NOTES

Effect of Incubation Conditions at 55 C on Moisture Loss from Agar Plates

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The percentage of moisture loss was least from plates incubated in plastic bags, sealed jars, or in a humid chamber. There was a stacking effect noted in plates incubated in plastic bags and sealed jars.

Standard methods for the microbiological analysis of dairy products and for dairy purposes are being drawn up in Australia, and a method for counting thermophiles in dairy products is among those being standardized. In dairy microbiology the thermophilic bacteria are considered those which have an optimum growth temperature above 50 C (3, 5). They are usually counted by incubating poured plates at 55 C for 1 to 3 days (2–5). Incubation of agar plates at 55 C presents special problems because of the rapid moisture loss that can occur at this temperature (1). Greater than 15% moisture loss from plates is considered unsatisfactory for most incubation procedures (3).

Methods suggested to minimize or counteract moisture loss during incubation at high temperatures include increasing the volume of agar (3, 4), placing a beaker of water in the incubator (2), incubating plates in a sealed jar, and wrapping plates tightly with plastic film (1).

In the experiments reported here, moisture loss was measured under different incubation conditions over the times of incubation usually employed in dairy microbiology. The conditions compared were: (i) no special precautions to prevent moisture loss; (ii) a 250-mL beaker of water in the incubator; (iii) a tray of water in the incubator; (iv) plates in plastic bags sealed with adhesive tape; (v) plates in sealed jars (anaerobic jars); (vi) plates in a humid chamber (over water in a desiccator).

Treatments (i) to (iv) were replicated in two incubators, one with and one without a small circulating fan, and treatment (v) was examined in the incubator without a fan. Moisture loss was measured from plates containing 10 and 20 mL of agar under these conditions. The incubation periods were 1, 2, and 3 days. In treatment (vi), plates containing 20 mL were incubated for 2 days only in the incubator without a fan.

The procedure adopted was as follows. The agar solution (1.5%) was cooled to 48 C, and 10 or 20 mL was dispensed into preweighed plastic petri dishes. After cooling, the lower part of the dish containing the agar was weighed, and its lid was replaced. Plates were then inverted and stacked for incubation.

For each incubation condition (treatments i to iii), for each incubator, and for plates containing either 10 or 20 mL, a total of 27 plates was used, comprising nine stacks of three plates arranged on three shelves in the incubator, three stacks per shelf. One plate was taken from alternate positions within the stacks after 1, 2, and 3 days, i.e., nine plates per day for each treatment per incubator. Plates were stacked six high in plastic bags with three bags per shelf. One bag was removed from each shelf on each day, i.e., 18 plates per day. In treatment (v), three jars each containing 15 plates were incubated, and one jar was removed each day. In treatment (vi), 20 plates were incubated on two occasions. In all treatments plates were cooled while inverted, and the dishes were weighed without lids and discarded.

Moisture loss was determined by difference and expressed as a percentage of mass dispensed.

The percentage of moisture loss was greater from plates containing 10 mL of agar than from plates containing 20 mL under the first four conditions of incubation (Table 1). Moisture loss after 3 days of incubation did not exceed 15% when plates were enclosed in sealed jars or plastic bags, regardless of agar volume. Incubation for 2 days in a sealed jar or a humid chamber resulted in the least moisture loss when compared with other incubation conditions for 2 days. Moisture loss after 3 days was also less than 15% in 20-mL plates incubated over a tray of water (Table 1), but the use of a tray was unsatisfactory because of excessive condensa-
TABLE 1. Effect of different incubation conditions in two incubators on mean percentage of moisture loss from agar plates

| Conditions          | ml/plate | Moisture loss (%) |           |           |           |
|---------------------|----------|-------------------|----------|----------|----------|
|                     |          | No fan            | Fan      | Fan      | Fan      |
|                     |          | 1* 2 3            | 1 2 3    | 1 2 3    |
| No water            | 10       | 20.2 40.9 60.0    | 23.8 48.2 78.3 |   |
|                     | 20       | 11.3 18.5 31.0    | 14.2 27.6 42.4 |   |
| Beaker of water     | 10       | 19.0 35.1 50.3    | 18.7 44.6 68.6 |   |
|                     | 20       | 9.1 17.8 29.0     | 12.8 24.4 40.1 |   |
| Tray of water       | 10       | 6.9 14.0 20.4     | 9.8 20.4 19.8 |   |
|                     | 20       | 5.9 7.4 10.0      | 7.0 10.9 10.0 |   |
| Plastic bags        | 10       | 5.2 8.1 10.4      | 6.2 8.5 13.6 |   |
|                     | 20       | 2.5 4.4 5.8       | 3.6 5.6 6.1 |   |
| Sealed jars         | 10       | 3.9 4.1 4.3       |   |   |   |
|                     | 20       | 2.2 2.5 2.8       |   |   |   |
| Humid chamber       | 20       | 2.5               |   |   |   |

* Number of days.

Moisture loss was greater than 15% in all other treatments.

The absolute moisture loss from plates depended on incubation conditions and not on agar volume.

Moisture loss was greater from plates in the incubator with a fan than in the incubator without a fan.

There was a stacking effect in plates incubated in plastic bags (Table 2) and in plates incubated in sealed jars. Moisture loss was greater from the top and bottom plates in the stack than from those plates in the center. Plates containing 10 ml, at the top of the stack in plastic bags, had lost in excess of 15% moisture after 2 and 3 days of incubation on some occasions. No plates in sealed jars lost in excess of 10% under the same conditions. This stacking effect may be due to differences in temperature between the agar in the plate and the plate lid. It is suggested that when the stack is first placed in the incubator, the top plate heats up more quickly than the plate immediately under it. Because the lid of the top plate is next to the cooler agar of the second plate, moisture from the hot plate condenses on the cool lid. The reason for high moisture loss from the bottom plates in the stack is unknown but may be due to moisture condensing on the lid of the petri dish while the stack is sitting on the bench and cooling, after being removed from the incubator. It is also evident that the polythene plastic bags used (those in which the sterile plates came) were permeable to water vapor.

It is concluded that plates containing 10 ml of agar can be incubated at 55 C in humid chambers, sealed jars, or in plastic bags to keep moisture loss within acceptable limits. For incubation times in plastic bags in excess of 1 day the top plate in the stack should be a blank.

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