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Plating medium *p*H as a Factor in Apparent Survival of Sublethally Stressed Yeasts[1]

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Sublethally stressed cells of 9 of 10 species of yeast were recovered at maximum levels when potato dextrose agar was adjusted to approximately *p*H 8. The optimum for *Candida utilis* was at approximately *p*H 10. At *p*H 3.5, as commonly employed with media selective for yeasts and molds, recovery of heat-stressed organisms ranged from essentially the same as at optimum *p*H to levels of 1% or less of the maximum count. The extent to which this may be of practical significance in assessing the microbiological quality of food products remains to be determined.

During the 1920's a number of investigators used media adjusted to various acid *p*H levels for selective enumeration of yeasts and molds in butter. In 1930, a committee of the American Dairy Science Association recommended that standardization on *p*H 3.5 be effected (1). White and Hood (16) subsequently reported that among 60 yeast cultures isolated from dairy products only a few gave lower counts when plated on media at *p*H 3.0 than at *p*H 3.4 and above. At *p*H 3.4, no detrimental effect on development of yeasts was observed when butter samples were plated. At *p*H 4.2 and above, bacterial colonies always developed, but not at *p*H 3.8 or lower. At the present time, potato dextrose agar (PDA) adjusted to *p*H 3.5 ± 0.1 with tartaric acid is recommended for detection of yeasts and molds in dairy products and other foods (2, 3). Malt agar or dextrose agar may be used for some foods (3).

Mace and Koburger (8) determined that a major factor in the higher counts Skidmore and Koburger (14) had obtained using the rose bengal-tetracycline medium of Cooke (4), in comparison with acidified PDA, was attributable to the less acid *p*H of the antibiotic-containing medium. When the latter was adjusted to below *p*H 5.0, its ability to recover yeasts and molds from a variety of processed foods was reduced considerably. The stressed state of the yeasts in many processed foods was considered to be a factor in the influence of *p*H, since organisms which had developed colonies on the medium at less acid *p*H nearly all grew on transfer to agar at *p*H 3.5. Koburger (6) subsequently found PDA, adjusted to *p*H 7.0 before autoclaving and to which 100 mg each of both chlortetracycline and chloramphenicol per liter subsequently were added, gave markedly greater counts than did acidified PDA. Bacteria were sometimes numerous when acidified PDA was used but never were found on the medium containing antibiotics. The possibility that the amount of sample added to the acidified medium so altered the *p*H that bacteria could grow, as Powers et al. (13) have shown, apparently was not explored.

Olson (J. Dairy Sci., **37**:643, 1954) reported unacidified PDA containing 100 mg of chlortetracycline/ml commonly gave counts slightly higher than did PDA acidified to *p*H 3.5. Olson and Bonner (11) subsequently used the antibiotic-containing medium successfully for analyzing cottage cheese. Overcast and Weakley (12) found that a peptone-dextrose medium of undesignated *p*H which relied upon 20 mg of rose bengal per ml and 20 mg of chlortetracycline per ml for suppression of bacterial growth gave essentially the same counts as did PDA acidified to *p*H 3.5, when 17 samples of cottage cheese were plated. Mossel et al. (9, 10) have reported favorably on use of oxytetracycline in place of low *p*H to suppress bacteria when enumerating yeasts and molds in food products.

Both because stressed bacteria are known to be more sensitive to adverse *p*H of the culture medium employed for enumeration (Nelson, Bacteriol. Proc. p. 5, 15, 40; 1956), and because the better recovery of yeasts from processed

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foods when media relying upon antibiotics for suppression of bacterial growth had been used has been attributed to the stressed condition of the microorganisms (8, 14), a series of experiments to determine possible effect of pH of recovery medium on apparent survival of sublethally heat-stressed yeasts was undertaken.

MATERIALS AND METHODS

Yeasts of 10 different species were obtained from James Sinski of the Department of Microbiology and Medical Technology, The University of Arizona. *Saccharomyces cerevisiae* Y 2034 was obtained from L. J. Wickerham of the Northern Regional Research Laboratory of the United States Department of Agriculture. The culture designations have been changed to conform to the nomenclature of Lodder (7). The yeasts were cultured in yeast maltose broth at approximately 24 C. Quiescent cultures incubated 48 hr were used in most cases, although a wrist-type shaker was used for *Endomyces magnusii*, *Candida albicans*, and *C. utilis* because of low cell yields from quiescent cultures. Broth culture in 2- to 5-ml amounts, depending on cell concentration, was diluted in 50 ml of sterile skim milk, and approximately 3 ml of this dilution was placed carefully in the bottom of a screw-cap tube (16 by 125 mm). After stressing for the required time following come-up in a thermostatically controlled, agitated water bath, the samples were cooled in an ice bath. Plates were poured with PDA (Difco), the pH of which was adjusted to the several levels by addition of NaOH or H_2SO_4 after cooling 47 C. Sulfuric acid was used in place of tartaric acid to minimize the chance that an added organic acid might have an effect other than pH. The pH values were determined electrometrically by thrusting electrodes into a control portion of solidified medium at room temperature. Incubation was at approximately 24 C for 5 days.

