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Recovery of Chill-Stressed *Vibrio parahaemolyticus* from Oysters with Enrichment Broths Supplemented with Magnesium and Iron Salts

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The effects of magnesium and iron salts on the recovery and growth of chill-stressed cells of *Vibrio parahaemolyticus* were studied. Supplementation of glucose salt Teepol (GST) broth with 20 to 100 mM of Mg^{2+} significantly ($P \leq 0.05$) increased the number of cells recovered from oyster homogenate stored at 3°C. Populations detected with supplemented GST were comparable to those obtained with Horie arabinose ethyl violet (HAE) broth, with or without Mg^{2+}. Recovery of *V. parahaemolyticus* from homogenates stored at −18°C was also improved when enrichment broths supplemented with Mg^{2+} were used. Ferric iron (added as $FeCl_3$) at 240 μM in GST and 240 or 960 μM in HAE significantly enhanced the extent of recovery of chilled cells. Ferrous iron was generally less effective. Teepol did not influence the growth of nonchilled cells, but significantly reduced the viable population in suspensions of chilled cells when used at a level of 0.4% in GST. The relatively high pH (9.0) of HAE caused a significant reduction in the number of viable, chill-stressed cells of *V. parahaemolyticus*. The overall results indicated that HAE broth is superior to GST for recovering *V. parahaemolyticus* from refrigerated and frozen oyster homogenates.

Confirmation of several outbreaks of food-borne infection caused by *Vibrio parahaemolyticus* in the United States during the past decade has encouraged increased research effort to define optimal conditions for detection and enumeration. The bacterium is found primarily in brackish coastal waters and sediment and on sea life taken from these environments. The methodology for enumerating the organism on seafoods has been therefore largely directed toward shellfish.

Several researchers have demonstrated that *V. parahaemolyticus* is readily inactivated at refrigeration and freezing temperatures (2, 5, 9, 16, 18, 22, 24, 25). Like many bacterial genera, cells of *V. parahaemolyticus* may undergo injury upon exposure to refrigeration or freezing conditions (3, 17). Cells have been shown to exhibit signs of damage after exposure to a temperature of 2°C for periods of time as short as 30 min. Cold-stressed cells are more sensitive than are nonstressed cells to selective conditions in most enrichment broths. In such media, stressed cells may fail to repair their injured sites and appear nonviable. Sublethal injury can be repaired, however, if cells are exposed to suitable recovery environments. In general, the repair process is characterized by restoration of altered permeability, synthesis of the lost intracellular pool, regeneration of ribonucleic acid (particularly ribosomal ribonucleic acid), or synthesis of protein and adenosine triphosphate (4).

Methods currently used for detecting *V. parahaemolyticus* recommend the use of several enrichment media which contain various types of selective agents (7, 12). Little is known about the effects of these selective conditions on resuscitation of chill-injured cells. Furthermore, no reports have been issued describing the recovery of cold-injured *V. parahaemolyticus* in enrichment broths supplemented with various cations, although magnesium has been reported to benefit the stability and resuscitation of heat-damaged *V. parahaemolyticus* (10, 11) and *Staphylococcus aureus* (13, 15). The extent of dependency of injured cells of *V. parahaemolyticus* on iron during the period in which increased rates of synthesis of adenosine triphosphate may occur as part of the repair process has not been reported. The purpose of this study was to evaluate the effects of selective conditions of enrichment broths and magnesium and iron salts on the recovery of chilled *V. parahaemolyticus*.

MATERIALS AND METHODS

Organism and growth conditions. *V. parahaemolyticus* strain 8700 (serotype O4:K11) was used throughout the study. Tryptic soy broth (Difco, De-

troit, Mich.) supplemented to contain 3.0% NaCl (TSBS, pH 7.3) served as the medium to culture cells for all subsequent experiments designed to evaluate efficacy of enrichment broths. Stationary-phase cells (16 h at 30°C, with agitation) were used as test inocula.

