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Enrichment Broths for Enumeration of *Vibrio parahaemolyticus*

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Eight enrichment broths were studied for enumeration of *Vibrio parahaemolyticus* in finfish and shellfish of marine and brackishwater origins employing the three tube most probable number method. Nutrient broth eosin-y, Horie's arabinose ethyl violet broth, alkaline peptone water and salt-water yeast extract broth were found equally efficient for enumeration of *V. parahaemolyticus*. Others were not very effective. Nutrient broth eosin-y gave consistent results and hence was the best.

Key words: Enrichment broth, *V. parahaemolyticus*, enumeration

Vibrio parahaemolyticus is a potential entero-pathogen. The recommended limit of this organism in fish products is 100 cfu/g. (Anon, 1974). Its enumeration in seafood and seawater is usually carried out by the most probable number (MPN) method.

Several enrichment broths are used for enumeration of *V. parahaemolyticus* from fish and fish products. Twedt (1984) recommended glucose salt teepol broth (GSTB). FDA subsequently modified it by substituting with alkaline peptone water (APW) or alkaline peptone salt broth (APSB) containing 3% sodium chloride (Elliot, *et. al.*, 1995). Salt polymyxin-B broth (SPB), a forerunner of salt polymyxin-E (Colistin) broth was used by Sakazaki, et. al. (1979). SPB has been recommended by ICMSF (Anon, 1978). Peterson (1979) found that Horie's arabinose ethyl violet broth (HAEB) yielded MPN values 10 times higher than GSTB. Kaneko and Colwell (1973) used salt-water yeast extract broth (SWYEB). Hofer and Silva (1984) found nutrient broth supplemented with 2% sodium chloride and 500 mg/100 ml eosin-y (NBEY) more effective. Thiosulfate citrate bile salts sucrose broth (TCBSB) was suggested by Taylor *et. al.* (1982).

The relative efficiencies of different enrichment broths for enumeration of *V. parahaemolyticus* in finfish and shellfish of marine and brackishwater origins are reported in this paper.

Materials and Methods

Eight enrichment broths, viz., glucose salt teepol broth, alkaline peptone water (Furniss *et. al.*, 1978), Horie's arabinose ethyl violet broth, salt polymyxin broth, nutrient broth eosin-y, salt water yeast extract broth, salt trypticase soy broth (STSB)

(Nakanishi and Murase, 1974) and thiosulfate citrate bile salts sucrose broth (Taylor *et. al.*, 1982) were prepared. Selective agents, NaCl content and pH of different enrichments are given in Table 1.

Table 1. Selective agents, NaCl content and pH of different enrichment broths.

Broth	Selective agent	NaCl, %	pH
GSTB	Teepol	3.0	7.4
STSB	Salt	7.0	7.6
APW	-	1.0	8.4-8.6
SPB	Polymyxin-B	2.0	8.6-9.0
HAEB	Ethyl violet	3.0	9.0
NBEY	Eosin	2.0	8.3
SWYEB	Salt	2.4	7.2-7.4
TCBSB	Sodium cholate	1.0	8.6

Nine fresh finfish and shellfish of marine and brackishwater origin were collected aseptically in separate polythene bags from local markets and landing centres (Table 2). Surface tissue of finfish and abdominal portion of shrimp with exoskeleton were the preferred cites. Three tube most probable number (MPN) method (Anon, 1995) was employed for enumeration of *V. parahaemolyticus*. All enrichment broths were used simultaneously for each sample. The characteristically large colonies (3-4 mm dia) with blue or green centre on TCBS agar plates were regarded as presumptive *V. parahaemolyticus* and were further subjected to biochemical tests (Sakazaki, 1973) for confirmation. Counts were subsequently derived from the MPN Table (Anon, 1969).

