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Accelerated Procedure for the Enumeration and Identification of Food-Borne *Staphylococcus aureus*

R. VICTOR LACHICA

Unified Food Control Laboratory, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

A procedure was developed for accelerating to 29 h the enumeration and identification of both healthy and stressed cells of *Staphylococcus aureus* in foods. Baird-Parker agar medium was incubated for 24 h; *S. aureus* was identified within 5 additional h by using a simplified thermonuclease test.

The present procedure for the enumeration of food-borne *Staphylococcus aureus* by direct plating requires 3 to 4 days for completion (2, 8). The Baird-Parker egg yolk-tellurite-pyruvate agar (BP) medium is the medium of choice for presumptive enumeration (2, 12, 14, 15, 17). It was originally designed to recover *S. aureus* in 24 h (3); however, subsequent investigation revealed that a longer incubation period (36 to 48 h) was needed to detect late egg yolk clearers (4). Experience was required in judging which colonies to count, and the need to subculture suspect colonies for definitive identification is cumbersome and time-consuming.

The present study reports a convenient procedure for the enumeration and identification of food-borne *S. aureus* in 29 h.

MATERIALS AND METHODS

Cultures, samples, and basal medium. The following enterotoxigenic strains of *S. aureus* were kindly provided by R. W. Bennett of the U.S. Food and Drug Administration, Washington, D.C.: 137, 196E, 243, 265, 472, 494, 587, and 790. These were stored at 7°C and transferred monthly on slants of nutrient agar (Difco Laboratories).

Food samples were either brought by food inspectors or purchased from retail outlets. Unless examined immediately after being obtained, samples of cheese and yogurt were stored at 7°C and the rest were kept at −10°C.

BP agar base and egg yolk-tellurite enrichment from Difco were flown to Guatemala under refrigeration and stored at 7°C upon arrival. From these components, plates of BP medium were prepared by the recommendations of the manufacturer. The plates were used within 5 days of preparation.

Coagulase and thermonuclease tests. For coagulase tests, reconstituted rabbit coagulase plasma plus ethylenediaminetetraacetic acid from Difco was mixed with 0.2 ml of brain heart infusion broth (Difco) culture in a test tube (10 by 75 mm). Examination for coagulation was made after incubation for 4 h at 37°C and after an additional 20 h of incubation at room temperature. Coagulation at the 3+ or 4+ level was considered a positive reaction (16).

The microslide method of Lachica et al. (10) was used for the detection of thermonuclease activity. Wells (2 mm in diameter) cut into the toluidine blue-deoxyribonucleic acid agar were filled with brain heart infusion broth cultures previously heated to boiling for 15 min. A positive reaction was indicated by a bright pink zone around the wells within 4 h at 37°C.

A modification of the thermonuclease test (STN test) was developed to permit the direct identification of *S. aureus* on the recovery medium in 5 h (9). It involved placing the agar plates with grown colonies in a 60°C incubator for 2 h to inactivate heat-labile nucleases. Subsequently, the plates were overlaid with 10 ml of molten toluidene blue-deoxyribonucleic acid agar and incubated for 3 h at 37°C. Colonies showing a bright pink halo were *S. aureus*.

Enumeration procedures. Food samples were homogenized in the usual manner (2, 8), decimal dilutions were made with 0.1% peptone (Difco), and 0.1-ml volumes were surface inoculated onto duplicate plates of BP medium. After incubation for 48 h at 37°C, suspect colonies were scored and counted. For definitive identification, at least one of the black, egg yolk-positive colonies and, if present, at least one of the black, egg yolk-negative colonies, were transferred to brain heart infusion broth. After incubation for 18 h at 37°C, the broth was tested for coagulase and thermonuclease activities.

The accelerated procedure differed from the traditional procedure in two respects: (i) shortened incubation of BP medium to 24 h and (ii) definitive identification of *S. aureus* in 5 h by the STN test. To verify the reliability of the STN test, at least one suspect colony from each food sample was subcultured before the heat treatment and examined for coagulase and thermonuclease production. No attempts were made to recover viable cells after the heat treatment of the BP plates.

RESULTS AND DISCUSSION

The efficacy of the accelerated procedure was initially investigated by using food samples experimentally inoculated with heat-stressed cells. Overnight brain heart infusion broth cultures of *S. aureus* preheated for 60 min in a 50°C water bath were inoculated to food samples at a rate of about 10^4 colony-forming units (CFU) per g.

The counts of *S. aureus* by the accelerated procedure were comparable to the traditional procedure regardless of strain or food menstruum used (Table 1). Many of the *S. aureus* colonies recovered by the accelerated procedure were smaller but produced sufficient amounts of thermonucleases.

