Inhibition of *Vibrio parahaemolyticus* by Sorbic Acid in Crab Meat and Flounder Homogenates

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ABSTRACT

The effect of sorbic acid on growth of three strains of *Vibrio parahaemolyticus* was studied using crab meat and flounder homogenate (pH 6.2). Addition of 0.05% sorbic acid resulted in delayed growth of all three strains of *V. parahaemolyticus* in crab meat and flounder homogenates. When 0.1% sorbic acid was incorporated into homogenates, no increase in numbers of the three strains occurred in the crab meat homogenate, and only slight increases occurred in the flounder homogenate.

*Vibrio parahaemolyticus* has been increasingly recognized as a cause of foodborne illness resulting from ingestion of contaminated seafood (9,14). Recent studies have reported the survival of *V. parahaemolyticus* in refrigerated and frozen seafood products (2,3,6,8,15). Rapid growth of *V. parahaemolyticus* in these products poses a potential health hazard if the product is subjected to temperature abuse.

Vanderzant and Nickelson (9) reported that *V. parahaemolyticus* can survive in a shrimp homogenate at pH values ranging from 6 to 10 without loss of viability. Robach and Hickey (10) demonstrated growth of three strains of *V. parahaemolyticus* in trypticase soy broth plus 2.5% NaCl at pH 5.5. Beuchat (1) demonstrated the growth of *V. parahaemolyticus* at pH 4.8 in trypticase soy broth plus 3.0% NaCl.

Chemical inhibition of growth of *V. parahaemolyticus* has focused mainly on the effect of NaCl concentration. Covert and Woodburn (3) demonstrated that NaCl has a protective effect on the viability of *V. parahaemolyticus* held at −18 C in Trypticase Soy broth. Gray and Muir (6) reported that *V. parahaemolyticus* survived in solutions of 0.5 M NaCl independent of temperature, but few organisms survived in a 0.01 M NaCl solution regardless of temperature. Emswiler and Pierson (4) found 100 mM potassium phosphate buffers (pH 6.7,8) without additional NaCl to be lethal to *V. parahaemolyticus*. Buffers containing 3% NaCl resulted in maximum survival.

Lee (7) reviewed Japanese work on the effect of 14 food preservatives against *V. parahaemolyticus* in a laboratory medium. He reported propylparaben to be effective at 0.05-0.1%. Robach et al. (12) reported that 50 ppm of butylated hydroxyanisole (BHA) inactivated *V. parahaemolyticus* 04:K11 in trypticase soy broth plus 2.5% NaCl, but 400 ppm of BHA were necessary to inactive the organism in a crab meat homogenate. Robach and Hickey (10) found that 0.2% potassium sorbate inhibited growth of three strains of *V. parahaemolyticus* in trypticase soy broth plus 2.5% NaCl at pH 6.0 and 0.05% potassium sorbate inhibited growth of the organisms at pH 5.5.

Sorbic acid and potassium sorbate, collectively known as the sorbates, have been used as antimicrobial agents in the food industry for over 30 years (5). Sorbates have been mainly used as antifungal agents in many foods and are GRAS (generally recognized as safe) food additives. Recently, sorbates have been found to exhibit antibacterial activity in fresh poultry (11) and in fish sausage (16). They also have been reported to inhibit growth of salmonellae in a cooked, uncured sausage (13) and in fresh poultry (11).

This study was designed to test the effectiveness of sorbic acid in inhibiting growth of three strains of *V. parahaemolyticus* in two different seafood homogenates incubated under conditions favorable to the rapid growth of the organism.

EXPERIMENTAL

Test organisms

Three strains of *V. parahaemolyticus* were used in this study: serotype 04:K11, ATCC 27519, and ATCC 17802. Stock cultures were transferred weekly by inoculating onto slants of Trypticase Soy agar (BBL) containing an additional 2.5% NaCl (TSAS; pH 7.0). Inoculum cultures were prepared by inoculating a 250-ml shake flask containing 50 ml of Trypticase Soy Broth with an additional 2.5% NaCl (TSBS) with a loopful of the slant culture and incubating for 15 h at 35 C.

