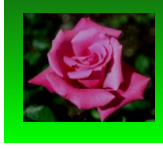
Evaluation of four new chromogenic Salmonella plating media



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Introduction

The widespread occurrence of Salmonella spp. requires the need for the rapid detection and identification in feed, food, environment and in clinical samples. Conventional plating media used to differentiate Salmonella spp. from other members of the family Enterobacteriaceae depend upon the ability of Salmonella to produce hydrogen sulphide coupled with the inability to ferment lactose. These are however inadequate methods with a significant number of species not exhibiting these characteristics. Furthermore, Proteus spp., Citrobacter spp. and H_2S -positive *E. coli* often exhibited false positive Salmonella spp. A second approach is the utilization of motility of Salmonella by use of semisolid media. Chromogenic Salmonella plating media have been developed for the rapid and more reliable identification. These media contain inhibitors for Gram-positive bacteria, the most of nonfermenters and some of the enterobacteriaceae. The chromogenic substrates should specifically enable Salmonella spp. to hydrolyse these substrates and producing well coloured Salmonella colonies, clearly differentiating from non-Salmonella colonies. This should allow rapid presumptive diagnosis of suspected Salmonella spp. with few additional tests only. Furthermore, fastidious Salmonella spp., e.g., *S.* Typhi, *S.* Gallinarum have to grow on the new chromogenic Salmonella plating media.

Material and Methods

Composition of the chromogenic Salmonella plating media

Excellent nutrients and selected inhibititors are prerequisites of these media. Oxoid Salmonella Chromogenic Medium (OSCM) and AES Salmonella Agar Plate (ASAP) contain two chromogenic substrates. Magenta-cap (5-bromo-6-chloro-3-indolylcaprylate) is utilized by Salmonella species resulting in purple to mauve colonies. The specificity of C8-esterase (cleaving magenta-cap) is 92 %, the sensitivity 100 %. X-gal (5-bromo-4-chloro-3-indolyl- -D-glactopyranoside) results in turquoise to blue colony growth in microorganisms that produce the enzyme -D-galactosidase (OSCM). ASAP works with X- -D-glucopyranoside as differentíal chromogenic substrate. -D-glucosidase producing microorganisms form turquoise to blue colonies, -D-glucosidase negative bacteria like *E. coli* or *Citrobacter* spp. grow as white colonies. The chromogenic substrates of BBL CHROMagar , Salmonella and BCM Salmonella (Biosynth) are not published up to now (patent pending). OSCM and ASAP plate form an opaque background. All chromogenic Salmonella plating media were kindly provided by the companies listed in Table 1.

Evaluation with pure cultures

Salmonella enterica, *Salmonella bongori* strains and contaminants were taken from the stock collection of the Robert Koch-Institut, Wernigerode. All strains were freshly cultivated, the grown colonies were suspended in phosphate buffered saline (PBS) and diluted to 10^5 to 10^6 cfu/ml for fractionated inoculation. Incubation at 36 1 °C for 24 hours.

Estimation of the recovery rate from the Salmonella plating media

S. Enteritidis 8298/99 and *S.* Typhimurium 8244/99 were freshly cultured, the growth suspended in PBS and diluted to 10^5 - 10^0 cfu/ml. 0.1 ml of that dilutions were spread over the Salmonella plating media and over nutrient agar. Incubation at 36 1 °C for 24 h. The recovery rate was estimated by the quotient of counts grown onto the selective plating medium and the counts onto nutrient agar (= 100 %).

Recovery of *Salmonella* Enteritidis and *Salmonella* Typhimurium from spiked stool samples using chromogenic and conventional salmonella plating media

Freshly cultivated strains of *S.* Enteritidis 8298/99 and *S.* Typhimurium 8244/99 were suspended in PBS and diluted to the counts/0.1 ml listed in Table 2. 0.1 ml of that suspensions added to 6 ml of selenite broth (Oxoid) containing 1 ml of a mixture of stool and PBS (1:1). Incubated at 37 °C for 20 hours. 0.1 ml of dilutions (10^{-5} - 10^{-6}) spread onto the Salmonella plating media listed (Table 2).

