Rapid Detection of Bacterial Growth by Infrared Spectrophotometry

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A rapid and sensitive method based on measurement of carbon dioxide released from a culture medium as a result of bacterial metabolism of glucose is reported. The rate of release of carbon dioxide was measured by recording the absorbance at 2353 cm^{-1} (4.25 μm) against time using a standard infrared spectrophotometer. Detection of growth from only five organisms of *Salmonella typhosa* was significant after just fifteen minutes. In about three hours a clear pattern of growth was obtained by this method. The results are reproducible with a standard deviation around ±0.5%.

Index Headings: **Rapid detection; Bacterial growth; Infrared spectrophotometry.**

INTRODUCTION

The methods for detecting and identifying micro-organisms on the basis of their biological activities are highly specific and sensitive, and a single cell can be detected. By these methods, micro-organisms are studied during their growth by monitoring the increase in their mass or number and by monitoring the evolution of their metabolic products. Several systems exist that measure such changes continuously.[1-6] One of these techniques involves the radiometric measurement of radio-labeled CO_2 liberated from growth medium containing ^{14}C-labeled glucose as the substrate. This is a rapid technique, but the radio-labeled sugars are not available in an ordinary laboratory. Moreover, it requires specialized instrumentation and equipment to measure the radioactivity. It would be desirable to develop a method by which the use of other CO_2-measuring instruments could be made to monitor the bacterial growth. It is well established that an ordinary infrared (IR) spectrophotometer can easily measure CO_2 at levels down to <0.5 ppm with high precision.[7] In the present work we have exploited this potential of the IR technique and have developed a method for the rapid detection of bacterial growth by monitoring the liberated CO_2 at 2353 cm^{-1} (4.25 μm).

MATERIALS AND METHODS

Approximately five organisms of *Salmonella typhosa* (from human infection) were transferred aseptically into a sterile culture vial containing glucose and enriched tryptic soy broth, type 6B (pH 7), for aerobic culture (Johnston Labs, Inc.). The number of organisms was established by the serial dilution technique. This vial and a control were incubated at 37°C with agitation. The control vial contained only the culture medium. The rate of CO_2 released by metabolic activity was recorded by measuring the absorbance at 2353 cm^{-1} (4.25 μm) using a double-beam Perkin-Elmer 882 IR spectrophotometer at 30-min intervals. A continuous scan was also obtained by using the "time drive" mode of the spectrophotometer. The concentration of carbon dioxide increases with an increase in bacterial growth, which is indexed by the absorbance recorded at 2353 cm^{-1}. Thus the absorbance measurement will be a direct measure of bacterial growth. The culture vial was connected to the IR gas cell (pathlength 10 cm) by means of Tygon tubing to form a closed system, as shown in Fig. 1. Measurements were taken for six samples of the same species from the same dilution to establish the reproducibility.

In order to compare the efficiency of this method with that of the radiometric technique, again five organisms of the same species were cultured under the same conditions as above and monitored as reported previously.[4] Radioactivity was measured with a commercially available automatic counter, BACTEC Model 460. This method is based on the detection of $^{14}CO_2$ evolved by bacterial metabolism of ^{14}C-labeled glucose in the medium. The radioactivity is translated to the index of growth. Comparison of the growth patterns obtained by the two techniques was made on a time scale.

RESULTS AND DISCUSSION

All the samples studied produced readily detectable quantities of CO_2. The initial rate of release of the gas

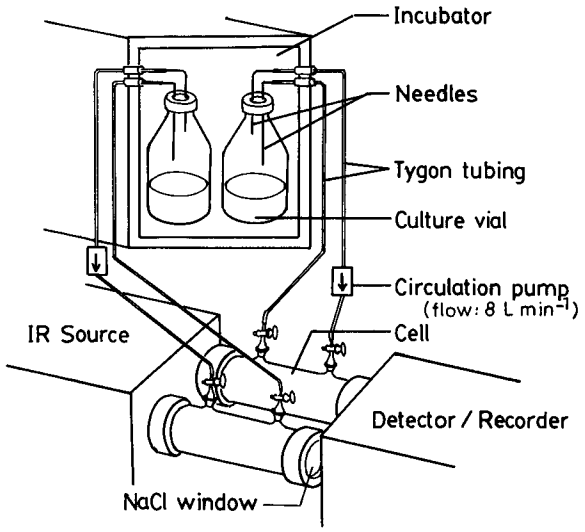

FIG. 1. Schematic diagram of the system.