Homogenate preparation

The crab meat and flounder homogenates were prepared by mixing 20 g of commercially pasteurized blue crab meat (*Callinectes sapidus*) for the crab meat homogenate and 20 g of fresh flounder fillet for
the flounder homogenate with 180 ml of distilled H₂O containing 3% NaCl. Mixtures were homogenized in a stomacher for 2 min at room temperature. Homogenates were then dispensed in 50-ml portions into 250-ml screw-capped Erlenmeyer flasks and autoclaved at 121 °C for 15 min. After cooling to room temperature, appropriate amounts of sorbic acid were aseptically added to the homogenates and the pH was adjusted to 6.2 with 8 N NaOH.

Growth studies

The growth flasks containing the sterile, pH 6.2 homogenates were inoculated with a 15-h culture of the appropriate test strain to an initial inoculum of approximately 10⁵ cells/ml. Flasks were incubated in a shaker water bath (American Optical, Buffalo, N.Y.) at 175 cycles per minutes and 35 °C. Samples of the homogenates were withdrawn at selected intervals, and serial dilutions were made in sterile 0.1 M potassium phosphate buffer (pH 7.2) containing 3.0% NaCl before pour plating with TSAS. After the agar had solidified, plates were overlaid with TSAS to prevent spreading and assure accurate counts. Plates were incubated at 35 °C and colonies were counted after 24 h.

RESULTS AND DISCUSSION

No growth of V. parahaemolyticus 04:K11 occurred for up to 48 h when 0.1% sorbic acid was added to the crab meat homogenate (Fig. 1). Addition of 0.05% sorbic acid to the crab meat resulted in a prolonged lag phase and subsequent growth of strain 04:K11 was slow (Fig. 1). An initial decrease in viable cells of strain 04:K11 through 8 h and slow growth through 30 h was observed when the flounder homogenate contained 0.1% sorbic acid (Fig. 2). A prolonged lag phase of 8 h occurred when 0.05% sorbic acid was added to flounder homogenate after which normal growth was observed (Fig. 2).

A sharp initial decrease in the number of viable V. parahaemolyticus 27519 was observed when 0.1% sorbic acid was incorporated into the crab meat homogenate (Fig. 3). Subsequent growth failed to reach the initial
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inoculum level of $1.8 \times 10^3$ cells/ml through 48 h. Addition of 0.05% sorbic acid to the crab meat resulted in a slight decrease of viable cells of strain 27519, but after 48 h of incubation counts were approaching those of the controls (Fig. 3). When 0.1% sorbic acid was added to the flounder homogenate, a decrease in viable cells of strain 27519 was observed through 30 h of incubation (Fig. 4). A decrease in number of viable cells of strain 27519 was also observed when 0.05% sorbic acid was incorporated into the flounder homogenate but growth was initiated after 24 h of incubation (Fig. 4).

An initial 2-log cycle decrease in the number of viable cells and no subsequent growth of V. parahaemolyticus 17802 was observed when 0.1% sorbic acid was added to the crab meat homogenate (Fig. 5). Addition of 0.05% sorbic acid to the crab meat resulted in a slight increase in the lag time of growth of strain 17802, but later growth was relatively fast (Fig. 5). Addition of 0.1% sorbic acid to the flounder homogenate resulted in an initial 1.5-log cycle decrease in the number of viable cells of strain 17802 (Fig. 6). Growth was initiated after 8 h of incubation, but it was slightly inhibited (Fig. 6). When 0.05% sorbic acid was added to the flounder homogenate, an extended lag phase for strain 17802 was observed (Fig. 6). Growth was initiated after 8 h of incubation but it also was slightly inhibited (Fig. 6).

Results obtained in this experiment indicate that sorbic acid has a definite antimicrobial effect against V. parahaemolyticus. Sorbic acid has been used for years as an effective antifungal agent, but only recently has its
antibacterial properties been reported (11, 13, 16). Results obtained here and in other studies show that sorbic acid and/or potassium sorbate are effective antibacterial agents even in substrates with pH values in the 6.0-6.3 range. While this research focused on two model seafood systems, further studies involving different food substrates and other microorganisms are under way.

REFERENCES