Proc. Natl. Acad. Sci. U.S. **40**:1064, 1954). The galactose mutations are of the same stability as the streptomycin-sensitivity gene. Some 60 other galactose mutants are similar to those reported in Table 1. No instances of mutability grossly higher than Gal_{16} or Gal_9 have been noted, and no certain cases of failure to revert have been found. Combinations of Gal mutations have been synthesized via lambda-phage transduction. Such combinations have never been observed to revert, and the combined independent probabilities (ca. 10^{-20}) indicate why this is so.

The author is a Career Development Awardee. This work was supported by U.S. Public Health Service grant E-1345 (C5) and National Science Foundation grant 14210.

SELECTION OF MUTANTS OF *ESCHERICHIA COLI* CONSTITUTIVE FOR TRYPTOPHANASE

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Received for publication 23 July 1962

In a study of the effect of low temperature on *Escherichia coli*, Ng, Ingraham, and Marr (J. Bacteriol. **84**:331, 1962) found that the induction of β-galactosidase was insensitive to glucose repression below 20 C. Another inducible enzyme, tryptophanase, was chosen to test the generality of this observation. Tryptophanase could not be induced at 13 C, and *E. coli* failed to grow at 13 C with tryptophan as the carbon source. It became apparent that a study of mutants constitutive for tryptophanase could decide whether the failure to produce tryptophanase at low temperature results from impairment of the induction mechanism or from inhibition of the enzyme-formation system.

Two methods were used to select mutants constitutive for tryptophanase: (i) pulse-feeding (Novick and Horiuchi, Cold Spring Harbor Symp. Quant. Biol. **26**:239, 1961) of subinducing amounts (0.1 μg/ml, final concentration) of L-tryptophan every 3 to 4 hr to uninduced cells in minimal medium; and (ii) incubating uninduced cells in medium 56 (Monod, Cohen-Bazire, and Cohn, Biochim. Biophys. Acta **7**:585, 1951) containing 0.1% L-tryptophan as the sole carbon source at 13 C for 6 weeks. Samples of cultures were plated on complex medium; colonies were picked and inoculated into liquid glycerol-minimal medium. After 24 hr of incubation at 30 C, the cultures were assayed for tryptophanase as described in Table 1.

Constitutive mutants isolated by either technique were able to grow at 13 C with tryptophan as the only carbon source. Thus, the failure of the wild type to form tryptophanase at low temperature does not result from an enzyme-forming system which is inactive at low temperature.

The mutants obtained by these two methods from three strains of *E. coli* are partial constitutives at 30 C (Table 1, column 3); i.e., the enzyme levels are increased by growth in the presence of inducer. Most of the mutants constitutively produced at least 10% as much enzyme as the induced parent (column 4); one strain, ML30 P19, constitutively produced 50%. If induced, all of the mutants except ML30 P22 produced 2.7 to 4.9 times more enzyme than the induced wild type (column 5). The degree of constitutivity does not correlate with the method of selection.

Partial constitutives which respond to inducer can result from mutation of an operator (Jacob et al., Compt. Rend. **250**:1727, 1960) or of a regulator cistron such as the R_1 regulator of alkaline phosphatase (Garen and Echols, J. Bacteriol. **83**:297, 1961).

The majority of the mutants, upon induction, produce higher levels of tryptophanase than the parental strain. This behavior is analogous to hyperproduction of β-galactosidase (Novick and Horiuchi, Cold Spring Harbor Symp. Quant. Biol. **26**:239, 1961) and to the strains which carry the structural cistron for β-galactosidase on episomal particles (Jacob and Monod, J. Mol. Biol. **3**:318, 1961). Since C6001 is an F^- strain,

TABLE 1. *Levels of tryptophanase in constitutive mutants of Escherichia coli**

Strain†	Units of enzyme/mg of cells‡	Uninduced/induced	Mutant uninduced/wild induced	Mutant induced/wild induced
		%	%	
C6001 W	24.6	2.3		
C6001 W (I)	1,053.3			
C6001 T	319.4	9.3	30.3	
C6001 T (I)	3,425.4			3.3
C6001 P	197.5	4.8	18.8	
C6001 P (I)	4,073.2			3.9
30SO W	163.4	5.1		
30SO W (I)	3,226.5			
30SO P	1,106.7	8.5	34.3	
30SO P (I)	12,955.9			4.0
ML30 W	3.4	0.4		
ML30 W (I)	890.2			
ML30 P22	368.7	41.7	41.4	
ML30 P22 (I)	884.0			0.99
ML30 P19	450.6	18.9	50.6	
ML30 P19 (I)	2,388.9			2.7
ML30 P27	130.5	3.7	14.7	
ML30 P27 (I)	3,561.0			4.0
ML30 T6	92.8	2.1	10.4	
ML30 T6 (I)	4,342.5			4.9
ML30 T11	85.1	2.3	9.6	
ML30 T11 (I)	3,696.6			4.2
ML30 T12	53.4	1.8	6.0	
ML30 T12 (I)	2,924.0			3.3

* Cells were grown at 30 C in medium 56, with glycerol as the carbon source and with or without 0.05% L-tryptophan as the inducer. The cells were filtered on a membrane filter, washed with 30 ml of 0.1 M phosphate buffer (pH 7.4), and resuspended in buffer to a density of 15 and 150 μg/ml of cells (dry wt) for induced and uninduced cells, respectively.

† The letters W, P, and T following the strain numbers denote wild type, mutants isolated by pulse-feeding, and mutants selected at low temperature, respectively. The (I) indicates that the cells were induced.

‡ Tryptophanase was assayed as described by Pardee and Prestidge (Biochim. Biophys. Acta **49**:77, 1961), with the following modifications: 0.5 ml of cells (which had been treated, without shaking, for 10 min with 0.25 ml of toluene at room temperature) were incubated with 0.25 ml of substrate for 30 min, after which 3 ml of Ehrlich's reagent were added. Units of enzyme were determined by diluting a suspension of induced 30SO cells and constructing a standard curve relating optical density at 568 mμ to the dilution factor and setting the activity of the undiluted suspension arbitrarily at 100 units.

episomal gene copies is an unlikely explanation for hyperproduction of tryptophanase. Genetic analyses of the constitutive mutants are now in progress.

This work was done while one of us (HN) held a predoctorate fellowship from the Whirlpool Corp. The authors are indebted to A. G. Marr and J. L. Ingraham for their interest in this study.

INTRACELLULAR α-AMYLASE IN *BACILLUS SUBTILIS*

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Received for publication 26 July 1962

Although some bacteria release considerable amounts of exoenzymes to the extracellular environment, the activities of most exoenzymes found in the cells is barely detectable, with the exception of penicillinase of *Bacillus subtilis*, recently studied by Kushner and Pollock (J. Gen. Microbiol. **26**:255, 1961) and by Pollock (J. Gen. Microbiol. **26**:239, 1961). In the course of study of the site of the synthesis and excretion of exo-α-amylase of *B. subtilis*, we have obtained a cell preparation in which exo-α-amylase remained.

B. subtilis strain K was grown aerobically at 30 C in 500-ml flasks containing 100 ml of medium of the following composition, per liter of tap water: glucose, 1.0 g; citric acid·H_2O, 3.0 g; Na_3-citrate·$2H_2O$, 6.0 g; Na-glutamate, 8.0 g; $MgSO_4$, 0.5 g; KCl, 1.5 g; $CaCl_2$, 0.1 g; $(NH_4)_2HPO_4$, 10.0 g; ethyl alcohol, 10 ml; yeast extract, 2.0 g; Zn^{2+} (as sulfate), 2 mg; Fe^{3+} (as chloride),