Stressed cells of *S. aureus* were also obtained by inoculating yogurt (pH 3.8) with overnight brain heart infusion broth cultures at a rate of about 10^8 CFU/g. A rapid decline in viability was observed after storage for 18 h at room temperature, and no cells were detected after 48 h. A similar observation was obtained by Minor and Marth (13). Survivors of *S. aureus* after 18 h were recovered with equal efficiency by the traditional and the accelerated procedures (Table 2). As with heat-stressed cells, the yogurt-stressed *S. aureus* strains grew as small colonies in 24 h. Nevertheless, they were easily detected by their bright pink halo of thermonuclease activity.

Hard cheese known locally as "queso duro" (81 samples) and cream (10 samples) were used to compare the traditional and the accelerated procedures. Both procedures gave comparable counts of *S. aureus* from hard cheese, regardless of the level of contamination (Table 3). Similar results were obtained with the cream samples (data not shown).

Egg yolk-negative strains were among the *S. aureus* population detected in over half the cheese samples and six cream samples in this study. In four samples of cheese, egg yolk-negative strains constituted >70% of the *S. aureus* population. Most of the black colonies with broad zones of precipitation on the 48-h plates turned out to be non-*S. aureus*. With samples containing low levels of *S. aureus* (<10^4 CFU/g or ml), heavy growth of concomitant microorganisms became a nuisance on the 48-h plates. With the accelerated procedure, the smaller colonies of *S. aureus* were easily detected by their pink halos. After only 1 h of incubation, many of the colonies of *S. aureus* exhibited a pink halo on overlaid plates of BP medium. An initial reading at this incubation period when zones of thermonuclease activity were still narrow facilitated easier counting of *S. aureus* colonies on crowded plates. A few colonies required an additional 2 h of incubation. Subcultures of suspect colonies identified as *S. aureus* by the coagulase and thermonuclease tests were all positive with the STN test.

Results of this study demonstrate the feasibility of accelerating the enumeration of foodborne *S. aureus* from 3 or 4 days to 29 h. An incubation period of 24 h was sufficient for stressed cells to recover, develop colonies, and produce thermonucleases on BP medium. And within an additional 5 h, colonies of *S. aureus* were detected directly on the recovery medium by means of the STN test. This is a considerable

TABLE 1. *Recovery of heat-stressed cultures of S. aureus experimentally inoculated into various foods*

Food sample	Strain	Recovery with the following procedure:[a]		% Recovery[b]
		Traditional	Accelerated	
Processed cheese	137	2.4×10^4	2.0×10^4	83
	196E	8.5×10^4	7.8×10^4	92
Sausage	243	4.2×10^4	4.6×10^4	110
	265	9.2×10^3	8.6×10^3	93
Salami	472	5.4×10^4	4.1×10^4	85
	494	2.5×10^5	2.2×10^5	88
Frankfurter	587	2.6×10^5	2.8×10^5	108
	790	5.8×10^5	4.5×10^5	76

[a] Each value represents (in CFU per gram) mean of two replicate runs in duplicate.

[b] Percent recovery was calculated by using CFU per milliliter from the traditional procedure as 100%. Mean for all experiments was 92%.

TABLE 2. *Recovery of S. aureus from experimentally inoculated yogurt after 18 h of storage*[a]

Strain	Recovery with the following procedure:[a]		% Recovery[b]
	Traditional	Accelerated	
137	3.8×10^4	3.2×10^4	84
196E	1.3×10^5	1.5×10^5	115
243	1.4×10^5	1.8×10^5	128
472	4.8×10^6	4.1×10^6	85
494	3.4×10^6	3.5×10^6	102
587	2.0×10^6	1.9×10^6	95
790	1.3×10^6	1.3×10^6	100

[a] See Table 1, footnote *a*.

[b] Mean of all experiments was 101%. See Table 1, footnote *b*.

TABLE 3. *Enumeration of S. aureus from naturally contaminated cheese samples at varying levels of contamination*[a]

Range of *S. aureus* contamination (CFU/g)	No. of samples	Recovery with the following procedure:		% Recovery[b]
		Traditional	Accelerated	
$(10^2$–9.9) $\times 10^2$	6	2.2×10^2	2.0×10^2	95
$(10^3$–9.9) $\times 10^3$	7	2.7×10^3	2.6×10^3	96
$(10^4$–9.9) $\times 10^4$	14	3.5×10^4	3.7×10^4	104
$(10^5$–9.9) $\times 10^5$	20	2.0×10^5	2.1×10^5	109
$(10^6$–9.9) $\times 10^6$	16	7.1×10^6	6.8×10^6	96
$(10^7$–9.9) $\times 10^7$	18	1.5×10^7	1.4×10^7	95

[a] Results represent (in CFU per gram) geometric means of *S. aureus* counts from samples comprising each range of contamination.