Demonstration of chill injury. A portion (10 ml) of stationary-phase culture was transferred to a 500-ml Erlenmeyer flask containing 190 ml of 0.1 M potassium phosphate buffer (pH 7.3) supplemented with 3% NaCl (wt/vol) and equilibrated at 3°C (treated) or 30°C (control). After continuous stirring for 30 min, 1 ml of suspension was transferred to 100 ml of TSBS and TSB containing 8% NaCl. Growth of *V. parahaemolyticus* in these media was followed by measuring the absorbance of cultures at 620 nm over a 13-h period of incubation at 30°C. The viable population of cells in chilled and control salt buffers immediately before recovery in TSB containing 3 and 8% NaCl was determined by surface plating appropriate dilutions on thiosulfate-citrate-bile salts-sucrose agar (TCBS; BBL Microbiology Systems, Cockeysville, Md.). The initial number of viable cells in both chilled and control suspensions was adjusted to from 1×10^7 to 4×10^7 or 5×10^7/ml before inoculating recovery broths.

Evaluation of enrichment broths. (i) Studies with pure cultures. Two basal media were evaluated as enrichment broths for supporting recovery of chilled *V. parahaemolyticus*. Glucose salt-Teepol broth (GST, pH 7.4) contained, in grams per liter: peptone 10; beef extract, 3; NaCl, 30; glucose, 5; methyl violet, 0.002; and Teepol (Shell Chemical Co., Houston, Tex.), 4. Horie arabinose-ethyl violet broth (HAE, pH 9.0) (12) contained, in grams per liter: peptone, 5; beef extract, 3; NaCl, 30; bromothymol blue, 0.03; ethyl violet, 0.001; and arabinose, 5. Arabinose solutions were filter sterilized and aseptically added to heat-sterilized basal media. Suspensions of chilled and control cells prepared as described above were serially diluted in salt buffer, and enumeration was done by a three-tube most-probable-number (MPN) technique. Cultures were observed for turbidity after 18 to 22 h at 35°C.

Since cold-stressed bacterial cells often show increased sensitivity to selective agents other than NaCl, the presence of Teepol (a surface-active agent) in GST and the relatively high pH of HAE (pH 9.0) were examined for their potential inhibitory effects against chilled and control cells of *V. parahaemolyticus*. Suspensions of test cells were enumerated by the MPN technique in GST broth containing 0, 0.1, 0.2, 0.3, and 0.4% of Teepol and in HAE broth adjusted to pH 7.0, 7.5, 8.0, 8.5, and 9.0.

Two magnesium ($MgCl_2$ and $MgSO_4$) and three iron [$FeCl_3$, $Fe_2(SO_4)_3$, and $FeSO_4$] salts were tested for their effects on growth of *V. parahaemolyticus* in enrichment broths. Magnesium salts were added to GST and HAE at final concentrations (Mg^{2+}) of 20, 50, 100, and 150 mM; iron salts were added to give final concentrations (Fe^{2+} or Fe^{3+}) of 120, 240, 480, and 960 μM. Salts were dissolved in distilled water, sterilized by autoclaving, and aseptically added to cooled GST and HAE to minimize thermally induced precipitation or other chemical reactions with constituents in the media. Chilled and control cells were enumerated in these media by the MPN procedure. Confirmation of the presence of *V. parahaemolyticus* in all MPN tests was done by streaking turbid cultures on TCBS and incubating plates at 35°C for 18 to 22 h.

