Effect of Water Activity on Enterotoxin A Production and Growth of *Staphylococcus aureus*

JOHN A. TROLLER

*Winton Hill Technical Center, The Procter & Gamble Company, Cincinnati, Ohio 45239*

Received for publication 31 January 1972

Previous studies indicated that enterotoxin B production by *staphylococci* was strongly inhibited by slight reductions in water activity ($a_w$) levels. Similar studies reported herein, employing an enterotoxin A-producing strain, indicated that this organism was capable of producing enterotoxin at a much lower $a_w$ level than that required for enterotoxin B production. *Staphylococcal* growth rates were slowed by decreased $a_w$ levels in all media tested; however, final cell counts did not drop below $10^4$/ml in the media with the lowest $a_w$ levels.

Scott (9) and others (1, 11, 13-15) have defined the water requirements for a number of species of bacteria in terms of water activity ($a_w$). Although the concept of water limitation as a means of preventing the growth of microorganisms in foods long has been recognized, it is only recently that studies have been reported on the effect of $a_w$ on growth and toxin formation by pathogens in food.

The effect of $a_w$ on the growth of *Staphylococcus aureus* has been thoroughly studied by Scott (8) and reviewed by Christian and Waltho (2). Genigeorgis et al. (3), McLean et al. (6), Markus and Silverman (5), and Hojvat and Jackson (4) related both *staphylococcal* growth and enterotoxin production to sodium chloride concentration. Troller (10) studied the effect of $a_w$ on the production of enterotoxin B by *S. aureus* 243 and found that toxin production was very sensitive to slight reductions in $a_w$ levels without a commensurate effect on growth.

The present report extends the work of Troller on enterotoxin B (10) to the production of enterotoxin A.

**MATERIALS AND METHODS**

**Cultures and growth conditions.** *S. aureus* strain 196E was used for the production of enterotoxin A. This culture was the gift of M.S. Bergdoll, Food Research Institute, Madison, Wis.

Stock cultures were stored at 5 C on porcelain beads, and working stock cultures were maintained at 5 C on plate count agar (Difco Laboratories, Inc., Detroit, Mich.) with monthly transfer. Plate counts were obtained in triplicate in plate count agar after 24 hr of incubation at 37 C.

The basal medium used in these studies was identical to a modification of the medium of Rieser and Weiss (7) described previously (10). As in the preceding study, the $a_w$ of the medium designated as A was adjusted by the supplementation of equal quantities of NZ Amine NAK (hydrolyzed protein; Sheffield Chemical, Division of National Dairy Products Corp., Norwich, N.Y.) and partially hydrolyzed protein (PHP; Mead Johnson and Company, Evansville, Ind.). Medium B was adjusted to various $a_w$ levels by the addition of NaCl, and KCl was used to adjust $a_w$ in medium C. Table 1 lists the concentrations of solutes employed to obtain various $a_w$ levels. All media were supplemented with filter-sterilized nicotinic acid (0.001%) and thiamine (0.00005%) after autoclaving. The initial pH was 6.8. When glycerol was also employed for the adjustment of $a_w$, results were virtually identical with those obtained with media A, B, and C. Therefore, these data are not included in this report.

**Enterotoxin production and analysis.** The procedures for harvesting medium and analyzing enterotoxin have been previously described (10). Cells were removed from 10.0 ml samples of culture medium by refrigerated centrifugation, at 45,000 x g, followed by dialysis of the supernatant fluid against distilled water at 5 C for 24 hr. The dialysate was then concentrated in dialysis tubing against a 50% solution of Carbowax 20M (Union Carbide Corp., Chicago, Ill.). The material remaining in the tubing after concentration was suspended in 2.0 ml of 0.37% brain heart infusion (BHI; Difco) broth and centrifuged, and the supernatant fluid was refrigerated until analysis.

