

PART II  
SCIENTIFIC AND TECHNICAL  
STUDIES ON ISOLATION OF SALMONELLA  
FROM SEA FOODS  
1. COMPARISON OF ENRICHMENT AND SELECTIVE  
MEDIA FOR RECOVERY OF SALMONELLAE  
FROM FISH.

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Three enrichment broths and six plating media were compared for efficiency of detection of *Salmonella* in the presence of high numbers of Coliforms ( $10^5$ /ml) and proteus ( $10^3$ /ml) from artificially inoculated fish samples. Recovery experiments with *Salmonella anatum*, *S. typhimurium* and *S. enteritidis* indicated that the two enrichment broths viz. Dulcitol Selinite (DSE) and Selinite Cystine (SC) were equally efficient. Further, the viability of *Salmonella*, inoculated into fish muscle and kept at 4° C for 48 hours, was found to be not affected by the low temperature storage. Selective plating media like Xylose Lysine Deoxycholate agar (XLD), Brilliant Green Sulphadiazine agar (BGS) and Brilliant Green agar (BG) were found to be superior in performance to *Salmonella-Shigella* agar: (SS) and Bismuth Sulphite agar (BiS).

#### INTRODUCTION

Testing of processed food materials for the presence or absence of enteropathogens like *Salmonella* has become a routine necessity in view of the increasing emphasis on consumer protection. This is more so in the case of sea food products where the product is prone to quick spoilage and extraneous contamination. Occurrence of *Salmonella* in sea foods has been reported by some workers (Liston *et. al.*, 1971; Anon, 1966) although one of the essential requirements in such foods is the total absence of salmonella in the raw and processed material.

With the need for means of detecting salmonellae in food, many workers (North and Bartram, 1953; Osborne and Stokes, 1955; Hajna and Daman, 1956; Hobbs, 1960; Montford and Thatcher, 1961; Jameson, 1962; Silliker *et. al.*, 1964; Raj, 1966; Galton and Morris, 1968) applied and compared the various methods normally followed for isolation and recovery of the salmonella organisms. They found that these media were not completely satisfactory as some of the food products showed adverse effects on the selectivity of the enrichment quality of the media. (Galton *et. al.*, 1968).

Since there exists a wide choice of media intended for salmonellae detection, it is hard to assess whether media and methods employed by different workers on varied food products at various geographical localities produced comparable results. Raj (1966) reported that many recommended media failed to isolate these organisms in frozen sea foods. This apparent lack of consistency in literature caused us to make a comparative study on the efficacy of the common enrichments viz. Selenite Cystine and Tetrathionate and the newer enrichment medium viz. DSE described by Raj (1966). Attempts were also made to compare the effectiveness of different selective media on the isolation of small numbers of salmonella in presence of high numbers of coliform and proteus artificially inoculated into fish substrates.

#### MATERIALS AND METHODS

##### Comparison of selective agar media:

Seven pure type cultures of salmonellae listed in Table I were used in this study. Cultures were grown in Brain Heart Infusion broth (Difco) at 37°C. The growths after suitable decimal dilution in normal saline were plated in various selective media viz. MacConkey agar (MC) Difco; Salmonella Shigella agar (SS) Difco; Bismuth Sulphite agar (BiS) Difco; Brilliant green agar (BG); Brilliant Green Sulphadiazine agar (BGS) (Morris and Dunn, 1970) and Xylose Lysine Deoxycholate agar (XLD) (Taylor, 1965). The counts were made after incubation of plates at 37°C for 48 hours and compared with those simultaneously grown on Triptone glucose extract agar where growth was taken as hundred percent.

##### Comparison of enrichment media:

Organisms were grown in Brain heart infusion broth for 24 hours at 37°C. The growth was, then decimally diluted with *n. saline* till the final dilution contained less than 10 cells/ml. Ten ml. of

this dilution were used for inoculations into the respective enrichment broth (90 ml) so that the final concentration of the cells of the enrichment medium came to less than 2/ml. Likewise suitable suspensions of *E. coli.* & *Proteus spp.* in *n. saline* were added to the broths such that the final concentration of these cells were  $10^5$  and  $10^3$ /ml respectively. The broths were incubated at 37°C for 24 to 48 hours. Suitable dilutions (1: 100 & 1: 1000) from the enrichment broths were streaked on the different selective media viz. MC agar, Bis agar, SS agar, BG agar, BGS agar, and XLD agar in duplicate.