(ii) Studies with inoculated oysters. Oysters (*Crassostrea virginica*) purchased from a local fish market were comminuted with a Toledo grinder equipped with an extrusion disk with holes 0.5 cm in diameter. Salt buffer suspensions of cells of *V. parahaemolyticus* with viable populations of 4×10^5 to 5×10^5/ml were added to oyster homogenate at a ratio of 1:10 (vol/wt). After thorough mixing, 25-g samples were deposited in sterile plastic bags and immediately placed in forced-air chambers at 3 and −18°C. Duplicate inoculated and uninoculated samples were withdrawn after selected storage periods, combined with 225 ml of salt buffer, and homogenized with a stomacher (Lab-Blender 400, Dynatech Laboratories, Inc., Alexandria, Va.) for 1 min. Two enrichment broths (GST and HAE), with and without various concentrations of magnesium and iron, were evaluated for their suitability to support the repair and growth of *V. parahaemolyticus*. The MPN technique was used; positive cultures were streaked on TCBS, and a minimal number of biochemical tests were employed to confirm the presence of *V. parahaemolyticus* (7).

Oyster homogenates stored at 3 and −18°C were also examined for total aerobic populations by plating an appropriately diluted portion on plate count agar. Colonies were counted after 5 days of incubation at 21°C.

Statistical analyses. Data presented represent averages of a minimum of two replications run in duplicate. Duncan's multiple range test (6) was used throughout the study to test for statistically significant differences ($P \leq 0.05$).

RESULTS AND DISCUSSION

Demonstration and chill injury. The double salt technique, i.e., the use of a nutrient medium containing two concentrations of NaCl to culture stressed and control cells, often is used to detect injury. In the present study, an increased sensitivity to 8% NaCl in TSB was noted for cells of *V. parahaemolyticus* which had been treated at 3°C for 30 min compared with control cells. Thus, a scheme was established to study the recovery of chill-stressed *V. parahaemolyticus* in original and modified formulas of GST and HAE broths.

Teepol concentration in GST. One reason for increased sensitivity of stressed cells is that permeability barriers are not totally functional in excluding the entry of antimetabolites. Surface-active agents in recovery broths at concentrations innocuous to healthy cells may, at the same concentration, be detrimental to injured cells (20). It was therefore decided that tests should be conducted to determine the effects of Teepol on recovery of *V. parahaemolyticus* in GST broth. Results are listed in Table 1. The addition of Teepol at concentrations up to 0.4%, the recommended level in the standard formula

TABLE 1. *Effect of Teepol on growth of nonchilled and chilled cells of V. parahaemolyticus in GST enrichment broth*

Treatment	Teepol concn (%)	Mean $\log_{10}$ MPN per ml
Nonchilled	0	7.745[abc]
	0.1	8.569[a]
	0.2	8.243[abc]
	0.3	8.312[ab]
	0.4	7.994[abc]
Chilled	0	7.502[c]
	0.1	7.857[abc]
	0.2	8.380[abc]
	0.3	7.639[bc]
	0.4	5.966[d]

[a-d] Values not followed by the same letter are significantly different ($P \leq 0.05$).

(7), had no significant effect on recovery of nonchilled cells. However, at a concentration of 0.4%, Teepol significantly inhibited the recovery of chilled cells. These observations suggest that GST broth, as formulated by standard procedures, may fail to detect some *V. parahaemolyticus* in refrigerated or frozen seafoods.

pH of HAE. The optimal pH for growth of *V. parahaemolyticus* is in the range of 7.5 to 8.5. Realizing that suboptimal environmental conditions would exert additional stress on cells during resuscitation, tests were run to determine the effects of pH on recovery of chill-injured and control cells in HAE broth. Although this broth does not contain selective chemicals, the relatively high reaction (pH 9.0) apparently is lethal to a portion of the chill-injured cells (Table 2). Data indicate that highest populations of nonchilled cells were detected in HAE broth at pH 7.0 to 8.5, whereas a significant reduction in MPN was observed at pH 9.0 compared with that at pH 7.0. Results indicate that chilled cells are more sensitive to both neutral pH and pH 9.0 than are nonchilled cells. Based on these findings, it is tempting to advise that the pH of HAE broth be lowered to 8.5 when used as an enrichment broth for enumerating *V. parahaemolyticus* in chilled seafoods. However, it should also be realized that some loss in selectivity will occur with this reduction in pH.

