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Convenient Method for Detecting $^{14}CO_2$ in Multiple Samples: Application to Rapid Screening for Mutants

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A procedure is presented for the rapid screening of bacterial colonies to detect mutants unable to produce $^{14}CO_2$ from a labeled precursor. The method is especially useful for mass screening for mutants that cannot be easily detected by their phenotypic characteristics.

The method described herein permits the rapid screening of many individual bacterial cultures for mutations in pathways producing $^{14}CO_2$ by trapping the $^{14}CO_2$ from each culture on paper saturated with barium hydroxide. This technique, combined with the procedures of Weiss and Milcarek (5) for the rapid isolation and cultivation of separate bacterial colonies in depression plates, permits the rapid screening of large numbers of separate colonies for the presence or absence of a given metabolic pathway producing CO_2. The use of $Ba(OH)_2$ paper takes advantage of the well-known insolubility of $Ba^{14}CO_3$, resulting in the fixation of $^{14}CO_2$ immediately above the colony in which it was formed. All of our studies have been with *Escherichia coli*, but the same method could be used for other bacteria, yeast, or mammalian cells in culture.

The method is as follows. (i) In each well of a Falcon 96-well plate (catalog no. 3040), place 100 μl of an appropriate minimal medium (with the use of a peristaltic pump) containing any necessary supplements and the appropriate ^{14}C-labeled substrate. (ii) Inoculate the plate with organisms grown in another Falcon plate, with the help of the 48-prong stamping device described by Weiss and Milcarek (5). (iii) Immediately cover the plate with filter paper that has been impregnated with $Ba(OH)_2$ and cover this with a Falcon plastic lid (catalog no. 3041). [The paper is prepared by wetting pieces of Whatman 3MM paper (11.7 by 7.5 cm) with saturated $Ba(OH)_2$, drying the papers by blotting with filter paper, and storing the papers in a desiccator over a KOH solution until use. Care must be taken to avoid prolonged contact of the paper with atmospheric CO_2. NaOH or KOH solutions cannot be used to wet the filter paper, as soluble carbonates are formed, which diffuse.] Secure the lid firmly on the dish with rubber bands. (iv) Wrap the plate in aluminum foil to limit evaporation, and incubate the cultures overnight at the desired temperature. (v) Remove the paper and immerse in acetone; remove the acetone by air-drying, followed by heating for 5 min at 110°C. (vi) Place the paper on Kodak RP/R-54 X-ray film for 1 to 7 days, depending on the number of counts used. (For a 24-h exposure, the specific activity of the ^{14}C-labeled substrate added should be sufficient to produce 1,000 dpm of $Ba^{14}CO_3$ above each well.)

A typical radioautogram showing the use of the above method is presented in Fig. 1. In this experiment L-[U-^{14}C]methionine was added to the growth medium to detect mutants deficient in adenosylmethionine decarboxylase. The black spots are above the wild-type colonies, and the gray spots are above the mutants.

To screen for temperature-sensitive mutants, the following modification is suggested. The organisms are grown overnight without labeled substrate at the permissive temperature with limiting glucose (0.02%). Additional glucose is then added (it can be added with the stamping device used above, transferring ca. 3 μl of 20% glucose per well). The plate is then incubated at the permissive temperature for 1 h and at the nonpermissive temperature (e.g., 43°C) for 30 to 60 min; radioactive substrate (5 μl) is then added to each well with a repeating Hamilton syringe, and the plate is immediately covered with a piece of $Ba(OH)_2$-impregnated paper. After further incubation for 2 h at the nonpermissive temperature, a radioautogram is prepared as described above.

This method can also be used to assay a reaction that does not produce $^{14}CO_2$, provided that the product of the reaction can be coupled to another reaction that does produce $^{14}CO_2$. For example, during growth on [*guanido*-^{14}C]arginine, [^{14}C]urea is produced by the sequential action of arginine decarboxylase and

