Radiometric Method for the Detection of Coliform Organisms in Water

 URIEL BACHRACH and ZELILAH BACHRACH

Department of Molecular Biology, Hebrew University, Hadassah Medical School, Jerusalem, Israel

Received for publication 11 March 1974

A new radiometric method for the detection of coliform bacteria in water has been described. The method is based on the release of $^{14}$CO$_2$ from [14C]lactose by bacteria suspended in growth medium and incubated at 37 C. The evolved $^{14}$CO$_2$ is trapped by hyamine hydroxide and counted in a liquid scintillation spectrometer. The method permits the detection of 1 to 10 organisms within 6 h of incubation. Coliform bacteria suspended in water for several days recover from starvation and may be quantitated by the proposed method. Bacteria from water samples may also be concentrated by filtration through membrane filters and detected by the radiometric assay.

MATERIALS AND METHODS

E. coli B was grown in synthetic M9 medium (1) to a turbidity corresponding to $10^8$ cells per ml. A sample of this culture was diluted (1:50) in sterile tap water and tested immediately or after keeping at room temperature for several days. The standard test was carried out as follows. To a sterile test tube (9.5 by 1.5 cm) the following materials were added: [14C]lactose, 0.1 ml (1 µCi/ml), 20 µCi/µmol, The Radiochemical Centre, Amersham, England), water sample (or membrane filters), 0.1 ml; nutrient broth (Difco) containing 2 × 10$^{-8}$ M lactose; and 0.001% bromophenol blue (pH 6.0 to 6.2), 0.2 ml. Tubes were immediately sealed with a rubber stopper supporting a polyethylene center well ( Kontes Glass Co. no. 88230) and shaken at 37 C in a water bath. At desired times, 0.2-ml quantities of hyamine hydroxide (Packard) were injected into the plastic center wells and incubation was continued for another 15 min. The reaction was finally stopped by injecting 0.2 ml of 0.5 N HCl into the reaction mixture; samples were agitated for an additional 15 min, to allow complete absorption of the evolved CO$_2$. The center well was removed, placed in a vial containing 10 ml of scintillation fluid (2), and assayed for radioactivity in a liquid scintillation spectrometer.

Bacteria were usually diluted in nutrient broth (Difco) containing 2 × 10$^{-8}$ M lactose and counted by plating on nutrient agar (Difco). Bacteria from natural environments and from artificial systems were also collected by filtration through membrane filters (HAWG, 047A0, HA 0.45 µm pore size, 47 mm, Millipore Corp.) and analyzed.

RESULTS

Preliminary studies indicated that bacteria suspended in buffer metabolized [14C]lactose very slowly. This finding can easily be explained by the low concentration of [14C]lactose in the reaction mixture, which did not permit induc-
tion of β-galactosidase. It has been well established that the uptake and metabolism of lactose by E. coli are subject to stringent regulatory mechanisms and that β-galactosidase can also be induced by IPTG (isopropyl-β-D-thiogalactopyranoside). We therefore tested the effect of this inducer on the metabolism of [14C]lactose by E. coli and showed that the fermentation of this compound increased markedly when cells were first incubated with 5 × 10^{-4} M IPTG. Best results were obtained when the incubation mixture contained nutrient broth instead of fermentation broth; this facilitated the multiplication of bacteria and rendered the test more sensitive. In subsequent experiments β-galactosidase was also induced by increasing the molar concentration of lactose in the nutrient broth medium. It has been shown that the addition of unlabeled lactose to the nutrient broth (at a final concentration of 2 × 10^{-5} M) caused β-galactosidase induction and the subsequent release of 14CO2 from the radioactive lactose. In some experiments CO2 was trapped by 15% KOH, but finally hyamine hydroxide was used because of better recoveries and reproducibilities. Since vapors of hyamine hydroxide may be toxic for bacteria, this trapping agent was injected into the center well at the end of the incubation period. In similar experiments, we found that best results were obtained when the pH of the nutrient broth was brought to 6.0 to 6.2. This was usually done by adding bromophenol blue as an indicator and adjusting the pH to the indicator inversion point. After finding the optimal conditions for the assay, we tested the effect of incubation time on the formation of 14CO2 from radioactive lactose by using a constant number of E. coli cells (8.6 × 10^6 cells per test tube). It may be seen (Fig. 1) that E. coli cells, at that concentration, released significant amounts of radioactive CO2, even after 15 min of incubation. The accumulation of 14CO2 progressed with incubation time in a linear fashion (Fig. 1).

To test the sensitivity of the method, a suspension of E. coli cells was diluted in lactose-nutrient broth and the various dilutions were assayed as described in the preceding paragraph, at various times. Figure 2 shows that 10^6 coliform cells may be detected after incubation with [14C]lactose for at least 2 h. At least 3 h of incubation are required for the detection of 10^8 cells, whereas 1 to 10 coliform bacteria form significant amounts of 14CO2 after 6 h of incubation. In the aforementioned experiment, freshly grown bacteria were employed. It is to be expected that in natural water sources starved E. coli cells may be encountered. This is mainly due to the lapse of time between the excretion of the organisms in the feces and their recovery from the water sample which obviously lacks nutrients. It was therefore decided to mimic the conditions which prevail in nature, and to test the activity of the bacteria after keeping in tap water for several days. The results of this experiment are illustrated in Fig. 3, which shows that E. coli cells starved at room temperature for 3 and 6 days were still capable of fermenting [14C]lactose—similar to freshly grown bacteria. Moreover, when the various preparations were incubated with [14C]lactose for 60 min, starved cells caused the release of slightly higher amounts of 14CO2, although the number of viable cells decreased by a factor of 0.5 to 1.0 log unit.

Because of the scarcity of coliform organisms in water, they are usually recovered by filtration of large volumes of fluid through membrane

---

**Fig. 1.** Effect of incubation time on the release of 14CO2 from radioactive lactose. (8.6 × 10^6 coliform organisms were used per test tube.)

**Fig. 2.** Release of 14CO2 from radioactive lactose after incubation with various numbers of coliform organisms. Coliform organisms—10^6 per test (●●●); 10^5 per test (○○○); 10^4 per test (△△△); 10^3 per test (□□□); 10^2 per test (□□□); 10 per test (●●●); 1 organism per test (○○○).
From the text, it seems to discuss a method for detecting coliform organisms in water using radioactive lactose. The method involves growing bacteria in radioactive lactose, detecting the radioactive lactose after incubation, and using this to count bacteria. The text mentions the use of filters and radioactive counting. The discussion section suggests that this method provides an important tool for early detection of fecal organisms in water, and it is used in laboratories for various organisms. The text also thanks others for their assistance in the experiments.