Selective Medium for Enumerating Erwinia Species Commonly Found in Vegetable Packinghouse Waters

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The incidence of postharvest decay of tomatoes (*Lycopersicon esculentum* Mill.) caused by *Erwinia carotovora* greatly increased following field washing of tomatoes after harvest (5). We found that carrots (*Daucus carota* L. var. *sativa* DC.), radishes (*Raphanus sativus* L.), celery (*Apium graveolens* L. var. *dulce* DC.), and peppers (*Capsicum frutescens* L.) had a higher incidence of bacterial soft rot after exposure to water in packinghouses than before; however, *E. carotovora* was seldom isolated from these waters on nonselective media. Therefore, a selective medium was required to determine the relation between populations of *E. carotovora* in these waters and decay incidence.

A selective medium for isolation of *E. rubrifaciens* from soil (4) was not specific for *E. carotovora* in packinghouse waters. This medium contained glycerol as the carbohydrate source. Substituting raffinose for glycerol modified the medium so that colonies of *E. carotovora* could be distinguished from colonies of other genera present in the water.

This medium contained raffinose, 10 g; dibasic potassium phosphate, 2 g; ammonium sulfate, 5 g; eosin yellow, 0.4 g; methylene blue, 0.065 g; actidione, 250 ppm; neomycin, 40 ppm; novobiocin, 40 ppm; agar, 15 g; and water, 1 liter (7). Raffinose was sterilized with propylene oxide before being added to the autoclaved medium (2).

In this medium, *E. carotovora* was characterized by raised reddish colonies having grainy texture and purple centers surrounded by a white transparent halo with an irregular margin. Colony size, ranging up to 2 mm, was dependent on plate populations. On crowded plates with more than 100-200 colonies, the small colonies did not have purple centers. Colonies of *Erwinia aroideae* and *E. chrysanthemi* had the same appearance as those of *E. carotovora*. We isolated *E. carotovora* cultures from carrots and tomatoes; *E. chrysanthemi* stock cultures were obtained from J. F. Knauss, University of Florida, Agricultural Experiment Station, Ridge Ornamental Laboratory, Apopka; *E. aroideae* stock cultures were obtained from C. Wehlberg, Division of Plant Industry, Florida Department of Agriculture, Gainesville.

For determining populations of *Erwinia* species, serial dilutions of the packinghouse waters were made in phosphate buffers (1) or, if chlorine was present, in 0.1 M sodium thiosulfate. The serial dilutions were plated on the solidified selective medium and incubated at 27 C for 4 days, when plate counts were made.

Pathogenicity of suspected colonies from wash water was determined by stab inoculations into carrot slices. Pectinolytic activity of the colonies was determined by liquefication of pectin media (3). Only colonies causing soft rot on carrot slices after 24 hr and liquefication of pectin media after 18 hr, both at 27 C, were considered confirmatory for *Erwinia*. Since only those colonies causing soft rot on carrot slices caused liquefication of the pectin media, pectinolytic activity was used as a presumptive confirmatory test. We tested three suspected *Erwinia* colonies from each plate for pectinolytic activity.

This method was used to estimate the population of pectinolytic *Erwinia* species in packinghouse waters used for dumping carrots; for washing carrots, radishes, tomatoes, peppers, and celery; and for hydrocooling radishes and celery. To determine the effect of *Erwinia* populations on incidence of bacterial soft rot, vegetable samples were collected before and after exposure to the packinghouse waters. Control samples collected after harvest and before exposure to packinghouse waters were washed under running tap water in the laboratory. All the vegetables except tomatoes were held for 4 days at 21 C before inspection. Tomatoes were held at 21 C up to 3 weeks or until they reached the red-ripe stage, with biweekly inspections.

The population of *Erwinia* cells per ml in wash water ranged from 1×10^4 for radishes to 5×10^5 for carrots and tomatoes. The population in the carrot dump tank water was 6×10^4, and in celery hydrocooler water, 1×10^4. Before exposure to these high *Erwinia* populations, the decay rates for radishes, celery, tomatoes, and peppers were 0%, 4%, 7%, 2%, and 0%, respectively, and after exposure, 84%, 45%, 47%, 26%, and 37%, respectively (Table 1).

TABLE 1. Concentrations of some *Erwinia* species in vegetable packinghouse waters and the effect of these concentrations on the incidence of soft rot

Vegetables and source of vegetables and water	% Decay after holding period[a]	*Erwinia* species populations (cells/ml water)
Carrots		
Control[b]	0	
Dump tank	68	6×10^4
Washer	84	5×10^5
Radishes		
Control[b]	4	
Washer	51	1×10^4
Hydrocooler	45	1×10^5
Celery		
Control[b]	7	
Washer	37	1×10^4
Hydrocooler	47	1×10^4
Tomatoes		
Control[b]	2	
Washer	26	5×10^5
Peppers		
Control[b]	0	
Washer	37	1×10^4

[a] Carrots, radishes, celery, and peppers were held 4 days at 21 C; tomatoes were held at 21 C until red-ripe stage with biweekly inspections.

[b] Washed in running water at laboratory.

The high population of *Erwinia* spp. present in the waters used during the commercial postharvest handling of vegetables has been demonstrated by the use of this selective medium. The high incidence of bacterial soft rot resulting from exposure to these high bacterial populations has been shown; thus, decay can be reduced by lowering the inoculum by recognized methods of sanitation such as chlorination of water (6, 8).

LITERATURE CITED

1. American Public Health Association, Inc. 1965. Standard methods for the examination of water and wastewater, New York, N. Y. 769 p.
2. Hansen, H. N., & W. C. Snyder. 1947. Gaseous sterilization of biological materials for use as culture media. Phytopathology 37:369-371.
3. Munnecke D. E., & M. P. Starr. 1967. Isolation of pathogens from diseased material, p. 15-17. Committee Amer. Phytopathol. Soc. Sourcebook of laboratory exercises in plant pathology. W. H. Freeman, San Francisco, Calif.
4. Schaad, N. W., & E. E. Wilson. 1970. Survival of Erwinia rubrifaciens in soil. Phytopathology 60:557-558.
5. Segall, R. H. 1967. Bacterial soft rot, bacterial necrosis, and Alternaria rot of tomatoes as influenced by field washing and postharvest chilling. Plant Dis. Reptr. 51:151-152.
6. Segall, R. H. 1968. Reducing postharvest decay of tomatoes by adding a chlorine source and the surfactant Santomerse F85 to water in field washers. Fla. State Hort. Soc. Proc. 81:212-214.
7. Segall, R. H. 1969. A differential medium for determining populations of Erwinia species causing soft rot. Phytopathology 59:1049 (Abstr.).
8. Splittstoesser, D. F., W. P. Wettergreen, & C. S. Pederson. 1961. Control of microorganisms during preparation of vegetables for freezing. I. Green beans. Food Technol. 15:329-331.