A METHOD FOR ISOLATING MUTANTS RESISTANT TO CATABOLITE REPRESSION[1]

N. Zwaig[2] and E. C. C. Lin[3]

Department of Biological Chemistry

Harvard Medical School

Boston, Massachusetts

Received January 13, 1966

Mutants in which the synthesis of an inducible enzyme is insensitive to catabolite repression have been selected by making a source of nitrogen available only to those cells able to produce the enzyme in the presence of glucose (Neidhardt, 1960; McFall and Mandelstam, 1963; and Loomis and Magasanik, 1965). Another procedure requires a starting strain incapable of growth on glucose but in which the sugar still exerts repression on other systems (Englesberg, 1959). We wish to report here an autoradiographic method for the detection of catabolite repression resistant colonies. With this technique a mutant of E. coli was obtained which produced high levels of glycerol kinase during glucose dissimilation.

Strain 7 (Koch, et al., 1964), a regulator constitutive de-

---

[1] This investigation was aided by grant GB-3527 from the National Science Foundation and grant GM-11983 from the U. S. Public Health Service.

[2] Research Fellow on leave from the Universidad de Buenos Aires, República Argentina.

[3] Supported by a Research Career Development Award from the U. S. Public Health Service.

rivative of E. coli K12 Hfr C, was used as our initial strain so that further resistance to repression required mutations other than those affecting the specific repressor. After treatment with the mutagen, N-methyl-N'-nitro-N-nitrosoguanidine (Adelberg, et al., 1965), surviving cells were grown for about 10 generations in simple glucose medium to select against auxotrophs and mutants impaired in glucose metabolism. About 1500 cells were sprayed on each agar plate containing: 1×10^{-6} M C^{14}-glycerol (26 curies/mole), 1% glucose, 1% tryptone, Bacto-neutral red (30 mg/L), Bacto-crystal violet (1 mg/L) and the appropriate inorganic components.

Since at low concentrations of glycerol, the rate of utilization of the compound was limited by the kinase (Hayashi and Lin, 1965), a mutant whose enzyme was less sensitive to catabolite repression should be detectable by its more rapid incorporation of the labeled glycerol in the presence of glucose. After 15 hours of incubation at 37°, prints of the colony topography were made with round sterile smooth shelf papers. The bulk of each colony was thus transferred physically to the paper and could be seen as a pink spot. After being dried, the paper was placed face down on an x-ray film and exposed overnight. The developed film was superimposed on the paper with the aid of position markers, and dark dots on the film (see Fig. 1 A) coinciding with pink spots on the paper were noted. The corresponding colonies on the agar were picked and streaked again on C^{14}-indicator plates alongside with cells of the parental strain as controls. Fig. 1 B shows the filter paper print of a mutant streaked in two opposite quad-