The data presented are results of individual experiments characteristic of the several runs made by using each organism.

RESULTS

Data on four cultures representative of several different types of response to various pH levels of the plating medium are shown in Fig. 1. The extremes of pH employed, whether acid or alkaline, had less effect on the counts of unstressed organisms than on the stressed organisms in all cases. As the degree of stress was increased, as shown for *S. cerevisiae* Y 2034 and *Kluyveromyces lactis*, the reductions in counts at pH extremes increased markedly.

Representative data for all cultures used for test purposes are summarized in Table 1. Although *S. cerevisiae* S was somewhat more heat resistant than *S. cerevisiae* Y 2034, recovery of both cultures at pH 3.5 was essentially the same percentage of the maximum recovery at pH 7.8 to 8.2. Recoveries at pH 3.5 ranged from only slightly less than maximum at optimum pH in the case of *C. vinii* to less than 1% of maximum for *K. lactis* stressed at close to the maximum temperature permitting any survival. *C. utilis* showed the highest pH for maximum recovery of stressed organisms. *C. albicans* had a somewhat higher optimum for recovery than did most of the other yeast cultures (commonly ca. pH 8.0, although some variation from this general figure was apparent). *C. vinii* showed no definite optimum.

DISCUSSION

The results indicate that at least some yeasts are similar to bacteria in that the pH of the recovery medium has a much greater effect upon sublethally heat-stressed cells than upon the unstressed control cells. In the range of heat treatment approaching complete kill, the reduction in count resulting from suboptimal pH of the plating medium is greatest. To what extent this applies to yeasts stressed by dessication, freezing, irradiation, and chemical means remains to be determined.

These data support the theory of Koburger and associates (6, 8) that the greater sensitivity to acid pH values of some physically stressed

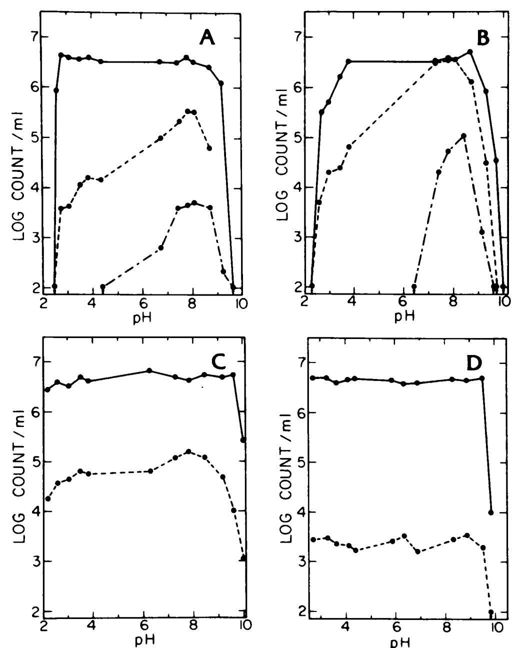

FIG. 1. *Relationship of pH of PDA plating medium to recovery of representative unstressed (——) and heat-stressed (------) yeasts. A, Saccharomyces cerevisiae Y 2034, stressed at 51 C for 20 min and at 51.5 C for 20 min (—-—); B, Kluyveromyces lactis stressed at 51 C for 20 min and 52 C for 15 min (—-—); Rhodotorula glutinis, stressed at 50 C for 5 min; D, Candida vinii, stressed at 56 C for 25 min.*

TABLE 1. *Influence of pH of PDA recovery medium upon apparent survival of heat-stressed yeasts*

Organism	Heat treatment	Optimum *p*H	Approximate % of maximum[a]
Saccharomyces cerevisiae S	53 C-17 min	7.8–8.2	4
S. cerevisiae Y 2034	51 C-20 min	7.8–8.2	3
S. cerevisiae	51.5 C-20 min	7.9–8.2	<2
Kluyveromyces lactis	52 C-15 min	8.0–8.4	0.1
	51 C-20 min	7.3–8.0	1
Pichia Kluyveri	51 C-5 min	7.5–9.2	20
Torulopsis glabrata	53 C-28 min	7.5–9.0	15
Rhodotorula glutinis	51 C-20 min	7.2–8.5	40
Endomyces magnusii	50 C-5 min	8.5	10–30
Hansenula anomala	51 C-9 min	6.8–8.0	40
Candida albicans	52 C-17 min	9.0–10.0	7
C. utilis	51 C-5 min	9.8–10.3	25
C. vinii	56 C-25 min		70–80

[a] Calculated as: (count at *p*H 3.5/count at optimum *p*H) × 100.

yeast cells is one reason why media which depend upon broad-spectrum antibiotics for suppression of bacteria give higher counts of fungi in some food samples than do media acidified to *p*H 3.5.