Table 1: Growth and colonial morphology of chromogenic Salmonella Plating Media incubated at 36 1 °C for 24 hours

Bacterial strains	Number of strains tested	BCM Salmonella (Biosynth)	BBL™ CHROMagar™, Salmonella (BD)	Oxoid Salmonella chromogenic medium (OSCM)	AES Salmonella Agar Plate (ASAP)
Salmonella (*S.*) spp. (subsp. I[1)], II, IV, VI)	60	reddish-pink; 1-3 mm, with/without pink halo	mauve; 1-2 mm; small mauve halos	purple to mauve, 1-3 mm; mauve halos	pink to purple; 1-2 mm
S. spp. (subsp. IIIa and IIIb)	16	reddish-pink to reddish-lilac; 1-3 mm with/ without pink halo	weak to fair growth; 1.5-2 mm; blue green with mauve or blue-lilac halo	red to blue-green; 3 mm; red lilac halo	pink to purple; 1-2 mm
S. Typhi	4	reddish-pink; 1-1.5 mm; with weak halo	mauve; pin-point to 1 mm; weak mauve halo	purple to mauve; 1 mm; weak mauve halos	pink to purple; 1 mm
S. Gallinarum	2	weak reddish-pink; pin-point	no growth to weak growth pinpoint; transparent to rose; rose halo	purple; 1 mm; purple halos	pink to purple; pinpoint
S. Pullorum	2	weak reddish-pink; pin-point	n.d.	purple; 0,5 mm; purple halos	pink to purple; pinpoint
S. bongori (subsp. V)	2	grey-greenish blue; 1-2 mm with no halo	mauve; 1-2 mm; small mauve halo	purple to mauve, 1-2 mm; mauve halos	light to pink; 2 mm
Escherichia coli	14	blue-green; 2-4 mm; with blue-green halo	bluish-lilac to dark turquoise; 1-2 mm; blue-violet halos	blue-green; 1-2 mm	light, white pinpoint to 2 mm
Escherichia coli	4	dark blue; 2-3 mm; with pink halo			
Escherichia coli O157:H7	2	blue-green; 1-3 mm; with green halo	n.d.	turquoise; 1.5 mm	light; 1 mm
Escherichia hermanii	2	colourless to blue-green pinpoint; to 1 mm with no halo	n.d.	purple to mauve; 2 mm	light-yellow to blue-green; 1.5 mm
Citrobacter diversus	2	greenish-blue pinpoint colony to 1 mm	no growth to good growth; 3 mm, blue-green with light mauve halos. Some strains grow with mauve colonies and small mauve halos.	blue-green; 1 mm	white; 2 mm
Citrobacter freundii	15	dark blue-green; 2-3 mm with no halo		blue-green; 1-2 mm	white; 2 mm
Citrobacter freundii	5	bluish-lilac; 1-2 mm with rose halo		bluish-lilac to mauve; 2 mm; lilac precipitate	white; 2 mm
Citrobacter amalonaticus	3	reddish-pink; 1-3 mm with/without pink halo		bluish-lilac; 1 mm; mauve precipitate	light grey to pink centred; 1 mm
Serratia marcescens	4	green; 2 mm with green halo	n.d.	purple to mauve; 2 mm	light-yellow; 1.5 mm
Shigella boydii, S. flexneri, S. dysenteriae	2 each	light; 1 mm	weak growth to fair growth; 1 mm; transparent with a very pale rose hue	light, pinpoint; 2 mm	light; 1 mm
Shigella sonnei	2	dark-turquoise; 3 mm	fair to good growth; 2 mm; blue-green with light mauve halos	blue-green; 2 mm	white; 2 mm
Yersinia enterocolitica	1	no growth	weak growth; 0.5 mm; transparent	light; 1 mm	light; 1 mm
Hafnia alvei	4	blue-green; pinpoint to 1 mm with blue-green halo	n.d.	dark turquoise; 1mm no halo	light; 1 mm
Enterobacter spp. (*E. agglomerans, E. cloacae, E. aerogenes, E. sakazaki*)	4	blue-green domed; 1-3 mm; with no halo	good to excellent growth; 1 - 2 mm; blue-green to blue	bluish-lilac; 2 mm; mauve halo	turquoise; 2 mm
Klebsiella ozenae	1	colourless to tan; 1-2 mm with no halo	fair to good growth; 3 mm; blue-green with light mauve halos	dark turquoise; 1-2 mm	turquoise; 2 mm
Klebsiella pneumoniae	4	blue-green; 2-3 mm with bluish precipitate	fair to good growth; 3 mm; blue-green with light mauve halos	dark turquoise; 1-2 mm	turquoise; 2 mm
Morganella morganii, Proteus mirabilis, P.vulgaris	1 each	colourless to cream; 1-2 mm with brownish precipitate in the medium	no growth to weak growth; 0.5 mm; transparent to tan	light; 1 mm; brownish precipitate	light; 2 mm
Providencia spp. (*P. rettgeri, P. alcalifaciens, P. stuartii*)	5	colourless to tan domed; pinpoint to 2 mm with brownish precipitate	n.d.	light to mauve; 1 mm brownish precipitate	light; 1 mm
Acinetobacter spp., *Pseudomonas aergunosa, P. fluorescens, P. putida; Stenotrophomonas maltophilia*	16	no growth	no growth to weak growth; pinpoint to 0.5 mm	no growth	no growth
Aeromonas hydrophilia, A. sobria, A. veronii, A. caviae	7	"	weak growth, mostly pinpoint to 0.5 mm; rose to mauve with rose to mauve halos	no growth to pinpoint purple	no growth to pinpoint
Staphylococcus aureus	2	"	no growth	no growth	no or very weak growth
Enterococcus faecalis	2	"	no growth to fair growth; pinpoint; blue-green	no growth	no or very weak growth
C. albicans	1	no growth	no growth	no growth	no growth