Magnesium and iron salts in enrichment broths. (i) Studies with pure cultures. Preliminary studies designed to monitor the growth of chilled cells of *V. parahaemolyticus* spectrophotometrically revealed that certain magnesium and iron salts appeared to stimulate the repair process. Tests were then conducted to determine the effects of selected salts added to GST and HAE broths on recovery of *V. parahaemolyticus* by the MPN technique. Results from studies with magnesium-supplemented salts are summarized in Table 3. Supplementation of GST with $MgCl_2$ or $MgSO_4$ did not result in significant improvement of recovery of chilled or nonchilled cells. In fact, 150 mM Mg^{2+} (added as $MgCl_2$) appeared to be toxic. In HAE broth not containing added Mg^{2+}, significantly lower numbers of chilled cells were detected compared with the nonchilled group. The addition of Mg^{2+} (100 and 150 mM as a sulfate salt) to HAE broth significantly improved the recovery of chilled

TABLE 2. *Effect of pH on growth of nonchilled and chilled cells of V. parahaemolyticus in HAE enrichment broth*

Treatment	pH	Mean $\log_{10}$ MPN per ml
Nonchilled	7.0	8.821[a]
	7.5	8.663[ab]
	8.0	8.243[abc]
	8.5	8.533[abc]
	9.0	8.088[bc]
Chilled	7.0	7.994[cd]
	7.5	8.055[bc]
	8.0	8.533[abc]
	8.5	8.474[abc]
	9.0	7.452[d]

[a-d] Values not followed by the same letter are significantly different ($P < 0.05$).

TABLE 3. *Effect of Mg^{2+} on growth of nonchilled and chilled cells of V. parahaemolyticus in GST and HAE enrichment broths*

Recovery medium	Treatment	Mg^{2+} concn (mM)	Mean $\log_{10}$ MPN per ml	
			$MgCl_2$	$MgSO_4$
GST	Nonchilled	0	8.200[abcd]	8.131[abc]
		20	8.074[abcdef]	8.581[ab]
		50	8.568[a]	8.821[a]
		100	8.175[abcde]	8.406[ab]
		150	7.371[fg]	8.566[ab]
	Chilled	0	7.795[bcdef]	8.106[abc]
		20	8.568[a]	8.243[abc]
		50	8.337[ab]	8.200[abc]
		100	8.306[abc]	8.312[abc]
		150	6.934[g]	8.312[abc]
HAE	Nonchilled	0	8.474[ab]	7.872[bc]
		20	8.107[abcdef]	8.474[ab]
		50	7.994[abcdef]	8.318[abc]
		100	8.169[abcde]	8.337[ab]
		150	8.312[abc]	8.243[abc]
	Chilled	0	6.988[g]	6.370[ef]
		20	7.561[cdefg]	6.711[def]
		50	7.543[defg]	6.336[f]
		100	7.420[efg]	7.371[cd]
		150	6.988[g]	7.654[bcd]

[a-g] Values in the same column not followed by the same letter are significantly different ($P \leq 0.05$).

cells. This implies that the level of Mg^{2+} in HAE is more critical than that in GST and that perhaps synthesis of ribosomal ribonucleic acid, a process requiring Mg^{2+}, is occurring during the repair of cells. Magnesium has been reported by others to act cooperatively with Na^{+} to prevent the lysis of slightly halophilic *V. alginolyticus* by providing sufficient mechanical strength to the cell membrane (23) and to be necessary for certain enzymes involved in repair processes as well as for the stability of cell walls, membranes, and ribosomes of normal and cold-shocked gram-negative bacteria (1, 21). Magnesium is also required for repair of sublethal-heat-damaged bacteria (13), and the possibility has been suggested that there is impairment of the system necessary for passing Mg^{2+} from the surface of the cell to the interior sites which need Mg^{2+} (14). The observation from experiments reported here with Teepol indicating that membrane or cell wall damage or both are a characteristic of chilled cells of *V. parahaemolyticus* would appear to give credence to the latter proposition, since it could be assumed that cells with membrane damage would benefit by higher concentrations of Mg^{2+} in the recovery medium.

