Simple, Reliable Cold Tray for the Recovery and Examination of Thermosensitive Organisms

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A simple chamber is described for the isolation and handling of thermosensitive bacteria on board ship and in conventional research or classroom laboratories.

Many investigators have examined marine habitats for the occurrence of thermosensitive microorganisms and often with varied results (1). ZoBell and Conn (8) and others (3, 5, 6) have shown that increased recovery rates of these organisms are often possible if the temperature of the primary isolation media is lowered, and numerous studies have been made demonstrating the extreme sensitivity of psychrophiles (1, 5, 6) and their enzyme systems (2, 4, 6) to temperatures of 15°C and above.

However, in many laboratories, on most ships, and in some field locations adequate cold room facilities are not available for processing and subsequent examination of thermosensitive bacteria. In addition, when cold rooms are available, they often become uncomfortable during long term use by investigators and may require the incorporation of specially designed isolation hoods or cabinets because of the aerosols of bacteria and fungi which are kept in suspension by air circulation systems.

During a recent Antarctic cruise (USNS Eltanin, cruise no. 38) we isolated large numbers of bacteria that grew well at 3°C. To examine, count, and isolate colonies, we developed a simple, reliable device for maintaining agar plates and tubes at less than 6°C while working with them in a warm (20°C or higher) shipboard laboratory. Subsequently we have found this device useful in our university laboratory for manipulation of additional thermosensitive bacterial isolates in other studies.

The isolates and original plates containing bacteria from the Polar Sea were shipped to our university laboratories in ice-filled freezer chests. These chests were re-iced at stopover points between Melbourne, Australia, and Athens, Georgia, and the temperature inside the chests was never observed to rise above 7.0°C. Preliminary examination of the 488 isolates (Table 1) indicates that 37.5% of these organisms will not grow at 15.0°C, whereas 63.5% will not survive at 20.0°C.

Although we do not have data comparing the isolation of the organisms with techniques other than the cold-tray procedure, we do feel that the high recovery rate of organisms growing below 15.0°C is at least indicative of its success. We have subsequently attempted to compare the use of the cold tray to procedures which use prechilled media, and we have found little difference in the two techniques when only a few plates or cultures must be examined. However, where many samples must be plated or a number of cultures examined, we have found that use of the cold tray allows for substantially higher recovery rates and fewer nonviable cultures than can be achieved with prechilled media alone. These results appear to occur randomly and are difficult to explain, but we find that often prechilled media warm and thus may kill susceptible organisms, whereas the cold tray maintains both culture and plating media at low temperature.

In testing the efficiency of the cold tray in maintaining working temperatures below 10°C, we have monitored the temperature of the stainless-steel plate surface and also various media routinely in use with a thermocouple (YSI). Temperatures on plate surfaces and in media remained stable (±0.5°C) for at least 4 hr, at which time the ice had sufficiently melted to allow the temperature to rise. Prechilled tubes and plated media quickly equilibrated and were maintained at 5.0 ± 0.25°C over the 4-hr period, whereas the stainless-steel plate was 1.25 ± 0.25°C over most of the surface. We did notice a temperature gradient near the outlet of the tray, but the temperature never rose above 7.0°C during the 4-hr period.

The present chamber consists of a tray 10 cm deep, 61 cm wide and 76 cm long (Fig. 1). Small chunk or preferably crushed ice is placed in a 5-cm deep layer, and a stainless-steel plate, ap-
TABLE 1. Growth range for Polar Sea isolates

| Depth (m) | No. of isolates growing at |
|-----------|---------------------------|
|           | 4 C | 15 C | 20 C |
| 2-10      | 116 | 91   | 66   |
| 200-400   | 123 | 63   | 30   |
| 1,000-1,500 | 128 | 76   | 49   |
| >3,000    | 121 | 74   | 33   |
| Total     | 488 | 304  | 178  |
| Per cent  | 100 | 62.5 | 36.5 |

FIG. 1. Cold tray used for the examination of marine thermosensitive microorganisms.

proximately 2 mm thick, 56 cm wide and 71 cm long, is placed on the ice surface. Any convenient size tray may be used, provided it is sufficiently deep and equipped with a small drainage hole. It is advisable to leave at least a 5-cm space above the metal plate to create a cold air pocket; this greatly reduces the warming of the petri dish lids. After the chamber has cooled, prechilled media can be placed agar side down in the metal surface and up to 30 (10 cm diameter) petri dishes can be accommodated with ease.

We experienced no difficulty in using a chamber such as this on the USNS Eltanin; even in rough weather, the petri plates did not slide on the metal plate. Sliding of petri dishes might present a problem on a ship with a quick roll but most large oceanographic vessels should be sufficiently stable.

One minor technical problem concerning the cold chamber should be noted. Marks made on glass petri dishes with a felt-tip marker pen often came off as the plates were handled. This problem was eliminated by placing small strips of masking tape on dry glass petri dishes and marking the tape surface. Such markings are not removable from plastic petri dishes since a permanent bond is formed between the marking pen ink solvent and the dish plastic.

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