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Isolation of *Salmonella* with the use of 100 ml of the R10 modification of Rappaport's enrichment medium

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SUMMARY

One hundred and eighty samples of pork sausages were examined after pre-enrichment in buffered peptone water (P medium), for the presence of salmonellas. From each pre-enrichment four enrichments were made: (1) 0·1 ml of P medium was inoculated into 10 ml of Rappaport's medium, formula R10 (R10/43 °C), (2) 1 ml of the P medium was added to 100 ml of R10 broth (R10/100 ml/43 °C), (3) 1 ml of P medium was inoculated into 10 ml of Muller–Kauffmann tetrathionate broth (MK medium) prepared in accordance with the International Standards Organization document ISO 3565 (MK/43 °C) and (4) 10 ml of P medium were added to 100 ml of MK broth (MK/100 ml/43 °C). All the enrichments were incubated at 43 °C for 48 h. Forty-six and 47 samples were found positive with the first two enrichment methods (R10/43 °C and R10/100 ml/43 °C), while only 16 samples were found positive with the method MK/43 °C, and 27 with the method MK/100 ml/43 °C. The superiority of either one of the two R10 procedures over either one of the two MK methods is statistically highly significant (paired χ^2; $P < 0{\cdot}001$ in all four comparisons). The superiority of procedure MK/100 ml/43 °C over the method MK/43 °C is also statistically significant ($P < 0{\cdot}005$). Both R10/43 °C and R10/100 ml/43 °C procedures had a much stronger inhibitory effect on the competing organisms (lactose- and sucrose-negative) than the two MK methods.

INTRODUCTION

We have recently reported that a modification of Rappaport's enrichment medium (R10 medium), inoculated from pre-enrichments in buffered peptone water (P medium) and incubated at 43 °C, was more efficient in the detection of salmonellas from samples of pork sausages, minced meat, sewage, and faeces of healthy pigs (Vassiliadis *et al.* 1977, 1978*a*, *b*, 1979*a*) than the Muller–Kauffmann tetrathionate broth prepared in accordance with the International Standards Organization document ISO 3565 (MK broth) (Edel & Kampelmacher, 1969, 1973; Anon. 1975). Another important advantage of the R10 broth (R10/43 °C), over the MK broth (MK/43 °C), which was consistently observed in all the above studies, is its strong inhibitory effect on the competing organisms (CO) which are

lactose- and sucrose-negative on brilliant green deoxycholate agar and may produce on this agar 'false positive' colonies. As evidence is accumulating in food and water bacteriology that a pre-enrichment procedure increases the number of salmonella isolations (Vassiliadis *et al.* 1972; Edel & Kampelmacher, 1973; Trichopoulos *et al.* 1975; Harvey & Price, 1977; Harvey, Price & Xirouchaki, 1979) the R10 medium, which is particularly suitable for enrichment after pre-enrichment, may assume a much greater importance.

Recently Harvey, Price & Xirouchaki (1979), have compared another modification of Rappaport's medium (R25 medium) which we introduced earlier (Vassiliadis *et al.* 1970), with the standardized MK broth and with selenite broth. They observed that the R25 broth was the most efficient medium in salmonella isolations from polluted river water. In our recent investigations we have found that the R10 medium was at least as efficient as the R25 medium in the isolation of salmonellas and that the R10 medium compared to the R25 broth has the advantage of inhibiting more considerably the competing organisms, especially those which are lactose- and sucrose-negative on brilliant green deoxycholate agar (Vassiliadis *et al.* 1977, 1978*a*, *b*, 1979*a*, *b*).

In our previous work we used both the R10 and the MK enrichment media in 10 ml quantities. In the present study we compare these media in 100 ml quantities, as this volume is recommended in the International Standards Organization document for the MK procedure (Anon. 1975).

MATERIALS AND METHODS

From November 1978 to July 1979, 180 samples of pork sausages were examined. Fifteen grams of pork sausage were pre-enriched as previously reported (Vassiliadis *et al.* 1977, 1979*b*).

Enrichment and selective media

(*a*) The Muller–Kauffmann tetrathionate broth was prepared according to the instructions given by Edel & Kampelmacher (1969) and the International Standards document (Anon. 1975), using the commercially available dehydrated base (Oxoid CM 343), and the brilliant green of Schmid GMBC & Co., Stuttgart-Unterturkhein. The batch of dehydrated medium was different from those used in previous studies.

The MK broth was distributed in test-tubes in 10 ml quantities and in small jars in 100 ml quantities, on the day of its preparation. Before inoculation the MK broths were preheated at about 45 °C.

(*b*) The modified Rappaport's medium formula R10 (Vassiliadis *et al.* 1976, 1978*b*), was prepared from solutions A, B and C described by Rappaport, Konforti & Navon (1956), using malachite green oxalate p.a. in the preparation of solution C (Papadakis *et al.* 1976).

