

IMPROVED FIXATION OF STAPHYLOCOCCUS AUREUS FOR ELECTRON MICROSCOPY

Susan B. Jones, Samuel A. Palumbo, and James L. Smith

Eastern Regional Research Center, USDA, ARS, Philadelphia, PA 19118

Published glutaraldehyde fixation protocols for bacteria, e.g. [1], were unsatisfactory in our hands when applied to S. aureus 196E. Cell interiors were either dense or lost from thin sections, especially in stationary phase cells. Osmium tetroxide as primary fixative (R-K fixation, [2]) preserved the integrity of the cytoplasm, but fine structure of wall/membrane interfaces was poor and artifactual membranes developed in the nucleoplasm. We thus sought an optimum aldehyde primary fixative for S. aureus and report here that excellent preservation was obtained by combining 10% formaldehyde with 0.5% glutaraldehyde, followed by osmium tetroxide and uranyl acetate.

METHODOLOGY. S. aureus 196E was cultured in tryptic soy broth (Difco) as described [3]. Cells were harvested by centrifugation at exponential (4-6 hr) or stationary (16-18 hr) phase of growth. Unwashed cells were suspended in freshly-prepared fixative (see Table 1) at 20 C. Glutaraldehyde was from EM Sciences, Ft. Washington, PA, or Polysciences, Warrington, PA, and formaldehyde was prepared from paraformaldehyde. After primary fixation, cells were rinsed in the vehicle buffer for 24 hr at 4 C. All trials included secondary fixation in 1% osmium tetroxide at 20 C overnight followed by 90 min in 0.5% uranyl acetate, both in veronal acetate buffer [2]. Silver-gray Epon sections were stained with uranyl acetate and lead citrate.

RESULTS. For exponential phase cells, the three fixations listed in Table 1 preserved ribosomes well. However, 1% glutaraldehyde, 1.5% formaldehyde resulted in variable retention of nucleoplasm (Fig. a) and 10% formaldehyde without glutaraldehyde produced mesosome-like structures in the nucleoplasm and diffuse walls that were poorly bonded to the cell membrane (Fig. b). Addition of 0.5% glutaraldehyde to 10% formaldehyde corrected these defects, as described below for stationary phase cells.

Stationary phase cells were more difficult to preserve than exponential phase cells. Holes occurred in cells fixed 1 hr in 4% glutaraldehyde (not shown) or 1% glutaraldehyde, 1.5% formaldehyde (Fig. c), despite extended rinsing before secondary fixation. Glutaraldehyde crosslinking of free amino groups [4] in the already highly crosslinked S. aureus cell wall [5] may restrict penetration of fixative or egress of unbound glutaraldehyde during rinsing. Reducing fixation time to 30 min improved retention of DNA (Fig. d) but cytoplasm was grainy and structureless. Results similar to the latter were obtained with 0.6% glutaraldehyde, 2.5% formaldehyde, 1 hr (not shown).

Fixation with 0.5% glutaraldehyde, 10% formaldehyde produced excellent structure of stationary (Fig. e) and exponential cells (not shown). Wall and membrane formed a compact unit. Cytoplasm was structured, but ribosomes were not vivid in stationary phase S. aureus, possibly reflecting the small polyribosome pool in stationary phase bacteria (20% of ribosomes clustered versus 80% in exponential cells [7]). The use of 10% formaldehyde, a nucleic acid and protein crosslinking agent [6], produced coherent nucleoids (Figs. b,e) similar in form to those produced by R-K fixation [2], in contrast to the dispersed nucleoplasm observed after glutaraldehyde fixation [3].

Table 1. Preservation of *S. aureus* 196E with various prefixation protocols¹

Growth phase	Fixative composition, duration ²			Quality of preservation		
	Glut, %	Form, %	Time, min	Cytoplasm	DNA	Wall/membrane
Exponential	1	1.5	30	++ ³	+	+++
	0	10	60	++	+	-
	0.5	10	60	+++	+++	+++
Stationary	4	0	60	-	-	+++
	1	1.5	60	-	-	+++
	1	1.5	30	++	+	+++
	0.6	2.5	45	++	+	+++
	0.5	10	30-60	+++	+++	+++

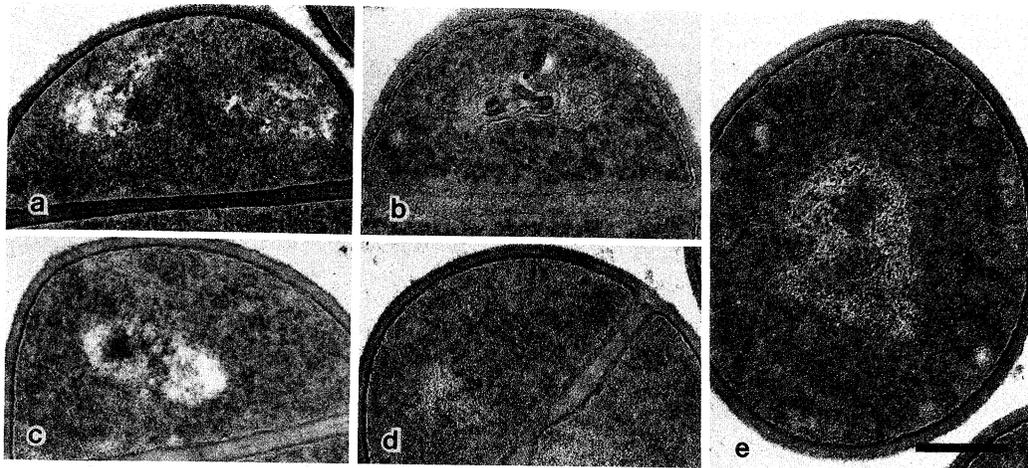
¹Aldehyde prefixation followed by OsO₄ and uranyl acetate. See text.

²Buffer: 50 mM Na cacodylate or phosphate, 0.15 M NaCl, 10 mM MgCl₂, pH 6.5.

³Excellent, +++; good, ++; adequate, +; poor, -.

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Figs. a-e. *S. aureus* 196E. Primary fixative for exponential cells was a, 1% glutaraldehyde, 1.5% formaldehyde, 30 min; b, 10% formaldehyde, 1 hr; and for stationary cells, c, 1% glutaraldehyde, 1.5% formaldehyde, 1 hr; d, 1% glutaraldehyde, 1.5% formaldehyde, 30 min; and e, 0.5% glutaraldehyde, 10% formaldehyde, 45 min. Bar = 0.2 μ m.