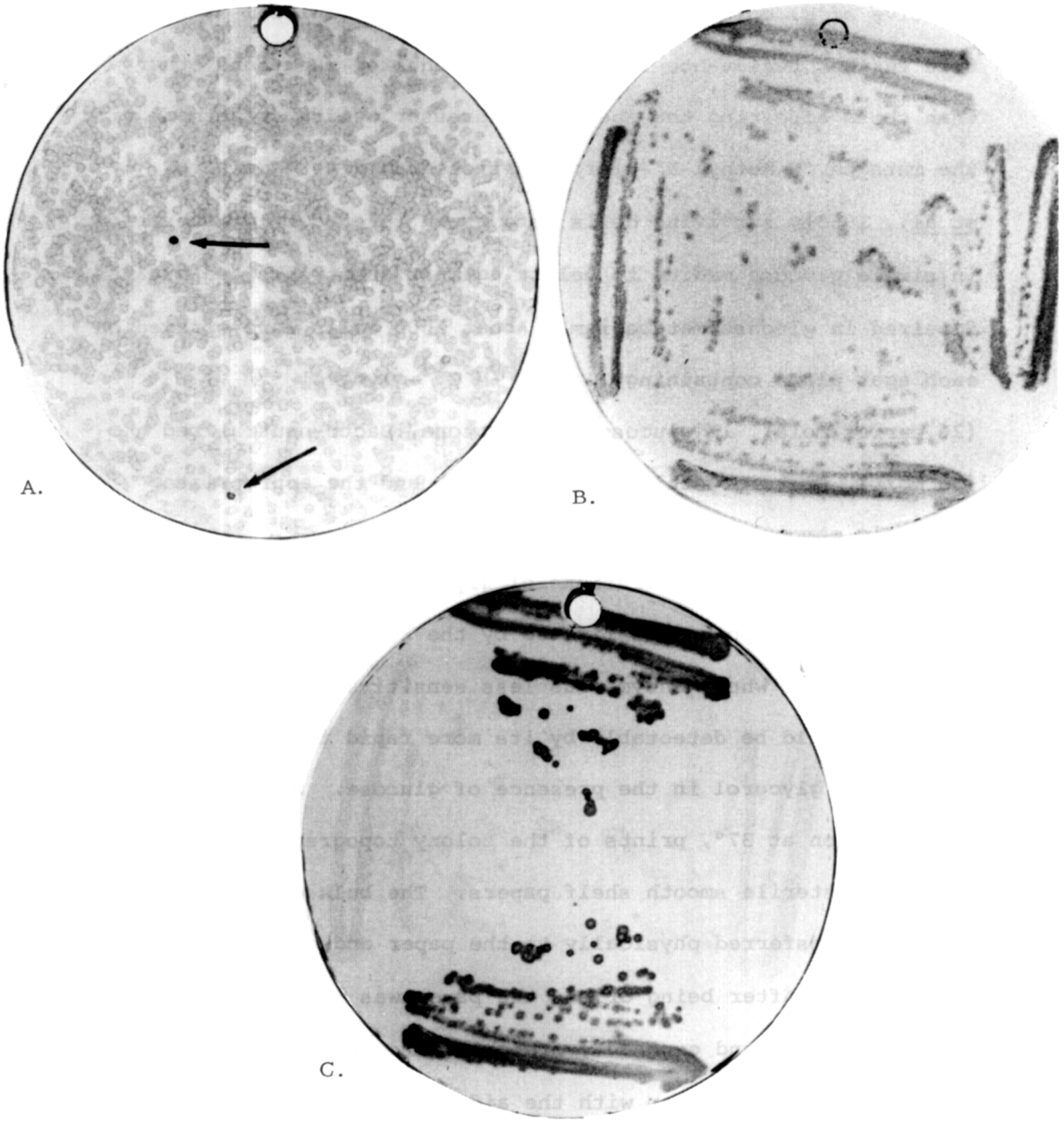

Fig. 1 A. Autoradiograph of colonies grown out from cells subjected to mutagenic treatment. The x-ray film (Kodak Blue Brand) was exposed for 20 days instead of the usual overnight period so that the positions of parental-type colonies could also be rendered visible. Mutant colonies are indicated by arrows. B. Print of restreaked colonies on paper. Mutant cells in top and bottom quadrants and parental-type cells in remaining quadrants. C. Autoradiograph of the print shown in B.

rants with the parental strain in the two remaining quadrants. Fig. 1 C shows the contrast in autoradiography of mutant and parental colonies. In a control experiment where glucose was omitted from the agar, both types of colonies were dark.

To test the repressibility of glycerol kinase directly, mutant (strain 30) and parental (strain 7) cells were grown in the presence and absence of glucose and their extracts assayed for the enzyme. The results summarized in Table I indicate that the kinase was much less sensitive to repression in the mutant. The nature of the genetic changes in this and other phenotypically similar mutants will be described in another communication.

TABLE I

Effect of Glucose on the Formation of Glycerol Kinase*

Strain	Carbon source for growth	Glycerol kinase
		relative activity
7	casein hydrolysate	100**
7	casein hydrolysate + glucose	9
30	casein hydrolysate	102
30	casein hydrolysate + glucose	70

*Conditions for growth and enzyme assay were essentially that described by Lin, et al., 1962. Carbon sources were each added at 1%.

**1.4 μmoles L-α-glycerophosphate formed per min per mg protein at 25°.

The method reported here also distinguished cells which were catabolite repression resistant in their lac operon from those which were not (Loomis and Magasanik, 1965). With appropriate modification, the procedure should be applicable for the detection of any mutant whose uptake or incorporation of a labeled compound is altered.

REFERENCES

Adelberg, E. A., Mandel, M., and Chen, G. C. C., Biochem. Biophys. Res. Communs. 18, 788 (1965).

Englesberg, E., Proc. Natl. Acad. Sci. 45, 1494 (1959).

Hayashi, S. and Lin, E. C. C., Biochim. Biophys. Acta 94, 479 (1965).

Koch,J. P., Hayashi, S., and Lin, E. C. C., J. Biol. Chem. 239, 3106 (1964).

Lin, E. C. C., Koch, J. P., Chused, T. M., and Jorgensen, S. E., Proc. Natl. Acad. Sci. 48, 2145 (1962).

Loomis, Jr., W. F., and Magasanik, B., Biochem. Biophys. Res. Communs. 20, 230 (1965).

McFall, E., and Mandelstam, J., Biochem. J. 89, 391 (1963).

Neidhardt, F. C., J. Bacteriol. 80, 536 (1960).