The R10 medium can be kept in the refrigerator for at least one month. When needed it was preheated to about 45 °C and distributed into test-tubes in 10 ml quantities and in small jars in 100 ml quantities.

(*c*) The brilliant green deoxycholate agar was prepared with the commercially available, dehydrated modified brilliant green agar (Oxoid CM 329), to which 2·5 g of sodium deoxycholate/l of medium was added (Vassiliadis *et al.* 1979*c*).

Methods

From each pre-enrichment four inoculations were made in the two R 10 and the two MK broths.

The tubes of 10 ml of R 10 medium received 0·1 ml of the P medium and were incubated at 43 °C for 48 h (R 10/43 °C), whereas the jars containing 100 ml of R 10 medium were inoculated with 1 ml of the P medium and incubated also at 43 °C for 48 h (R 10/100 ml/43 °C).

The MK broths were inoculated with a ten times heavier inoculum and incubated at 43 °C for 48 h. Thus the tubes containing 10 ml of MK broth received 1 ml of the P medium (MK/43 °C) and the jars containing 100 ml of MK broth were inoculated with 10 ml of the P medium (MK/100 ml/43 °C). This difference in the inocula is inherent to the two enrichment broths, as it was observed earlier (Rappaport *et al.* 1956; McCoy, 1962; Harvey, Price & Hall, 1973; Vassiliadis *et al.* 1974; Van Schothorst *et al.* 1977).

From all these enrichment media, subcultures were made on selective BGD agar medium in Petri dishes, after 24 and 48 h incubation. The Petri dishes were then incubated at 37 °C for 24 h. From each BGD agar plate showing suspicious growth, four colonies, when available, were examined.

RESULTS

Positive samples, serotypes and strains of Salmonella *in relation to the procedure used*

The total number of samples from which salmonellae were isolated by at least one of the enrichments used, as well as the number of samples found positive for salmonellae by each of the procedures employed, are shown in Table 1. In the same table the number of serotypes and strains isolated by each enrichment method is also indicated. Table 2 relates the various serotypes isolated and the number of strains of each to the four procedures of enrichment.

Inhibition of competing organisms in relation to the enrichment method used

In Table 3 the proportion of CO lactose- and/or sucrose-positive, or lactose- and sucrose-negative, on BGD agar is separately indicated. In Table 4, the proportion of colonies of CO lactose- and sucrose-negative and of true salmonella colonies on BGD agar streaked from the four enrichment media, is shown.

DISCUSSION

The results of the present study confirm earlier observations that enrichment in 10 ml of R 10 medium, after pre-enrichment in buffered peptone water, yields a significantly higher number of salmonellas than the enrichment in 10 ml of MK broth. In this study, however, we have also shown that enrichment in 10 ml of R 10 medium is superior to enrichment in 100 ml of MK broth (paired χ^2 15·7; $P < 0{\cdot}001$) and is not inferior to enrichment in 100 ml of R 10 medium (Table 1) (paired χ^2 0·2; $P > 0{\cdot}30$). If this finding were confirmed with different types of foods and feeds it would greatly simplify routine laboratory work, since the R 10 medium

Table 1. *Salmonella isolations from pork sausages with the use of four procedures of enrichment (after pre-enrichment)*

Positive samples	Enrichment procedures* R10/43 °C	MK/43 °C	R10/100 ml/ 43 °C	MK/100 ml/ 43 °C
Positive after 24 h and 48 h	42	9	38	10
Positive after 24 h only	3	4	4	4
Positive after 48 h only	1	3	5	13
Total positive	46	16	47	27
Percentage positive	25·6	8·9	26·1	15·0
No. of serotypes isolated	19	12	18	16
No. of strains isolated	55	16	53	28
No. of samples examined	180	180	180	180
Total positive by at least one of the enrichments:	samples	48 (26·7 %);	serotypes 19;	strains 59

* R10/43 °C = enrichment at 43 °C, in 10 ml of final R10 medium, containing only 10 ml of the 0·4 % (w/v) solution of malachite green oxalate (Merck)/1110 ml of final medium; R10/100 ml/43 °C = same as above but enrichment in 100 ml of R10 medium, inoculated with 1 ml of pre-enrichment medium; MK/43 °C = enrichment at 43 °C, in 10 ml of standardized Muller–Kauffmann tetrathionate broth; MK/100 ml/43 °C = same as above but enrichment in 100 ml of MK medium, inoculated with 10 ml of pre-enrichment broth; (the R10/43 °C and MK/43 °C media in 10 ml volumes are inoculated with one-tenth of the inoculum made in the 100 ml media).

