a chemical difference, or whether it will be necessary to resort to some explanation based on "permeability".

These observations are reported by Doudoroff, M., Hassid, W. Z., Putnam, E. W., and Lederberg, J., "Direct" Utilization of Maltose by E. coli, accepted for publication in the Journal of Biological Chemistry.

2. Gene Recombination in E. Coli. The isolation and behavior of diploid heterozygotes.

The usual course of sexual reproduction in E. coli K-12 is shown on the top line of the enclosed figure. Cells occasionally fuse, forming the diploid zygote, which undergoes immediate reduction, (See Fig. 1). In the diagram (Fig. 2) the crossover pattern that would result in a lactose-negative prototroph is shown. Of course, in other zygotes, a crossover between Lac and B might result in a lactose-positive prototroph, but in any event, a single prototroph colony would be either pure Lac- or pure Lac+.

Stocks have now been found which produce zygotes which occasionally continue to proliferate as diploids for an extended period of time as illustrated in Fig. 2. However, in about one division in eight or ten, a diploid may undergo segregation, with crossing-over, along the same patterns as the standard. However, different segregations will be Lac- and Lac+, accordingly, the colonies produced will be mosaics of + and - rather than pure + or -. Since the diploid can be isolated as a prototroph, and then transferred to complete medium for segregation, we are not restricted to the recovery of prototroph recombinants, but can also secure types such as the "multiple mutant" which is shown as A-B- in the top line of the diagram. This was not previously possible, as crosses had to be conducted on minimal medium, (which permits only prototrophs to develop), in order to suppress the parental cells which are present in excess. Now, the selection of prototrophs can be used to secure the intermediate diploid stage, which can then be plated out freely on complete medium.

In collaboration with M. R. Zelle of Cornell University, it has been shown that single cells can be picked individually under the microscope and that they will later segregate into the various genetic types. This observation further completes the proof for sexual fusion in this bacterium. It is hoped to continue with cytological comparisons of diploid heterozygotes and haploids, with the continued aid of the present grant, and with assistance from the Research Committee of the University of Wisconsin.

Some of these experiments have been published as "Aberrant Heterozygotes in Escherichia coli", which is due to appear in the April 1949 number of the Proceedings of the National Academy of Sciences.

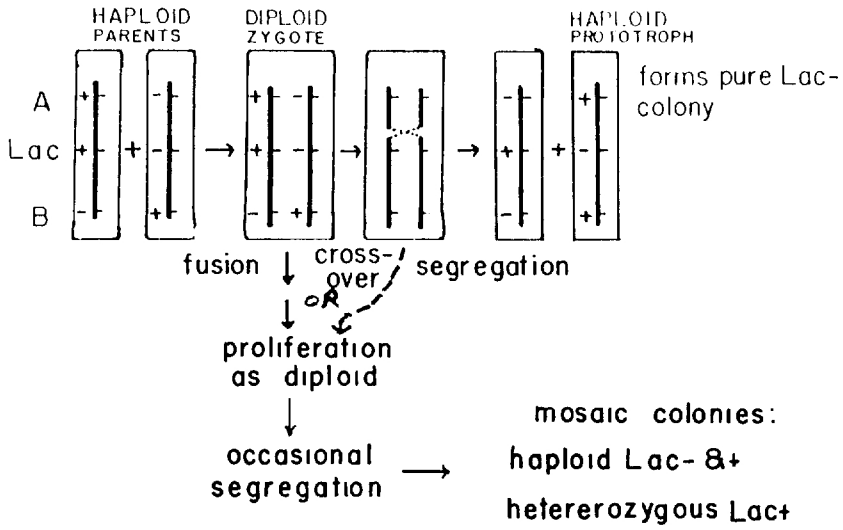

Fig. 2 Behavior of persistent heterozygotes

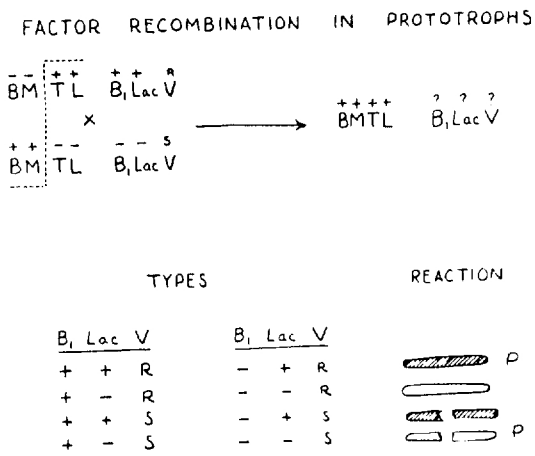

Fig. 1 Recombination to form prototrophs