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NOTES

Tolerance of Bacteria to High Concentrations of NaCl and Glycerol in the Growth Medium

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When compared at similar levels of water activity, glycerol was more inhibitory than sodium chloride to relatively salt-tolerant bacteria and less inhibitory than salt to salt-sensitive species.

Scott (9) showed that the inhibitory effects of sucrose and of salts on the growth of staphylococci were very similar if compared on the basis of their influence on the water activity (a_w) of the growth medium. This was confirmed for other bacteria important in foods including salmonellae (3), *Clostridium botulinum* (10), and *C. perfringens* (5). However, Christian (2) observed that high concentrations of glycerol were much less inhibitory to salmonellae than either sucrose or salts when the solutes were compared on an a_w basis. The relative ineffectiveness of glycerol as a growth inhibitor has since also been demonstrated for *C. botulinum* (1) and *C. perfringens* (5). However, these three species all have rod-shaped cells and relatively high a_w requirements for growth. To determine whether greater resistance to glycerol than to NaCl is a general property of nonhalophilic bacteria, it was necessary to examine the sensitivities to these solutes of a wider range of species, more diverse in both morphology and water requirement.

The 16 species tested (5 gram-positive cocci, 6 gram-positive rods, and 5 gram-negative rods) are listed under Fig. 1. Most were used previously in a study of the relationship between water requirement and cell potassium content (4). The basal media used were Trypticase-yeast extract-glucose broth (0.997 a_w) for *C. botulinum* and quarter-strength brain heart broth (0.998 a_w) for all other organisms. These a_w values were determined from freezing point depression and isopiestic equilibration measurements, respectively (9). The a_w levels of both media were adjusted to a range of values in steps of 0.01 by additions of NaCl or glycerol calculated from published data (7, 8). Checks on freezing points of final media showed that a_w was within 0.002 of the nominal value at the lowest a_w levels, the error being proportionately less at higher levels.

The inoculum for *C. botulinum* was 10^4 spores suspended in 0.1 ml of water, and for all other bacteria it was 0.02 ml of an early stationary-phase culture. *C. botulinum* was grown in 10 ml of medium in evacuated, sealed glass ampoules, and the other organisms were grown in 10-ml volumes in 100-ml screw-capped bottles which were sealed with paraffin wax and incubated lying flat. All cultures were incubated at 30 C for 28 days.

The results are shown in Fig. 1. In NaCl, the limiting a_w levels were in the range 0.97 to 0.83, whereas in glycerol the range was 0.97 to 0.89 a_w. Only three species responded identically to both solutes. Those more sensitive to NaCl than to glycerol were exclusively rods, whereas the cocci were predominantly more sensitive to glycerol than to NaCl.

The anomalous position of *Vibrio metchnikovi* is in keeping with its requirement for a medium of relatively high ionic strength and its marked sensitivity to nonelectrolytes generally (6). The mean minimum a_w for all 16 species is 0.92 in NaCl and 0.93 in glycerol. If the data for *V. metchnikovi* are omitted, the mean value in glycerol is also reduced to 0.92 a_w. The general trend of the results in Fig. 1 might then be explained if, for any individual species, glycerol was less inhibitory than NaCl at a_w above about 0.92 and more inhibitory than NaCl below 0.92 a_w. Measurements of growth rates of *Staphylococcus aureus* at a_w levels between 0.96 and 0.90, controlled by either NaCl or glycerol, showed that this was not so: at each a_w, the inhibition of growth rate was about 10% greater in glycerol than in NaCl.

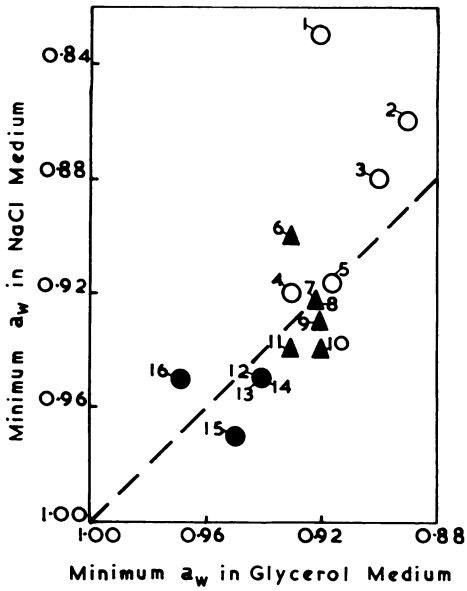

FIG. 1. *Relation between the minimum a_w levels for growth of 16 species of bacteria in NaCl-adjusted medium and in glycerol-adjusted medium. Symbols:* ○, *gram-positive cocci;* ▲, *gram-positive rods;* ●, *gram-negative rods. The numbers identify data for individual species: 1, Micrococcus sp. MR1; 2, Staphylococcus aureus; 3, S. albus; 4, M. lysodeikticus; 5, Sarcina sp. 2b; 6, Bacillus subtilis W168; 7, B. sphaericus; 8, B. cereus C3; 9, B. cereus 5B; 10, B. megaterium KM; 11, Clostridium botulinum type A; 12, Salmonella newport; 13, S. oranienburg; 14, Escherichia coli; 15, Pseudomonas fluorescens; 16, Vibrio metchnikovi. Points for species equally sensitive to NaCl and glycerol fall on the dashed line.*

Thus for some bacteria, particularly cocci, glycerol can be much more inhibitory than NaCl at concentrations equivalent in terms of a_w. Unfortunately, rod-shaped bacteria having no NaCl requirement but with the salt tolerance of *Micrococcus* MR1 are rare. Studies with such bacteria might show whether the relationship reported here is a general phenomenon or whether it is linked in some way to morphology.

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