Iron is an essential component of the cytochrome system, acting as an electron acceptor and donor in the electron transport scheme to produce adenosine triphosphate. It might also play a critical role in the recovery of chill-injured *V. parahaemolyticus*, because increased energy requirements via adenosine triphosphate production have been noted to be necessary for repair of injured cells (19). Tests were therefore conducted to determine the effects of supplementing GST and HAE broths with iron salts on recovery of chilled *V. parahaemolyticus*. Results are presented in Table 4. Micromolar concentrations of Fe^{3+} (added as $FeCl_3$) gave trends similar to those observed in tests with millimolar concentrations of Mg^{2+}, whereas $Fe_2(SO_4)_3$ and $FeSO_4$ were generally ineffective. Concentrations of Fe^{3+} higher than 960 μM, added as chloride or sulfate salts, appeared to be toxic to both chilled and nonchilled cells. The transfer of iron across the cell membrane is accomplished by active transport systems. The smaller and more highly charged Fe^{3+} has higher affinity to bind with the carrier protein in the membrane, thus facilitating more rapid uptake from the external environment compared with Fe^{2+}. This may explain the apparent beneficial effect of Fe^{3+} compared with Fe^{2+} in the repair of chill-injured *V. parahaemolyticus*. Results suggest that cells of *V. parahaemolyticus* require increased energy production immediately after injury if maximum recovery is to be achieved in HAE broth.

TABLE 4. *Effect of Fe^{3+} and Fe^{2+} on growth of nonchilled and chilled cells of V. parahaemolyticus in GST and HAE enrichment broths*

Recovery medium	Treatment	Fe^{3+} or Fe^{2+} concn (μM)	Mean $\log_{10}$ MPN per ml		
			$FeCl_3$	$Fe_2(SO_4)_3$	$FeSO_4$
GST	Nonchilled	0	7.745^{ab}	7.857^{abc}	7.953^{a}
		120	8.106^{ab}	8.226^{abc}	7.904^{a}
		240	8.238^{a}	8.243^{abc}	7.651^{a}
		480	8.038^{ab}	8.380^{ab}	7.502^{a}
		960	7.857^{ab}	7.772^{bc}	7.458^{a}
	Chilled	0	8.057^{ab}	7.642^{bc}	7.623^{a}
		120	8.106^{ab}	7.661^{bc}	8.063^{a}
		240	8.200^{a}	8.117^{abc}	7.842^{a}
		480	7.937^{ab}	7.842^{abc}	8.088^{a}
		960	8.103^{ab}	7.910^{abc}	7.655^{a}
HAE	Nonchilled	0	7.890^{ab}	8.157^{abc}	8.169^{a}
		120	7.334^{abc}	8.269^{abc}	7.904^{a}
		240	7.814^{ab}	8.601^{a}	8.041^{a}
		480	7.994^{ab}	8.446^{ab}	8.041^{a}
		960	7.759^{ab}	8.175^{abc}	7.835^{a}
	Chilled	0	6.185^{c}	7.524^{c}	7.259^{a}
		120	6.995^{abc}	7.977^{abc}	8.124^{a}
		240	6.852^{bc}	7.994^{abc}	7.724^{a}
		480	7.208^{abc}	7.627^{bc}	7.826^{a}
		960	7.549^{ab}	8.175^{abc}	7.745^{a}

[a-c] Values in the same column not followed by the same letter are significantly different ($P \leq 0.05$).

