A Research Note

Evaluation of Four Media for Recovery of Heat-Stressed Streptococci

C. A. MAGNUS, A. R. McCURDY and W. M. INGLEDEW*

Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0

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ABSTRACT

Two strains of Streptococcus faecium and one strain of Streptococcus faecalis were subjected to heat stress in a ham broth and recovered on all purpose tween agar; deMan, Rogosa, Sharpe agar; tryptone-dextrase-yeast extract-meat extract-peptone agar; and tryptic soy agar. Survival curves for the organisms recovered on each agar were constructed and D values (death rates) were calculated. Differences in death rates were noted for each organism between different media. The greatest recovery of cells that had received sub-lethal heat treatment occurred using all purpose tween agar.

Bacterial spoilage (souring and greening) of semi-preserved cured hams is often caused by a number of species of hetero- and homofermentative Lactobacillus spp. and group D Streptococcus spp. Streptococcus faecium and Streptococcus faecalis are commonly isolated, and both are quite heat resistant (5,9,11,12). These microbes have now been studied in buffers, ham broth and in a simulated industrial process, and comparative heat resistance data has been reported (11,12).

The ability of a micro-organism to withstand thermal stress is markedly affected by environmental influences (7), and numerous reviews have been written on factors affecting thermal resistance (1,2,6,17). Some of the factors which influence the resistance of a particular organism in a specific environment include conditions of incubation, composition of growth and recovery media, physiological age of the culture and experimental technique.

The recovery of heat-stressed organisms has also been widely investigated (14,15). Three features of sub-lethal injury that affect the recovery of heat stressed microorganisms by normal enumeration procedures include increased lag time in optimal medium, the delay or complete inhibition of microorganisms in nutritionally insufficient medium, and the prevention of growth on selective culture media (2,14,16). Sub-lethally damaged cells are quite capable of growth but only after incubation in a suitable environment during which repair may occur. The damaged cells often regain the characteristics of the undamaged organisms.

To study the thermal resistance of micro-organisms, the most suitable medium for recovery of the heat-stressed organisms has to be carefully chosen in order to maximize the numbers of cells capable of repair and recovery and in order to obtain reliable recovery data that would approach the recovery which could be obtained in a food system. In this study, four media which have often been recommended for the growth of such microbes were evaluated for their ability to support the recovery of heat-stressed Streptococcus faecium and Strept. faecalis cells.

MATERIALS AND METHODS

Cultures

Streptococcus faecalis P-2A and Streptococcus faecium P-1A were isolated from commercially processed hams produced in Saskatchewan (11). Streptococcus faecium E-20 was obtained from Dr. J. H. Houben (Utrecht, Holland).

Media

Four nutritionally-rich media were chosen for this study. All purpose tween (APT) (Difco) and deMan, Rogosa and Sharpe (MRS) (Oxoid) were selected as they are designed to satisfy the complex nutritional requirements of lactic acid bacteria. Tryptone-dextrase-yeast extract-meat extract-peptone agar (TDYMP consisting of per L: 15 g tryptone, 3 g meat extract, 5 g yeast extract, 1 g dextrose, and 1.5 g peptone milk - pH 7.0) was recommended by Dr. J. H. Houben as a medium to enhance recovery of streptococci (9). Tryptic soy agar (TS) (Difco) was selected because it is a medium which can be used for the cultivation of fastidious organisms like streptococci, and it had been used in earlier work as a recovery medium (3).

Experimental conditions

The three organisms were cultured and subjected to heat stress following the method outlined by Magnus et al. (11). This "attemperated dilution blank method" (13,18) allowed the continuous heat treatment of a known concentration of microorganisms with the withdrawal of samples at definite intervals.
Heat up time in such a system is almost instantaneous, and death can be followed over four to five logarithms. One-ml portions of the prepared bacterial suspension of approximately 10^10 cells/ml were micropipetted into 99 ml of the menstrum, mixed vigorously for 5 sec and the bottle was immediately resubmerged into the water bath (Haake D3-19 circulator monitored in a second flask with a thermocouple connected to a Cole Parmer Model 850 Z digital display) set at 66°C for both *S. faecium* E-20 and *S. faecium* P-1A, and at 60°C for *S. faecalis* P-2A. Variations in the temperatures of the bottles under operating conditions were always less than 0.5°C; subsequent sampling was done without removing the bottle from the bath.

RESULTS AND DISCUSSION

Figure 1a, 1b, and 1c shows the survival curves of each organism as obtained after the recovery of the heat-stressed micro-organisms on each of the four tested media. In all cases, greatest survival was obtained when the heat-injured cells were recovered on APT agar. Table 1 gives the D values obtained for each reported survival curve. The D value is a measurement of the heat resistance -more precisely the time in minutes at a constant temperature to bring about one decimal reduction (90% reduction) of the microorganism present. The D values obtained for organisms recovered on APT agar were greater than those D values obtained on the other tested media. *S. faecium* E-20 had a D value of 7.3 min when exposed to 66°C (D_66) and recovered on APT agar, compared to a D_66 of 4.0 min when MRS was used as the recovery medium. This demonstrates dramatically how the choice of a medium can influence the results and interpretations which can be gained from otherwise excellent results. Similar differences in D values were noted for the other two organisms tested. D values for the organisms recovered on APT agar are higher because more viable (but stressed) micro-organisms were able to repair themselves, and effectively reproduce better on this medium than on the other three. TDYMP and TS agars show lesser degrees of recovery while MRS agar provided the least desirable conditions for recovery. This is in part due to the lower pH of the latter medium (pH 5.2) as heat stressed organisms are more sensitive to other environmental stresses, pH being one example (10).

KF agar and m-Enterococcus agar, the traditional media recommended for detection and enumeration of the faecal streptococci (4) were not included in this recovery study. Hoadly and Cheng (8) have found that these media effectively hindered recovery of injured bacteria. They also found that while recoveries of pure untreated cultures of *Streptococcus faecalis* on KF agar and m-Enterococcus agar were comparable, counts on these two media were still less than 50% of counts obtained on tryptic soy agar.

This study provides additional support that the medium for recovery of injured bacteria must be carefully evaluated in order to obtain valid cell enumeration data and information on the effect of heat on the death of such microbes. The choice of a nutritionally rich, non-selective recovery medium is critical when determining the most

| Table 1. D values (min) of heat-stressed organisms recovered on each of 4 test media. |
|-----------------------------------------------|--------|--------|--------|
| S. faecium E-20 (66°C)                        | 7.3    | 7.0    | 4.0    | 5.0    |
| S. faecium P-1A (66°C)                        | 7.5    | 7.5    | 5.8    | 6.6    |
| S. faecium P-2A (60°C)                        | 6.4    | 5.9    | 4.0    | 4.9    |

Figure 1. Survival curves for *Streptococcus faecalis* P-2A (1a), *Streptococcus faecium* E-20 (1b), and *Streptococcus faecium* P-1A (1c) employing four different recovery media.
accurate possible thermal resistance characteristics of a particular organism in a specific environment. The experimentally derived survival curves and subsequent calculation of the death rate (D value) and thermal death times all rely on reliable cell counts. In essence, by using the best medium possible for cell recovery, the most accurate data is obtained which can then be extrapolated for industrial use. These values are used to determine the processing parameters for a particular food - in this case canned ham. Misleading recovery data could lead to underprocessing of a food resulting in food spoilage and economic loss.

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REFERENCES