The single-diffusion method in agar, as modified by Weirether (12), was used to estimate enterotoxin...
The amounts from increased using ious rectly ysis and at 0.965 0.98 0.902 0.91 0.945 Bergdoll. The above tive pressure measurement. Water activity measurements are expressed as \( a_w \) or the ratio of the vapor pressure of a given solution to that of pure water. The \( a_w \) can also be expressed as the equilibrium relative humidity, divided by 100, of an atmosphere above a given material in a closed chamber.

Measurements of \( a_w \) were carried out with Hygrosensor elements (no. 4-4822; Hygrodynamics, Inc., Silver Spring, Md.) mounted in the lids of 4-oz jars. Each jar contained approximately 15 ml of the solution to be measured. Jars were allowed to equilibrate at 27 C for approximately 2 hr before measurement. Sensors were calibrated against saturated KNO\(_3\) solutions prior to use. Sensors showing \( a_w \) levels of the calibrating solutions with variation of less than ±0.002 were considered suitable for use.

**RESULTS AND DISCUSSION**

**Effect of \( a_w \) on growth.** The effects of various \( a_w \) levels on the growth rates of *S. aureus* 196E in media A, B, and C are shown in Fig. 1. As reported earlier (10) with an enterotoxin B-producing strain, \( a_w \) levels of the growth medium profoundly affected the generation times of staphylococci growing in the various media. The generation time of the 196E strain increased from 48 to 309 min with a shift in \( a_w \) from .98 to .89 in medium A and from 40 to 130 min with an \( a_w \) shift from .99 to .91 in medium C. Medium B appeared to support the most rapid growth of the three media tested with a generation time of 37 min in the control medium (\( a_w = .99 \)). Growth rates at all \( a_w \) levels were more rapid in medium B.

The lowest \( a_w \) levels tested reduced the maximal numbers (Fig. 2) of staphylococci from 3 \( \times 10^{10} \) per ml in the control cultures (\( a_w = .99 \) or .98) to approximately 4 \( \times 10^{8} \) per ml in cultures adjusted to minimal \( a_w \) levels. Attempts to determine growth and enterotoxin production were not performed at the \( a_w \) level reported in the literature (8) to be limiting (.86 \( a_w \)). The lag time of only the 196E strain grown in medium A was appreciably extended with decreasing \( a_w \) levels, an effect reported for several bacterial species by Wodzinski and Frazier (13–15). Slight lag time extension was noted in all media at the lowest \( a_w \) levels tested.

**Effect of \( a_w \) on enterotoxin production.** The production of enterotoxin A is markedly inhibited by decreases in \( a_w \), although its production is relatively less sensitive to \( a_w \) than is that of enterotoxin B (10). While the maximal amount of enterotoxin measured was directly related to \( a_w \) level (Fig. 3), the response was considerably less than that for enterotoxin B. A reduction of the \( a_w \) of medium A from .99 to .945 produced a 60% drop (Fig. 3) in enterotoxin content. A similar reduction in the \( a_w \) of medium B brought about a 55% decrease in maximal enterotoxin levels. Previous data (10)

| Table 1. Relationship between solute content of basal medium and water activity (\( a_w \)) |
|-----------------------------------------------|---------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Medium A | Medium B | Medium C |
| Hydrolyzed protein (%) | \( a_w \) | NaCl (%) | \( a_w \) | KCl (%) | \( a_w \) |
| 6 (Control) | 0.99 | 0      | 0.99 | 0      | 0.99 |
| 23      | 0.98 | 3.0    | 0.97 | 4      | 0.98 |
| 33      | 0.965| 5.0    | 0.94 | 8      | 0.97 |
| 43      | 0.945| 7.5    | 0.92 | 15     | 0.95 |
| 50      | 0.89 | 10.0   | 0.90 | 20     | 0.91 |
showed that reductions in $a_w$ levels from .99 to .97 or .98 reduced enterotoxin B levels by 90 to 99%, depending on the solute employed for $a_w$ adjustment. As with enterotoxin B, the production of detectable levels of enterotoxin A appeared to occur mostly during late logarithmic and early maximum stationary phases of growth. These results agree with those of Markus and Silverman (5) who reported that most enterotoxin A production by *S. aureus* S-100 occurred during the exponential phase of growth; however, 20% was secreted during the early stationary phase.