Suspected salmonella colonies were carefully picked and streaked in Triple Sugar Iron agar (TSI). The cultures positive on TSI agar were inoculated heavily into urea agar, lactose, dulcitol and lysine iron agar. Only cultures showing biochemical conformity with the genus salmonella were confirmed serologically by agglutination with polyvalent 'O' antiserum Recovery from fish substrates:

Low numbers of three species of salmonella were separately inoculated into 25 g. of fresh minced fish muscle having a general bacterial load of  $10^4$ /g. The flasks containing the inoculated muscle were kept at a temperature of 4°C for 48 hours after which 25 ml of sterile standard phosphate buffer at pH 7.2 were added to each flask and shaken thoroughly for 10 mts. Fifty ml of double strength enrichment broth were added to each flask, one set was kept at 37°C and another set at 43°C for 24 hours. The higher temperature of incubation was employed because of its reported superiority for salmonella enrichment (Carlson *et. al.*, 1967; Harvey and Price, 1968; Morris and Dunn, 1970). The enriched culture was suitably diluted and streaked on the six different selective media described earlier. The growth of the salmonellae in different media was qualitatively assessed. (See Table 3 & 4).

TABLE NO. 1 PERCENTAGE RECOVERIES OF DIFFERENT STRAINS OF SALMONELLA USED ON VARIOUS SOLID MEDIA.

(Growth on TGE Agar taken as 100%)

Test organism*	Selective media					
	SS agar	BG agar	BGS agar	Mac- Conkey agar	XLD agar	BS agar
<i>S. typhimurium</i>	59	82	87	75	85	67
<i>S. anatum</i>	62	90	92	81	90	61
<i>S. typhi</i>	69	84	89	82	89	87
<i>S. paratyphi</i> A.	64	80	83	71	81	68
<i>S. paratyphi</i> B.	61	81	82	73	81	69
<i>S. paratyphi</i> C.	67	84	85	75	87	70
<i>S. enteritidis</i>	63	81	80	78	86	73

\* Strains received from National Salmonella and Escherichia Centre, Central Research Institute, Kasauli.

#### RESULTS AND DISCUSSION

The data presented in Table I compare different selective plating media which include new plating medium XLD and other conventional ones. The results given are of average percentage recoveries as compared with 100% count obtained on TGE agar. Taking TGE Agar as a completely non-inhibitory medium, it is found that the percentage recovery in other media was less than 100. Obviously the selective media used here for the detection of salmonella have slight inhibitory effect. The variation of the inhibitory effect may be due to the ingredients present. Maximum recovery (ca. 90%) was observed in BG, BGS, and XLD. No significant difference could be found between the new medium viz. XLD and the conventional BGS. Both media were observed to be better in performance than BiS agar, SS agar and other selective media.

The growth of salmonella is readily detected within 24 hours on BG agar and BGS agar by its characteristic pink coloured colonies which can be easily recognised. However, it was found that beyond 24 hours incubation, the entire plate became pink, thus making it difficult to distinguish the characteristic pink colonies of salmonella from colonies of *Proteus*.

With regard to the comparative efficiency of the three enrichment broths (Table 2) with very low numbers of salmonella in presence of high numbers of Coliforms, it was observed that DSE medium shows the maximum isolation with the seven salmonella strains artificially inoculated. Among the other two enrichment broths, selenite cystine performs better. The inhibition of Coli-form and proteus in DSE was comparatively more than with the other two as seen from

TABLE II ISOLATION OF SALMONELLA FROM VARIOUS ENRICHMENT BROTHS AND SELECTIVE PLATING MEDIA IN THE PRESENCE OF *E. COLI.* ( $10^5/m$ ) AND *PROTEUS MIRABILIS* ( $10^3/ml$ ).

Test organism	Tetrathionate enrichment							Selenite Cystine enrichment						Dulcitol selenite enrichment.					
	Inoculum cells per ml.	MacConkey agar	B. G. agar	B. G. S. agar	Bi S. agar	S. S. agar	X. L. D. agar	MacConkey agar	B. G. agar	B. G. S. agar	Bi. S. agar	S. S. agar	X. L. D. agar	MacConkey agar	B. G. agar	B. G. S. agar	Bi S. agar	S. S. agas	X. L. D. agar
<i>S. typhimunium</i>	12	+	+	+	-	-	+	+	+	+	+	-	+	++	++	++	+	-	++
<i>S. para A.</i>	9	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>S. anatum</i>	10	-	-	+	+	-	-	-	+	+	-	+	+	+	+	++	+	+	++
<i>S. para B.</i>	19	-	-	+	-	-	+	-	+	+	+	+	+	+	+	+	-	-	+
<i>S. para C.</i>	7	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	+
<i>S. enteritidis</i>	14	-	-	+	-	-	+	-	+	++	+	-	+	-	+	++	+	+	++
<i>S. typhi</i>	6	-	-	-	-	-	+	-	+	+	+	-	+	-	+	+	+	-	+

+ = moderate growth; ++ = good growth; - = nil growth.

the recovery of the cultures in selective media. It is probable that Dulcitol favours the growth of the salmonellae under study.