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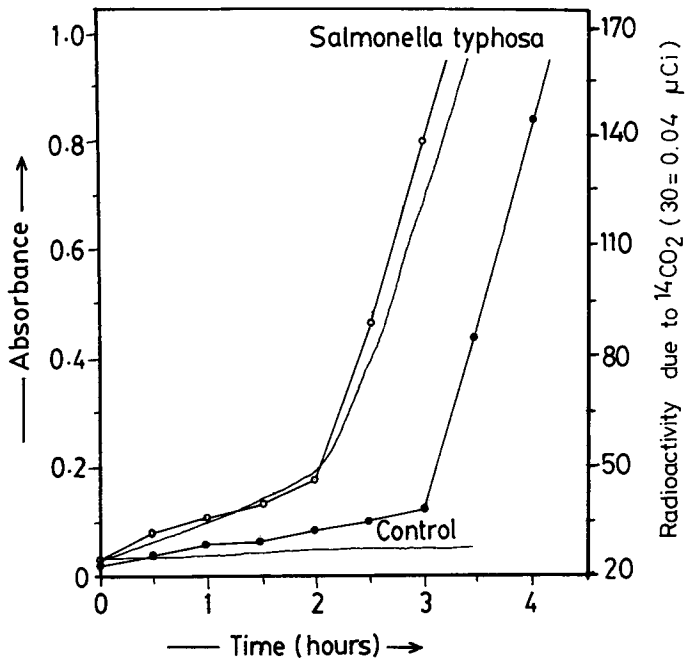

FIG. 2. Detection of CO_2 released from the cultures of *Salmonella typhosa*. (——) Trace obtained in the "time drive" mode; (—O—) Plot of absorbance vs. time; each point is a mean of six measurements. (—●—) Plot of radioactivity vs. time; each point is a mean of six measurements.

was relatively low, increasing sharply after two hours (Fig. 2). Detection of growth from five organisms of *Salmonella typhosa* was significant after just 15 min. A clear pattern of growth was obtained in 3 h. The standard deviation in absorbance measurements after ½-hour interval for six samples of the same species was found to be around ±0.5%. The internal precision of the spectrophotometer was 0.2%. The traditional methods usually require 1–2 days for significant detection of the growth of bacteria. The IR and the radiometric methods produced comparable results (Fig. 2), except that the former allowed early detection of growth.

Usually the release of CO_2 is affected by the change in pH of the media with time. It has been observed in the case of thioglycolate broth that the pH remains constant for 5 h.[4] In our case it remained unchanged for about 6 h. Keeping in mind these observations and assuming that such changes may start taking place after 4 h, we can infer that the results obtained by our method will not be affected because of its rapidity. The standard infrared instruments are usually double-beam; therefore, the measurement and procedural errors are automatically cancelled out by recording the absorbance of the sample and the control simultaneously. This is evident from the control curve in Fig. 2, which is almost parallel to the time axis. By making use of the "time drive" facility of the Perkin-Elmer 882 IR spectrophotometer (and most of the other microprocessor-controlled IR instruments) we obtained an automatic record of CO_2 released against time, as shown in Fig. 2. This is achieved by keeping the cells in the path of the sample and reference beams and the vials in the incubator, as shown in Fig. 1, while recording the absorbance at a fixed wavenumber against time.

We have demonstrated that the newly developed IR method is more sensitive and rapid than most of the available techniques. The sensitivity could be further enhanced by the use of long-path cells. An early detection of bacterial growth by this method will solve the problems associated with very sensitive organisms which are usually lost before they can be detected because of the long time periods involved in conventional detection methods. Its high sensitivity will also help to detect growth in samples such as breath gas,[8] which are small in size and carry fewer bacteria.

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