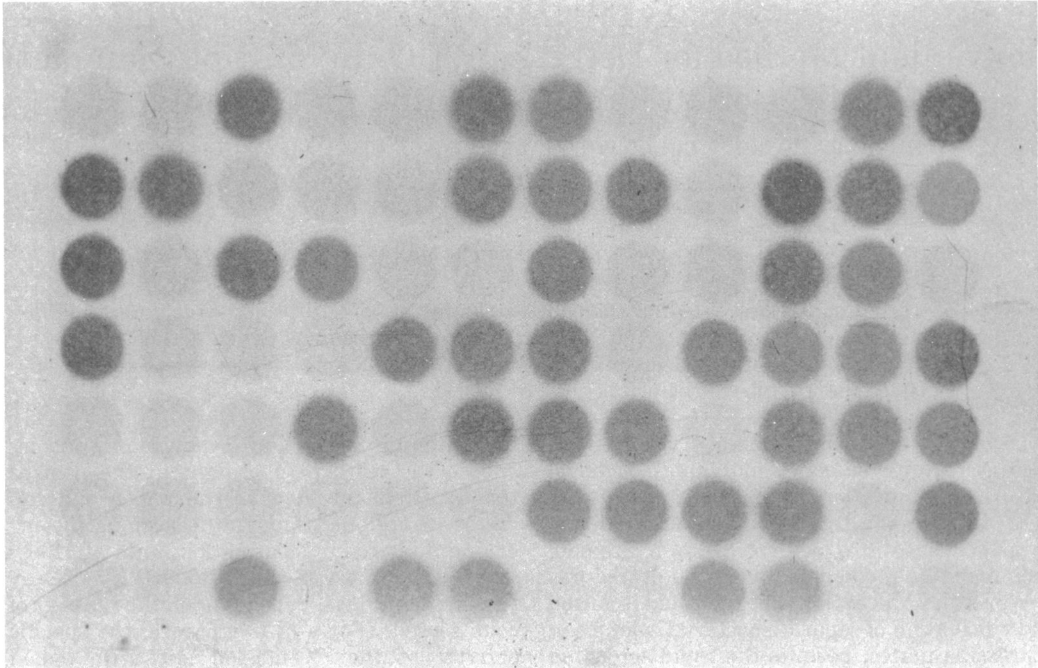

FIG. 1. *Radioautogram illustrating the results with E. coli cultures that produce $^{14}CO_2$ from L-[U-^{14}C]methionine (black spots) or that are deficient in this capability (light spots) because of a deficiency in adenosylmethionine decarboxylation. The culture medium contained 67 nmol of L-[U-^{14}C]methionine (specific activity ≅ 2,000 dpm/nmol) per ml; incubation was at 37°C overnight. Exposure of the filter paper to the X-ray film was for 1 week. The formation of $^{14}CO_2$ is a result of two reactions, i.e., the conversion of L-[U-^{14}C]methionine to adenosyl-[U-^{14}C]methionine and decarboxylation of the latter compound (6). The mutant strain had a 95% decrease in adenosylmethionine decarboxylase activity. In other experiments, higher-specific-activity methionine was used, and overnight exposure of the radioautogram was sufficient. (Details of these studies will be reported separately [manuscript in preparation].)*

agmatine ureohydrolase, and the [^{14}C]urea is excreted into the medium (2, 3). If urease is added to the growth medium, $^{14}CO_2$ is formed (2). We have used this method for detection of strains containing known mutations in agmatine ureohydrolase and have obtained patterns similar to those seen in Fig. 1.

The method has also been used for the qualitative assay of a reaction producing $^{14}CO_2$ in vitro. For this purpose, extracts are prepared in each well by either the lysozyme-ethylenediaminetetraacetic acid method of Weiss and Milcarek (5) or by treating the cells with toluene (4). With the latter technique, we have assayed for ornithine decarboxylase in ornithine decarboxylase-deficient mutants (1) in transduction experiments, with results similar to those in Fig. 1. This method may also be convenient for monitoring column eluants during the purification of any enzyme producing $^{14}CO_2$ either directly or in coupled reactions.

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LITERATURE CITED

1. **Cunningham-Rundles, S., and W. K. Maas.** 1975. Isolation, characterization, and mapping of *Escherichia coli* mutants blocked in the synthesis of ornithine decarboxylase. J. Bacteriol. 124:791–799.
2. **Morris, D. R., and K. L. Koffron.** 1967. Urea production and putrescine biosynthesis by *Escherichia coli*. J. Bacteriol. 94:1516–1519.
3. **Morris, D. R., and A. B. Pardee.** 1966. Multiple pathways of putrescine biosynthesis in *Escherichia coli*. J. Biol. Chem. 241:3129–3135.
4. **Moses, R. E.** 1974. DNA synthesis in toluene-treated cells of *Escherichia coli*, p. 135–149. *In* R. B. Wickner (ed.), Methods in molecular biology, vol. 7, DNA replication. Marcel Dekker Inc., New York.
5. **Weiss, B., and C. Milcarek.** 1974. Mass screening for mutants with altered DNases by microassay techniques, p. 180–193. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. XXIX, part E, Nucleic acids and protein synthesis. Academic Press Inc., New York.
6. **Wickner, R. B., C. W. Tabor, and H. Tabor.** 1970. Purification of adenosylmethionine decarboxylase from *Escherichia coli* W: evidence for covalently bound pyruvate. J. Biol. Chem. 245:2132–2139.