(ii) Studies with oysters. Based on results obtained from pure culture studies, selected levels of Mg^{2+}, Fe^{3+}, and Fe^{2+} were tested for their effects on recovery of *V. parahaemolyticus* from refrigerated and frozen oyster homogenates. The organism was not detected in oysters purchased from the fish market. Data showing the effects of adding Mg^{2+} to GST and HAE enrichment broths on the recovery of *V. parahaemolyticus* from homogenates stored at 3°C are listed in Table 5. A significant reduction in population occurred during the 6-day storage period, regardless of the enrichment broth used for enumeration, confirming the known sensitivity of *V. parahaemolyticus* to refrigeration temperatures. This observation was also made in trials with GST and HAE broths supplemented with iron salts.

Comparison of subscript letters for mean values within each column of data for $MgCl_2$-supplemented broths revealed some differences in the performance of media. After 3 days of storage, the presence of 20 and 100 mM Mg^{2+} in GST broth significantly increased the number of cells recovered to a level comparable to that

TABLE 5. *Effect of Mg^{2+} in GST and HAE enrichment broths on recovery of V. parahaemolyticus from oyster homogenate stored at 3°C*

Test salt	Recovery medium	Mg^{2+} concn (mM)	Log₁₀ MPN per g of oyster			
			Day 0	Day 1	Day 3	Day 6
$MgCl_2$	GST	0	$^{a}4.769_{a}$	$^{a}4.476_{ab}$	$^{b}1.540_{d}$	$^{b}0.602_{b}$
		20		$^{a}6.026_{a}$	$^{b}3.633_{a}$	$^{c}1.118_{ab}$
		100		$^{a}5.301_{ab}$	$^{b}2.586_{bc}$	$^{b}0.900_{b}$
	HAE	0	$^{a}6.405_{a}$	$^{b}4.633_{ab}$	$^{c}3.665_{a}$	$^{d}1.269_{ab}$
		20		$^{a}4.998_{ab}$	$^{b}3.362_{ab}$	$^{b}2.301_{a}$
		100		$^{a}3.998_{ab}$	$^{b}1.982_{cd}$	$^{b}1.862_{ab}$
$MgSO_4$	GST	0	$^{a}4.769_{a}$	$^{a}4.476_{b}$	$^{b}1.540_{b}$	$^{b}0.602_{a}$
		20		$^{a}6.269_{a}$	$^{b}3.498_{a}$	$^{b}1.638_{a}$
		100		$^{a}6.072_{ab}$	$^{b}3.165_{a}$	$^{c}0.900_{a}$
	HAE	0	$^{a}6.405_{a}$	$^{b}4.633_{ab}$	$^{c}3.468_{a}$	$^{d}1.269_{a}$
		20		$^{a}4.998_{ab}$	$^{ab}3.362_{a}$	$^{b}2.301_{a}$
		100		$^{a}4.780_{ab}$	$^{b}3.362_{a}$	$^{b}2.133_{a}$

[a-d] Comparisons of mean values for significant differences are made only within test salts. Values in the same row which are not preceded by the same superscript are significantly different at $P \leq 0.05$. Values in the same column which are not followed by the same subscript are significantly different ($P \leq 0.05$).

observed in HAE broth, with or without $MgCl_2$. The addition of $MgSO_4$ to GST broth also enhanced recovery. Significant increases were noted after 1 and 3 days of storage. The addition of $MgSO_4$ to HAE broth did not appear to enhance recovery of *V. parahaemolyticus*.

Table 6 lists data from experiments run to determine the effect of Mg^{2+} on recovery of *V. parahaemolyticus* from oyster homogenate stored at −18°C. The effect of storage time indicates that cells were extremely sensitive to freezing conditions. The presence of 20 mM Mg^{2+} (either as $MgCl_2$ or $MgSO_4$) in GST broth had a significantly beneficial effect. The overall results indicate that HAE broth performed better than did nonsupplemented GST broth for recovering *V. parahaemolyticus* from frozen oyster homogenate.

