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 *Salmonellaanatum 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 Sul phite 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; Montfored 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 conparable results. Raj (1966) reported that many recommended media failed to isolate these organisms in frozen sea foods. This apparent lack of cosistency in literature caused us to make a comparative study on the efficacy of the common enrichments viz. Selinite Cystine and Tetrathionate and the newer enrichment medium viz. DSF 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 brothers 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 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).

		Tetrathionate enrichment						Selenite Cystine enrichment						Dulicitol selenite enrichment.					
Test organism	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
S. typhimunium	12	+	+	+	−	−	+	+	+	+	+	−	+	++	++	++	+	−	++
S. para A.	9	−	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
S. anatum	10	−	−	+	+	−	−	−	+	+	−	+	+	+	+	++	+	+	++
S. para B.	19	−	−	+	−	−	+	−	+	+	+	+	+	+	+	+	−	−	+
S. para C.	7	−	−	−	−	−	−	−	+	+	−	−	−	−	+	+	−	−	+
S. enteritidis	14	−	−	+	−	−	+	−	+	++	+	−	+	−	+	++	+	+	++
S. typhi	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 procedure 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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REFERENCES:

Anon. 1966. -- Communicable disease centre, Salmonella surveillance Report. U. S. Department of Health, Education and Welfare -- Atlanta. Georgia 53. 4-6.

Carlson V. L., Snoeyenbos; G. H., Mekie, B. A. and Smyser C. F. 1967. -- Avian Dis. 11, 217—225.

Galton M. M., Lowery, M. D. and Hardy, A. V. 1954. *J. Infectious Diseases. 95*, 232.

Galton M. M. 1961. -- *Proc. U. S. Hivestock San. Assoc. 65, 434.*

Galton M. M., Morris, G. K. and Marton, N. T. 1968. *"Review of isolation methods and recommended procedures"* PHS publication. National communicable disease centre., Atlanta.

Hobbs, B. 1962. *"Chemical and Biological hazards in Foods"*. Symposium Food protection. Dowa State University Ames.

Hajna, A. A. and Damon, S. R. 1956. Appl. *Microbiol. 4*, 341—345.

Harvey, R. W. S. and Phillips W. P. 1961. *J. Hyg.* 59, 93—103.

Jameson, J. E, 1962. *J. Hyg. Camb. 60*, 193—207.

Leifson, E. 1936. Am. J. *Hyg. 24*, 423—432.

Leistner, L. R., Diebel, H., Johantges, J. and Niven, C. F. Jr. 1963. *Am. Meat. Inst. Found. Bull.* No. 56.

Listen, J., Matches, J. R. and Baross, J.

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

		Tetrathionate enrichment						Selenite Cystine enrichment						Dulcitol selenite enrichment					
Test organism	Inoculum cells/flask	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.
S. anatum	32	—*	—	+	—	—	—	—	++	++	+	—	++	+	+	++	+	—	++
S. enteritidis	22	—	—	—	—	—	—	—	+	++	—	—	++	—	+	++	—	+	++
S. typhimunium	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.

		Tetrathionate enrichment						Selenite Cystine enrichment						Dulcitol selenite enrichment					
Test organism.	Inoculum cells/flask.	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
S. anatum	32	—*	—	+	—	+	+	—	++	++	—	—	++	—	+	++	+	—	++
S. enteritidis	22	—	+	—	—	—	—	+	+	++	+	—	++	+	++	++	—	—	++
S. typhimunium	37	+	+	+	+	—	+	—	++	++	+	+	++	+	+	++	+	+	++

* Vide Table No. 3.

1971. F. A. O. Symposium. *Fish. Inspection and Quality Control*, p. 246.

Montford, J. and Thakeler F. S. 1961. *J. Food Sci. 26*, 510—517.

Morris, G. K. and Dunn, C. G. 1970, *Appl. mycrobiol 20*, 192—195.

Morris, G. K., Kochler, J. A., Gangarosa, J. and Sharar, R. G. 1970. *Appl. Microbiol. 19*, 434—437.

McCoy, J. 1962. *J. Appl. Bact. 25*, 213-224.

MacConkey, A. 1908. *J. Hyg. 8*, 322-334

McCarthy, M. D. 1966. *N. J. Med. hab. Technol. 20* (3).

North, W. R. J. R. 1961. *Appl. Microbiol. 9*. 188—195.

North, N. R. and Bastram, M. T. 1953. *Appl. Microbiol.* 1, 130—134.

Osborne, W. W. and Stokes, J. L. 1955. *Appl. Microbil.* 3, 295—299.

Raj, H. and Liston, J. 1961. *Food Technol. 15*, 423—434.

Raj, H. 1966. *Appl. Microbiol 14*, 12-20.

Report: 1966. Report of a subcommittee on sampling and methodology for salmonellae in Eggs. *Food. Technol. 20*, 473—481.

Ross, A. D. and Thatcher, F. S. 1958. *Food. Technol. 22*, 369.

Silliker, J. H., Deibel, R. H. and Fagon, P. T. 1964. *Appl. Microbiol. 12*, 224—228.

Silliker, J. H. and Taylor, W. I. 1958. *Appl. Microbiol. 6*. 228—282.

Smith, H. W. 1956. *J. Hyg. 57*, 266—273.

Smith, H. G. 1959. *J. Gen. Microbiol., 21*, 61—71.

Sugiyama, H., Dack, G. M. and Lippity, G. 1960. *Appl. Microbiol. 8*, 205—209.

Taylor, W. I. 1961. *Appl. Microbiol. 9*, 487—490.

Taylor, W. I. and Silliker, 1961. *Appl. Microbiol 9*

Taylor, W. I., 1965, *J. Clin. Path. 44.*, 471—475.

Taylor, W. I., Hobbs, B. C. and Smith, M. E., 1964. *Appl. Microbiol. 12*, 53—55.

Thatcher, F. S. 1963., Report of an Inernational Committee, *J. Appl. Bact. 26*, 266—285.

Thomson, S. 1955. *J. Hyg. 53*, 217—224,

Wilson, W. J. and Blair, E. M. Mev. 1931. *J. Hyg. 31*, 138—161.