Table 2. *V. parahaemolyticus* counts in 9 fish/shellfish samples using eight enrichment broths by MPN method

Sample	*V. parahaemolyticus* counts MPN/g using							
	GSTB	STSB	APW	SPB	HAEB	NBEY	SWYEB	TCBSB
Sardine, *Sardinella longiceps**	61	N.D.	$2.4x10^7$	N.D.	$4.2x10^5$	$5.3x10^4$	$2.4x10^7$	N.D.
Tilapia, *Oreochromis massambicus***	$7.5x10^3$	$9.3x10^5$	$2.0x10^5$	14	$1.1x10^6$	$1.1x10^6$	$6.4x10^5$	N.D.
Prawn								
*Penaeus indicus**	$2.4x10^5$	$1.6x10^5$	$4.6x10^6$	$1.5x10^5$	$2.4x10^7$	$4.6x10^6$	$1.1X10^6$	N.D.
*P. indicus**	$4.3x10^5$	$1.5x10^5$	$2.4x10^6$	93	$2.4x10^6$	$2.4x10^6$	$4.6x10^6$	N.D.
*P. indicus**	$4.3x10^4$	$1.6x10^5$	$1.6x10^6$	$2.3x10^3$	$4.6x10^5$	$9.3x10^5$	$2.1x10^6$	$2.3x10^4$
*P. indicus**	$3.4x10^3$	$2x10^5$	$7.5x10^3$	$3.6x10^3$	$1.1x10^5$	$7.5x10^5$	$4.6x10^5$	$4.3x10^3$
*P. indicus***	$2.1x10^5$	$7.5x10^3$	$2.4x10^5$	240	$2.3x10^4$	$1.5x10^5$	$2.4x10^4$	390
*Parapeneopsis stylifera**	360	$3.6x10^5$	$4.6x10^5$	$6.4x10^3$	$7.5x10^5$	$2.9x10^4$	$1.5x10^6$	$9.3x10^4$
*Macrobrachium rosenbergii***	$2.7x10^3$	$2.3x10^4$	$3.6x10^3$	$7.5x10^3$	$4.6x10^4$	$1.5x10^5$	$3.6x10^4$	240

* Marine, ** Brackishwater, N.D = Not detected

Results and Discussion

V. parahaemolyticus counts in the samples are given in Table 2. Higher counts were observed with NBEY, HAEB, SWYEB and APW. Statistical analysis of the logarithms of the counts using ANOVA technique (Table 3) showed no significant difference between the above four enrichment broths. The coefficient of variation for NBEY was the least (Table 4) and gave more consistent results than the other three.

Table 3. ANOVA of the counts obtained using NBEY, HAEB, SWYEB and APW

Source	ss	df	ms	F
Total	209.7900	43	-	-
Broth	0.7219	3	0.2406	<1
Error	209.0681	40	5.2267	

Table 4. Coefficient of variation of the four efficient enrichment broths

Broth	CV (%)
NBEY	38.03
HAEB	41.85
SWYEB	52.50
APW	55.18

Eyles *et. al.* (1985) studied the efficiency of APW, GSTB and HAEB for enumeration of *V. parahaemolyticus* from oyster and found APW more efficient. Nakanishi & Murase (1974) demonstrated the superiority of SPB over GSTB and STSB. Sakazaki *et. al.* (1979) showed that SPB was more effective than GSTB for enumeration of *V. parahaemolyticus* in naturally contaminated seafood. Hofer & Si va (1984) evaluated the efficiency of seven enrichment broths and got best results with NBEY. The present results agree with those of Hofer & Silva (1984). Karunasagar *et. al.* (1986) found that direct plating on TCBS agar gave better results than MPN techniques for samples with high count (10^4-10^6 cells/ml of homogenate) of *V. parahaemolyticus.* But Eyles *et. al.* (1985) reported that MPN technique with APW as the enrichment broth gave better recovery of *V. parahaemolyticus* than spread plating on TCBS. The present study proves that NBEY is the most efficient enrichment broth among those studied for enumeration of *V. parahaemolyticus* from finfish and shellfish of marine and brackishwater origin.

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