Data from tests with GST and HAE supplemented with iron salts showed that 240 μM Fe^{3+} (added as $FeCl_3$) in GST broth and 240 or 960 μM in HAE broth resulted in the recovery of significantly higher numbers of cells from refrigerated oyster homogenate after 1 day of storage compared with nonsupplemented broths (Table 7). Tests with $Fe_2(SO_4)_3$ and $FeSO_4$ revealed that neither had a significant effect on the recovery of *V. parahaemolyticus* from refrigerated oyster homogenate when compared with respective controls. Overall, HAE broth supplemented with $Fe_2(SO_4)_3$ performed better than did nonsupplemented GST broth.

The addition of iron to GST and HAE broths did not result in significant increases in numbers of *V. parahaemolyticus* detected in frozen oysters compared with respective nonsupplemented

TABLE 6. *Effect of Mg^{2+} in GST and HAE enrichment broths on recovery of V. parahaemolyticus from oyster homogenate stored at −18°C*

Medium	Mg^{2+} concn (mM)	Log₁₀ MPN per g of oyster			
		$MgCl_2$		$MgSO_4$	
		Day 0	Day 8	Day 0	Day 8
GST	0	$^{a}4.769_{a}$	$^{b}0.841_{b}$	$^{a}4.769_{a}$	$^{b}0.841_{b}$
	20		2.998_{a}		3.072_{a}
	100		2.165_{ab}		2.665_{ab}
HAE	0	$^{a}6.405_{a}$	$^{b}3.468_{a}$	$^{a}6.405_{a}$	$^{b}3.468_{a}$
	20		3.633_{a}		3.618_{a}
	100		2.362_{ab}		2.998_{a}

[a-b] Values in the same row within each test salt which are not preceded by the same superscript are significantly different ($P \leq 0.05$). Values in the same column which are not followed by the same subscript are significantly different ($P \leq 0.05$).

broths (Table 8). Again, however, the overall results indicate that HAE broth was superior to GST broth.

The recovery of *V. parahaemolyticus* from oyster homogenate with various enrichment broths is influenced not only by factors such as surface-active agents, pH, and inorganic salts, but also by the microflora naturally present at the time of analysis. Certain species of *Pseudomonas* isolated from oysters have been shown to repress the growth of *V. parahaemolyticus* (8). Total aerobic plate counts for refrigerated oyster homogenates used in the present study increased significantly during the 6-day storage period; counts for frozen homogenates decreased significantly during 8 days of storage. No attempts

TABLE 7. *Effect of Fe^{3+} and Fe^{2+} in GST and HAE enrichment broths on recovery of V. parahaemolyticus from oyster homogenate stored at 3°C.*