Although Overcast and Weakley (12) reported no significant difference in count when antibiotic-containing medium was compared with an acidified medium for plating cottage cheese samples, Olson and Bonner (10) have reported slightly higher counts on the former medium. Some of the difference in experience may be explained by the different samples used by the several investigators. Koburger and associates (6, 7, 14) used a variety of foods, and their data indicate the increased counts obtained using the antibiotic-containing media were considerably greater with nondairy foods than with dairy products. Cottage cheese particularly might be expected to contain actively growing yeasts relatively free from stress conditions.

The adverse effect of *p*H 3.5, as commonly used in media employed for enumeration of yeasts in foods, unquestionably will vary from sample to sample. The type(s) of organism present and the physiological state(s) will be major factors in determining how important a factor *p*H is. Two other problems associated with use of acid conditions are precipitation of acid-coaguable components of some foods (11, 12) and the tendency for the *p*H to be raised to a level not inhibitory to bacteria when a considerable amount of sample, sometimes with buffered diluent, must be added to plates in making counts on products with low levels of yeasts (13). Under some circumstances, bacteria have grown in such numbers on acidified plates as to make the counts unreliable (6). The effects of media containing broad-spectrum antibiotics (and possibly rose bengal) on recovery of stressed yeasts should be determined, as such media offer a potentially desirable alternative to adjustment of reaction to *p*H 3.5.

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LITERATURE CITED

1. American Dairy Science Association. 1930. Suggested methods for the microbiological analysis of butter. J. Dairy Sci. 13:394–405.
2. American Public Health Association. 1966. Recommended methods for the microbiological examination of foods, 2nd ed. American Public Health Association, Inc., New York.
3. American Public Health Association. 1967. Standard methods for the examination of dairy products, 12th ed. American Public Health Association, Inc., New York.
4. Cooke, W. B. 1954. The use of antibiotics in media for the isolation of fungi from polluted water. Antibiot. Chemother. 4:657–662.
5. Heather, C. D., and C. Vanderzant. 1957. Effects of temperature and time of incubating and pH of plating medium on enumerating heat-treated psychrophilic bacteria. J. Dairy Sci. 40:1079–1086.
6. Koburger, J. A. 1970. Fungi in foods. I. Effect of inhibitor and incubation temperature on enumeration. J. Milk Food Technol. 33:433–434.
7. Lodder, J., (ed.). 1970. The yeasts—a taxonomic study, 2nd ed. North-Holland Publishing Co., Amsterdam.
8. Mace, F. E., and J. A. Koburger. 1967. Effect of pH on recovery of the fungi from foods. Proc. W. Va. Acad. Sci. 39:102–106.
9. Mossel, D. A. A., A. M. C. Kleynen-Semmeling, H. M. Vincentie, H. Beerens, and M. Catsaras. 1970. Oxytetracycline-glucose-yeast extract agar for selective enumeration of moulds and yeasts in foods and clinical materials. J. Appl. Bacteriol. 33:454–457.
10. Mossel, D. A. A., M. Visser, and W. H. J. Mengerink. 1962. A comparison of media for the enumeration of moulds and yeasts in food and beverages. Lab. Pract. 11:109–112.

11. Olson, H. C., and M. P. Bonner. 1957. Methods for analysis of cottage cheese. Okla. Agr. Exp. Sta. Tech. Bull. T-71.
12. Overcast, W. W., and D. J. Weakley. 1969. An aureomycin-rose bengal agar for enumeration of yeast and mold in cottage cheese. J. Milk Food Technol. 32:442-445.
13. Powers, E. M., C. C. Ay, C. A. Eckfeldt, and R. B. Rowley. 1971. Modification of the American Public Health Association procedure for counting yeast and mold in cottage cheese. Appl. Microbiol. 21:155-156.
14. Skidmore, N. C., and J. A. Koburger. 1966. Evaluation of Cooke's rose bengal agar for enumeration of fungi in food. Proc. W. Va. Acad. Sci. 38:63-68.
15. Thomas, W. R., G. W. Reinbold, and F. E. Nelson. 1966. Effect of pH of plating medium on enumeration of pasteurization-resistant bacteria in milk. J. Milk Food Technol. 29:156-160.
16. White, A. H., and E. G. Hood. 1931. A study of methods for determining numbers of moulds and yeasts in butter. I. The relation of the pH of the medium. J. Dairy Sci. 14:463-476.