Additional studies on the response of enterotoxin C-producing strains to reduced $a_w$ levels are currently in progress. Preliminary data, not reported in this study, indicate that the effect of $a_w$ on enterotoxin C production is similar to that on enterotoxin A.

Unlike staphylococcal strains producing enterotoxins A and C, enterotoxin B-producing strains are rarely implicated in outbreaks of food poisoning. This could be caused by the sensitivity of B-producing strains to slightly reduced $a_w$ levels. Many of the foods which offer opportunities for the production of staphylococcal enterotoxins do not exceed $a_w$ levels of .95 to .96, levels which would easily prevent the production of enterotoxin B but not the production of enterotoxins A or C. Experiments on foods adjusted to various $a_w$ levels are currently in progress. However, present extrapolation of the results reported herein to foods would indicate that reliance cannot be placed on the reduction of $a_w$ alone as a means of preventing the formation of all enterotoxins.

**ACKNOWLEDGMENTS**

I thank J.V. Stinson for his excellent technical assistance and J.G. Voss and W.C. Frazier for their helpful comments and suggestions during preparation of the manuscript.

**LITERATURE CITED**

1. Burcik, E. 1960. Über die Beziehungen zwischen Hydratur und Wachstum bei Bakterien und Hefen. Arch. Mikrobiol. 15:203–235.
2. Christian, J. H. B., and J. A. Waltho. 1962. The water relations of staphylococci and micrococci. J. Appl. Bacteriol. 25:369–377.
3. Genigeorgis, C., and W. W. Sadler. 1966. Effect of sodium chloride and pH on enterotoxin B production. J. Bacteriol. 92:1383–1387.
4. Hojvat, S. A., and H. Jackson. 1969. Effects of sodium chloride and temperature on the growth and production of enterotoxin B by *Staphylococcus aureus*. Can. Inst. Food Technol. J. 2:56–59.
5. Markus, Z. H., and G. J. Silverman. 1970. Factors affecting the secretion of staphylococcal enterotoxin A. Appl. Microbiol. 20:492–496.
6. McLean, R. A., H. D. Lilly, and J. A. Alford. 1968. Effects of meat-curing salts and temperature on production of staphylococcal enterotoxin B. J. Bacteriol. 95:1207–1211.
7. Rieser, R. F., and K. F. Weiss. 1969. Production of staphylococcal enterotoxins A, B, and C in various media. Appl. Microbiol. 18:1041–1043.
8. Scott, W. J. 1963. Water relations of *Staphylococcus aureus* at 30°C. Aust. J. Biol. Sci. 6:549–564.
9. Scott, W. J. 1957. Water relations of food spoilage microorganisms. Advan. Food Res. 7:83–127.
10. Troller, J. A. 1971. Effect of water activity on enterotoxin B production and growth of *Staphylococcus aureus*. Appl. Microbiol. 21:435–439.
11. Walter, H. 1924. Plasmaquelle und Wachstum. Z. Bot. 16:353–417.
12. Weirether, F. J., E. E. Lewis, A. J. Rosenwald, and R.
E. Lincoln. 1966. Rapid quantitative serological assay of staphylococcal enterotoxin B. Appl. Microbiol. 14: 284–291.

13. Wodzinski, R. J., and W. C. Frazier. 1960. Moisture requirements of bacteria. I. Influences of temperature and pH requirements of Pseudomonas fluorescens. J. Bacteriol. 79:572–578.

14. Wodzinski, R. J., and W. C. Frazier. 1961. Moisture requirements of bacteria. II. Influence of temperature, pH, and malate concentration on requirements of Aerobacter aerogenes. J. Bacteriol. 81:353–358.

15. Wodzinski, R. J., and W. C. Frazier. 1961. Moisture requirements of bacteria. III. Influence of temperature, pH, and malate and thiamine concentration on requirements of Lactobacillus viridescens. J. Bacteriol. 81:359–365.