[b] Mean of 81 samples tested was 100%. See Table 1, footnote *b*.

improvement over the traditional procedure, not only in terms of reduction of the time factor but also in the ease of manipulation. Little experience was required for satisfactory performance.

Although egg yolk has no diagnostic significance in the accelerated procedure, it was retained because the efficiency of recovery of BP medium was reduced in the absence of egg yolk (unpublished data). In addition to pyruvate (5, 12), egg yolk also contributes to the efficiency of the BP medium in recovering stressed cells of *S. aureus* (17). Lecithin appears to be the component responsible for the stimulatory effect of egg yolk (1).

The present results are consistent with reports of others (6, 7) in substantiating the reliability of the STN test (9). Although the existence of thermonuclease-negative strains of *S. aureus* has been described (6, 11, 16), their occurence is rare.

LITERATURE CITED

1. **Andrews, G. P., and S. E. Martin.** 1978. Modified Vogel and Johnson agar for *Staphylococcus aureus*. J. Food Protect. **41**:530–532.
2. **Association of Official Analytical Chemists.** 1975. Section 46. *In* Official methods of analysis of the A.O.A.C., 12th ed. Association of Official Analytical Chemists, Washington, D.C.
3. **Baird-Parker, A. C.** 1962. An improved diagnostic and selective medium for isolating coagulase positive staphylococci. J. Appl. Bacteriol. **25**:12–19.
4. **Baird-Parker, A. C.** 1962. The performance of an egg yolk-tellurite medium in practical use. J. Appl. Bacteriol. **25**:441–444.
5. **Baird-Parker, A. C., and E. Davenport.** 1965. The effect of recovery medium on the isolation of *Staphylococcus aureus* after heat treatment and after storage of frozen or dried cells. J. Appl. Bacteriol. **28**:390–402.
6. **Boothby, J., C. Genigeorgis, and M. J. Fanelli.** 1979. Tandem coagulase/thermonuclease agar method for the detection of *Staphylococcus aureus*. Appl. Environ. Microbiol. **37**:298–302.
7. **Chopin, A., S. Tesone, J.-P. Vila, Y. Le Graet, and G. Mocquot.** 1978. Survive de *Staphylococcus aureus* au cour de la préparation et de la conservation de lait écrémé en poudre. Problèmes posés par le denombrement de survivants. Can. J. Microbiol. **24**:1371–1380.
8. **International Commission on Microbiological Specifications for Foods.** 1978. Microorganisms in foods. I. Their significance and methods of enumeration, 2nd ed. University of Toronto Press, Toronto.
9. **Lachica, R. V. F.** 1976. Simplified thermonuclease test for rapid identification of *Staphylococcus aureus* recovered on agar media. Appl. Environ. Microbiol. **32**: 633–634.
10. **Lachica, R. V. F., C. Genigeorgis, and P. D. Hoeprich.** 1971. Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. Appl. Microbiol. **21**:585–587.
11. **Lachica, R. V. F., K. F. Weiss, and R. H. Deibel.** 1969. Relationships among coagulase, enterotoxin, and heat-stable deoxyribonuclease production by *Staphylococcus aureus*. Appl. Microbiol. **18**:126–127.
12. **Martin, S. E., R. S. Flowers, and Z. J. Ordal.** 1976. Catalase: its effect on microbial enumeration. Appl. Environ. Microbiol. **32**:731–734.
13. **Minor, T. E., and E. H. Marth.** 1972. Fate of *Staphylococcus aureus* in cultured buttermilk, sour cream, and yogurt during storage. J. Milk Food Technol. **35**:302–306.
14. **Niskanen, A., and M. Aalto.** 1978. Comparison of selective media for coagulase-positive enterotoxigenic *Staphylococcus aureus*. Appl. Environ. Microbiol. **35**: 1233–1236.
15. **Rayman, M. K., J. J. Deyovod, U. Purvis, D. Kusch, J. Lanier, R. J. Gilbert, D. G. Till, and G. A. Jarvis.** 1978. ICMSF methods studies. X. An international comparative study of four media for the enumeration of *Staphylococcus aureus* in foods. Can. J. Microbiol. **24**: 274–281.
16. **Rayman, M. K., C. E. Park, J. Philpott, and E. C. D. Todd.** 1975. Reassessment of the coagulase and thermostable nuclease tests as means of identifying *Staphylococcus aureus*. Appl. Microbiol. **29**:451–454.
17. **Stiles, M. E., and P. C. Clark.** 1974. The reliability of selective media for the enumeration of unheated and heated staphylococci. Can. J. Microbiol. **20**:1735–1744.