Table 2. *List of* Salmonella *serotypes and strains isolated with the use of the different enrichment procedures used*

Serotypes	Total no. of strains	Enrichment procedures* R10/43 °C								
		MK/43 °C	+	+	−	+	−	+	−	−
		R10/100 ml/43 °C	+	+	−	+	+	−	+	+
		MK/100 ml/43 °C	+	−	−	+	−	+	−	+
S. agona	7		.	.	.	.	4	.	1	2
S. anatum	2	1		.	1	.	.	.	.	.
S. bovis-morbificans	1	1		.	.	.	.	.	.	.
S. braenderup	1	.		.	.	.	.	.	.	1
S. brandenburg	3	.		.	.	.	.	.	.	3
S. bredeney	2	1		.	.	.	1	.	.	.
S. derby	6	1		.	.	1	3	.	.	1
S. heidelberg	1	.		.	.	.	1	.	.	.
S. infantis	4	.		.	1	.	2	.	.	1
S. kedougou	1	.		.	1	.	.	.	.	.
S. livingstone	1	1		.	.	.	.	.	.	.
S. london	7	1		.	1	.	2	.	1	2
S. muenchen	2	1		.	.	.	.	.	.	1
S. newport	4	1		.	.	.	1	.	.	2
S. tennessee	1	1		.	.	.	.	.	.	.
S. typhimurium	8	1		.	1	1	5	.	.	.
S. typhimurium var. *copenhagen*	2	.		.	.	.	2	.	.	.
S. uphill	1	1		.	.	.	.	.	.	.
S. westerstede	5	1		1	.	.	2	1	.	.
Total no. of strains isolated	59	12		1	5	2	23	1	2	13

* See footnote to Table 1.

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Table 3. *Growth of competing organisms lactose- and/or sucrose-positive on brilliant green deoxycholate (BGD) agar, or lactose- and sucrose-negative (sugar(−)) on BGD agar according to the enrichment procedure used*

Growth of the two groups of competing organisms	Enrichment procedures*			
	R 10/43 °C	MK/43 °C	R 10/100 ml/43 °C	MK/100 ml/43 °C
Lactose and/or sucrose(+) only	130 (72·2)†	80 (44·4)	134 (74·4)	76 (42·2)
Lactose and sucrose(−) only	1 (0·6)	9 (5·0)	1 (0·6)	12 (6·7)
Organisms of both groups	13 (7·2)	75 (41·7)	19 (10·6)	72 (40·0)
Samples negative for competing organisms	36 (20·0)	16 (8·9)	26 (14·4)	20 (11·1)
Total of samples examined	180 (100·0)	180 (100·0)	180 (100·0)	180 (100·0)

* See footnote to Table 1. † Percentages in parentheses.

Table 4. Salmonella *and 'false positive' colonies on brilliant green deoxycholate agar streaked from four enrichment media*

	Enrichment procedures*			
	R 10/43 °C	MK/43 °C	R 10/100 ml/43 °C	MK/100 ml/43 °C
No. of samples with suspicious colonies	55	87	61	94
No. of colonies examined	299	301	301	341
No. of *Salmonella* colonies	274	55	262	87
Percentage of *Salmonella* colonies	91·6	18·2	87·0	25·5
No. of 'false positive' colonies	25	246	39	254
Percentage of 'false positive' colonies	8·4	81·7	13·0	74·5

* See footnote to Table 1.

could be used in 10 ml quantities in test-tubes rather than in 100 ml quantities in jars. As in previous studies, it was again observed that the use of the R10 procedures allowed the detection of significantly more samples with more than one serotype per positive sample than the use of the methods involving enrichment in MK broth.

In our previous work on pork sausages, minced meat, sewage, and faeces of healthy pigs (Vassiliadis *et al.* 1977, 1978*a*, *b*, 1979*a*) we had observed that the R10/43 °C procedure, inhibited much more the CO of any sort (CO lactose- and/or sucrose-positive and CO lactose- and sucrose-negative) than the MK/43 °C method. In the present investigation the majority of the CO which grew in MK broth at 43 °C were lactose- and sucrose-negative and often produced 'false positive' colonies (Tables 3 and 4). The strong inhibition of the R10 medium on the CO lactose- and sucrose-negative covers many species of this category of CO, including *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and varieties of *Escherichia coli*, *Enterobacter* and *Citrobacter* not fermenting sugars on BGD agar.

It should be added that the use and preparation of R10 medium is very simple. Once prepared it is ready for use for at least one month. It is also cheap and, unlike some commercial enrichment media (Harvey, Price & Crone, 1975), the different laboratory-prepared lots are uniform in performance, provided malachite green oxalate (Merck) p.a. and $MgCl_2 . 6H_2O$ extra pure or p.a. are used.

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