[1)] among them *Salmonella enterica* subspecies *enterica* (I): *S.* Abony; *S.* Agona; *S.* Augustenborg; *S.* Blegdam; *S.* Bovismorbificans; *S.* Choleraesuis; *S.* Dublin; *S.* Enteritidis; *S.* Hadar; *S.* Panama; *S.* Paratyphi B; *S.* Paratyphi C; *S.* Rostock; *S.* Saintpaul; *S.* Senftenberg; *S.* Typhimurium; *S.* Wassenar; *S.* Wayne and two strains each of *Salmonella* subsp. II, IV, VI.

Results and discussion

Salmonella enterica strains of subsp. I, II, IV and VI grew on all tested chromogenic Salmonella plating media with typically coloured colonies (Table 1). Salmonella strains of subsp. IIIa and IIIb (formerly *S.* Arizonae and *S.* Diarizonae) grew identically to these Salmonella spp., mentioned above, on BCM Salmonella and on ASAP.

The most strains of *S.* IIIa and *S.* IIIb appear blue-green to lilac on BBL™ CHROMagar, Salmonella and on Oxoid Salmonella chromogenic medium. *S.* Typhi, *S.* Paratyphi (B, C), *S.* Gallinarum and *S.* Pullorum grew on all chromogenic plating media tested with small to normal sized colonies. *Salmonella bongori* (formerly subsp. V, two strains tested) grew on BCM Salmonella and on ASAP with colonies, different of the colonies of *Salmonella enterica*. However, colonies of *Salmonella bongori* appear similar to, e.g., *S.* Typhimurium, on the two other chromogenic plating media. The contaminants *S. aureus; E. faecalis; Pseudomonads, Aeromonads, Alcaligenes, Acinetobacter, Stenotrophomonas maltophilia* are widely to completely inhibited on all media tested. Strains of the tribe Proteus-Providencia-Morganella are partly inhibited or grew colourless with a brownish precipi-tate. *Shigella boydii, S. dysenteriae, S. flexneri* formed light, small colonies; *S. sonnei* grew with blue-green colonies on X-gal containing chromogenic media and light on ASAP. *E. coli* strains grew clearly distinguishable from Salmonella spp. by formation of blue-green or light colonies. Citrobacter spp. grew on ASAP with white colonies because on not possessing -D-glucosidase activity. Most strains of Citrobacter