Recently, many workers (Carlson et al 1967, Morris and Dunn 1970) reported that the performance of enrichment broths incubated at higher temperature (43°C) was significantly superior to that at 37°C. But in our present study with the fish substrates, it has been found that there is no significant difference between the results obtained at these two temperatures. Both DSE medium and SC medium proved to be comparatively effective than the tetrathionate for the recovery of salmonella from fish (Vide Tables 3 and 4). It is probable that fish muscle has an adverse effect on the performance of Tetrathionate medium. The low temperature storage at 4°C for 48 hours of the salmonella inoculated fish sample did not affect its recovery. Raj (1966) demonstrated that DSE medium was highly selective in the presence of larger numbers of other micro-organisms in the fish homogenate. We, on the other hand, found that both DSE & Selenite Cystine broths are equally efficient in the recovery of low numbers of salmonella from fish substrates.

Tables 3 and 4 indicate the relative efficiency of the enrichment broths and the differential plating media. The performance of the various media indicate that BGS and XLD are superior. Recently, different workers (Taylor 1965, McCarthy 1966, Rollender et al 1969, and Morris et al. 1970) found that XLD medium performs better than the other traditional media. The advantage of XLD over the other media may be on its more differential system. Most of the salmonella organisms utilize xylose rapidly whereas the coliform, proteus and other non-lactose fermenting organisms do not utilize this carbohydrate. Hence, the preferred pro-

cedure for isolating small numbers of salmonella from fish is to use either DSE or selenite cystine as enrichment medium and the BGS or XLD agar, as the selective plating medium.

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TABLE III ISOLATION OF SALMONELLA ORGANISM IN THE FISH MUSCLE FROM VARIOUS ENRICHMENT BROTHS AND SELECTIVE MEDIA INCUBATED AT 37°C.

Test organism	Inoculum cells/flask	Tetrathionate enrichment						Selenite Cystine enrichment						Dulcitol selenite enrichment					
		Mc. agar	B.G. agar	BGS agar	BiS. agar	S. S. agar	XLD agar	Mc. agar	B. G. agar	BGS agar	BiS. agar	S. S. agar	XLD agar	Mc. agar	B.G. agar	BGS agar	BiS. agar	S. S. agar	XLD agar
<i>S. anatum</i>	32	—*	—	+	—	—	—	—	++	++	+	—	++	+	+	++	+	—	++
<i>S. enteritidis</i>	22	—	—	—	—	—	—	—	+	++	—	—	++	—	+	++	—	+	++
<i>S. typhimunium</i>	37	—	+	+	+	—	—	—	+	++	—	+	++	—	+	++	—	+	++

\* Recovery results; (++) or (+) or (—) indicate good, moderate or absence of Salmonella.

TABLE IV EFFECT OF HIGHER INCUBATION TEMPERATURE 43°C ON THE ISOLATION OF SALMONELLA ORGANISM IN FISH MUSCLE FROM ENRICHMENT BROTHS.

Test organism.	Inoculum cells/flask.	Tetrathionate enrichment						Selenite Cystine enrichment						Dulcitol selenite enrichment					
		Mc. agar	B.G. agar	BGS agar	BiS. aggr	S. S. agar	XLD agar	Mc. agar	B. G. agar	BGS agar	BiS. agar	S. S. agar	XLD agar	Mc. agar	B.G. agar	BGS agar	BiS. agar	S. S. agar	XLD agar
<i>S. anatum</i>	32	—*	—	+	—	+	+	—	++	++	—	—	++	—	+	++	+	—	++
<i>S. enteritidis</i>	22	—	+	—	—	—	—	+	+	++	+	—	++	+	++	++	—	—	++
<i>S. typhimunium</i>	37	+	+	+	+	—	+	—	++	++	+	+	++	+	+	++	+	+	++

\* Vide Table No, 3.

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