Test salt	Recovery medium	Fe^{3+} or Fe^{2+} concn (μM)	Log_{10} MPN per g of oyster			
			Day 0	Day 1	Day 3	Day 6
$FeCl_3$	GST	0	$^{a}6.072_{a}$	$^{b}4.405_{d}$	$^{c}1.962_{a}$	$^{d}0.477_{b}$
		240		$^{a}5.618_{bc}$	$^{b}2.837_{a}$	$^{c}0.477_{b}$
		960		$^{a}4.801_{cd}$	$^{ab}2.618_{a}$	$^{b}0.477_{b}$
	HAE	0	$^{a}6.665_{a}$	$^{b}5.665_{bc}$	$^{c}3.362_{a}$	$^{d}1.011_{a}$
		240		$^{a}5.968_{ab}$	$^{b}3.801_{a}$	$^{c}1.158_{a}$
		960		$^{a}6.871_{a}$	$^{b}3.301_{a}$	$^{c}1.269_{a}$
$Fe_2(SO_4)_3$	GST	0	$^{a}6.072_{a}$	$^{b}4.405_{b}$	$^{c}1.962_{c}$	$^{d}0.477_{c}$
		240		$^{b}5.362_{ab}$	$^{b}2.609_{abc}$	$^{c}0.477_{c}$
		960		$^{a}5.208_{ab}$	$^{b}2.118_{bc}$	$^{b}0.477_{c}$
	HAE	0	$^{a}6.665_{a}$	$^{b}5.665_{ab}$	$^{c}3.362_{abc}$	$^{d}1.011_{b}$
		240		$^{a}6.072_{a}$	$^{b}3.665_{ab}$	$^{c}1.269_{ab}$
		960		$^{a}6.133_{a}$	$^{a}3.998_{a}$	$^{b}1.645_{a}$
$FeSO_4$	GST	0	$^{a}4.998_{a}$	$^{a}4.862_{ab}$	$^{b}1.827_{a}$	$^{b}0.477_{a}$
		120		$^{a}4.665_{b}$	$^{ab}1.723_{a}$	$^{a}0.477_{a}$
		480		$^{a}4.658_{b}$	$^{a}2.055_{a}$	$^{a}0.477_{a}$
	HAE	0	$^{a}6.405_{a}$	$^{a}6.112_{ab}$	$^{a}4.665_{a}$	$^{b}1.419_{a}$
		120		$^{a}6.301_{ab}$	$^{ab}4.498_{a}$	$^{b}1.919_{a}$
		480		$^{a}6.362_{a}$	$^{a}5.165_{a}$	$^{b}1.837_{a}$

[a-d] Comparisons of mean values for significant differences are made only within test salts. Values in the same row which are not preceded by the same superscript are significantly different ($P \leq 0.05$). Values in the same column which are not followed by the same subscript are significantly different ($P \leq 0.05$).

TABLE 8. *Effects of Fe^{3+} and Fe^{2+} in GST and HAE enrichment broths on recovery of V. parahaemolyticus from oyster homogenate stored at −18°C*

Test salt	Recovery medium	Fe^{3+} or Fe^{2+} concn (μM)	Log_{10} MPN per g of oyster	
			Day 0	Day 8
$FeCl_3$	GST	0	$^{a}6.072_{a}$	$^{b}1.176_{b}$
		240		1.534_{ab}
		960		1.223_{b}
	HAE	0	$^{a}6.665_{a}$	$^{b}3.165_{ab}$
		240		3.665_{a}
		960		3.801_{a}
$Fe_2(SO_4)_3$	GST	0	$^{a}6.072_{a}$	$^{b}1.176_{b}$
		240		1.034_{b}
		960		1.222_{b}
	HAE	0	$^{a}6.665_{a}$	$^{b}3.165_{a}$
		240		3.665_{a}
		960		3.665_{a}
$FeSO_4$	GST	0	$^{a}4.998_{a}$	$^{b}1.407_{b}$
		120		1.176_{b}
		480		1.362_{b}
	HAE	0	$^{a}6.405_{a}$	$^{b}3.497_{a}$
		120		3.497_{a}
		480		3.665_{a}

[a-b] Comparisons of mean values for significant differences are made only within test salts. Values in the same row which are not preceded by the same superscript are significantly different ($P \leq 0.05$). Values in the same column which are not followed by the same subscript are significantly different ($P \leq 0.05$).

were made to determine the rates of growth of natural microflora in GST and HAE broths; however, the growth response of various genera to constituents in the two media may have been different and consequently may have influenced the ability of injured *V. parahaemolyticus* to undergo repair and eventual cell division.

In summary, the beneficial effects of adding Mg^{2+} and Fe^{3+} to enrichment broths on recovery of cold-stressed cells of *V. parahaemolyticus* have been demonstrated. Omission of Mg^{2+} or Fe^{3+} or both from enrichment broths may lead to an underestimation of the extent of contamination in refrigerated and frozen seafoods. Further studies are needed to establish optimal levels of Mg^{2+} and Fe^{3+} in GST and HAE broths which would give maximal recovery of *V. parahaemolyticus.*

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