spp. grew on BCM Salmonella and Oxoid Salmonella chromogenic medium (OSCM) with blue-green colonies, some bluish-lilac and few, especially all three *C. amalonaticus* strains tested, similar to Salmonella spp., Citrobacter spp. are partly inhibited on BBL™ CHROMagar™, Salmonella. Thus, the last mentioned medium showed the lowest recovery rate for *S.* Enteritidis and *S.* Typhimurium (Fig. 1). Oxoid Salmonella chromogenic medium (OSCM) and ASAP behaved in this measure similar to Hektoen Enteric Agar (BD); BCM Salmonella showed the highest recovery rate and ranks between Hektoen Enteric Agar and XLD. Recovery of Salmonella from spiked stool samples under-lined this but pointed also on different selectivity of the three chromogenic media (Table 2).

Fig. 1: Recovery rate (%) of *S.* Enteritidis 8298/98 and *S.* Typhimurium 8244/98 from various chromogenic and conventional Salmonella plating media

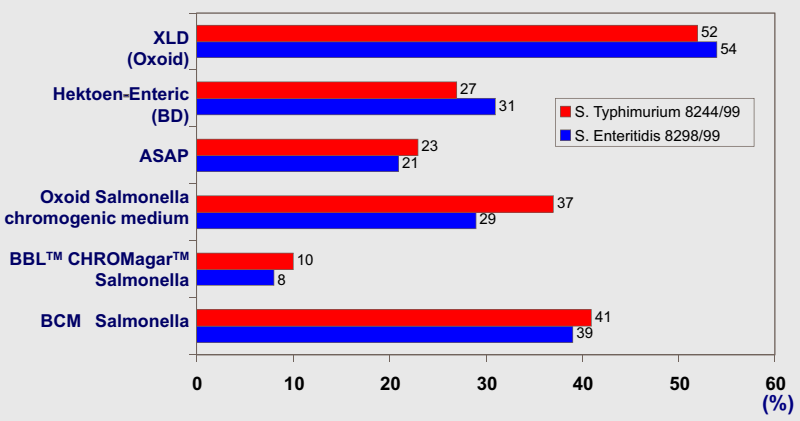

Table 2: Recovery of *S.* Enteritidis 8298/98 and *S.* Typhimurium 8244/98

Cfu inoculated	OSCM (Oxoid)	ASAP (AES)	BCM Salmonella	XLD	Hektoen Enteric
***Salmonella* Enteritidis**					
4	no Salm./13 non-Salm.	no growth	no Salm./27 non-Salm.	no growth	no growth
9	14 Salm./4 non-Salm.	no growth	7 Salm./51 non-Salm.	no growth	3 Salm.
11	47 Salm./8 non-Salm.	6 Salm.	69 Salm./72 non-Salm.	36 Salm.	41 Salm.
***Salmonella* Typhimurium**					
3	54 Salm./6 non-Salm.	10 Salm.	56 Salm./32 non-Salm.	9 Salm.	23 Salm.
5	53 Salm./1 non-Salm.	18 Salm.	40 Salm./4 non-Salm.	61 Salm.	37 Salm.
11	1 Salm./1 non-Salm.	1 Salm.	2 Salm./10 non-Salm.	2 Salm./1 non-Salm.	no growth

Conclusion

All tested chromogenic Salmonella plating media enable a good recognition of Salmonella spp., in particular of Salmonella strains of subsp. I occurring to an extend of ca. 98 % of the isolates. The well coloured colonies could be easily detected also in mixed cultures. Isolation by enrichment in selenite broth from stool samples significantly improved the detection using the chromogenic plating media.

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