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Detection of Salmonellae by Using Rambach Agar and by a C8 Esterase Spot Test

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In food microbiology, Rambach agar facilitates the differentiation of non-typhi *Salmonella* through a specific red pigmentation of the colonies. The usefulness of Rambach agar was examined relative to its usefulness to the field of clinical microbiology. Of 170 non-typhi *Salmonella* strains, 92 and 97% gave bright red colonies after 24 and 48 h of incubation, respectively, while 100% of 112 other members of the family *Enterobacteriaceae* were of a different color (blue, green, beige, or colorless). Red colonies were also found with five of five *Acinetobacter* isolates and one of three *Pseudomonas* isolates. To further detect *Salmonella typhi* and the rare beige or colorless colonies atypical of *Salmonella* isolates, a C8 esterase detection spot test was carried out. With UV light, that test revealed fluorescent colonies for all *Salmonella* isolates tested.

A new plate medium to facilitate the differentiation of salmonellae has been described by Rambach (4) and has been proposed in particular for the detection of food-borne salmonellosis outbreaks (2) and in the control of veterinary diseases. By the use of this propylene glycol (PG) medium, metabolization of *Salmonella* strains (non-typhi *Salmonella* spp.) yielded colonies with a distinct bright red pigmentation, while colonies of other members of the family *Enterobacteriaceae* were colorless or beige or were blue, green, or violet for β-galactosidase negative (β-gal$^-$) and β-galactosidase-positive (β-gal$^+$) isolates, respectively. Since the investigator tested only 100 *Salmonella* strains and 8 strains of other members of the family *Enterobacteriaceae* and since the medium is commercially available (Technogram, Paris, France), we extended these results in particular to the field of clinical microbiology by streaking onto the medium various bacteria isolated from patient feces: strains of *Salmonella*, other members of the family *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter*.

Moreover, it has been established that *Salmonella* spp. possess an esterase activity, specifically, C8 esterase (C8E) activity, that is not present in the other lactose-negative members of the family *Enterobacteriaceae* (1); however, information is lacking on the C8E activities of *Pseudomonas* and *Acinetobacter* spp. Thus, the presence of this enzyme was assayed by a laboratory-made spot test on β-gal$^-$ colonies grown on Rambach agar.

The study was carried out on 358 strains: 190 *Salmonella* strains, 112 strains of other members of the family *Enterobacteriaceae*, 51 *Pseudomonas* strains, and 5 *Acinetobacter* strains (see Tables 1 and 2). The *Salmonella* strains belonged to 28 different serotypes; they were isolated between 1987 and 1990 from clinical samples and were identified by a conventional procedure: API 20E or API 20NE (BioMérieux/API, La Balme les Grottes, France). They were also seroidentified (Diagnostic Pasteur, Paris, France). Bacterial strains were stored on storage agar (Diagnostic Pasteur, Paris, France) at 4°C and streaked onto purple-lactose agar (3) (BioMérieux, Marcy l'Etoile, France). The other strains were also of clinical origin. After isolation on purple-lactose agar, all the strains were streaked onto Rambach agar contained in 90-mm-diameter petri dishes.

Rambach agar has been described elsewhere (4). Briefly, it contains PG together with a pH color indicator which turns red when it is acidified and a substrate for β-gal (5-bromo-4-chloro 3-indolyl β-galactopyranoside) that yields blue-pigmented colonies of metabolizing bacteria. PG$^-$ β-gal$^-$

TABLE 1. Pigmentation of 190 *Salmonella* colonies (28 serotypes) on Rambach agar after 24 h of incubation

Salmonella serotype	Total no.	No. of red colonies
Agona	1	0[a]
Blockley	3	3
Bovis-morbificans	3	3
Brandeburg	2	2
Bredeney	3	3
Derby	3	3
Dublin	3	2 (1)[b]
Enteritidis	24	23 (1)
Goldcoast	1	1
Hadar	2	2
Haifa	1	1
Hartford	1	1
Heidelberg	4	4
Infantis	6	6
London	4	3 (1)
Mbandaka	1	1
Nagoya	1	1
Newport	4	4
Ohio	2	2
Panama	8	8
Paratyphi A	3	0
Paratyphi B	8	8
Saint-paul	3	2 (1)
Schwarzengrund	2	2
Typhi	20	0
Typhimurium	58	55 (2)
Virchow	16	15 (1)
Wien	3	2 (1)
Total	190	157 (8)

[a] The non-red colonies were beige.
[b] The number of colonies that required 48 h of incubation to become red is given in parentheses.

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TABLE 2. Pigmentation of 168 gram-negative bacteria colonies on Rambach agar and the C8E test for β-gal⁻ strains after 24 h of incubation

Strain	No. of strains	Color[a]	No. of colored strains	No. of strains C8E test positive
Pseudomonas aeruginosa	50	Red	17	17
		Beige	33	33
Pseudomonas maltophilia	1	Beige	1	1
Acinetobacter spp.	5[b]	Red	5	5
Proteus spp.	39[c]	Colorless, beige	39	0
Citrobacter spp.	18[d]	Blue	18	NT[e]
Klebsiella spp.	11[f]	Blue	11	NT
Enterobacter cloacae	6	Blue	6	NT
Serratia spp.	4[g]	Blue	4	NT
Escherichia coli	8	Blue	8	NT
Escherichia coli β-gal⁻	5	Beige	5	0
Shigella dysenteriae	2	Beige	2	0
Shigella flexneri	1	Beige	1	0
Shigella sonnei	1	Green	1	NT
Hafnia alvei	1	Green	1	NT
Morganella morganii	8	Colorless, beige	8	0
Providencia stuartii	5	Colorless, beige	5	0
Yersinia spp.	3[h]	Beige	3	0
Total	168			

[a] Colony pigmentation did not change after 48 h of incubation.
[b] *Acinetobacter* spp. included two *A. baumanii*, one *A. calcoaceticus*, one *A. junii*, and 1 *A. lwoffi*.
[c] *Proteus* spp. included 34 *P. mirabilis*, 4 *P. vulgaris*, and 1 *P. rettgeri*.
[d] *Citrobacter* spp. included 14 *C. freundii* and 4 *C. diversus*.
[e] NT, not tested.
[f] *Klebsiella* spp. included eight *K. pneumoniae* and three *K. oxytoca*.
[g] *Serratia* spp. included two *S. liquefaciens* and two *S. marcescens*.
[h] *Yersinia* spp. included one *Y. pseudotuberculosis*, one *Y. enterocolitica*, and one *Y. intermedia*.

bacterial colonies are colorless or beige, PG⁻ β-gal⁺ bacterial colonies are blue or green, PG⁺ β-gal⁺ bacterial colonies are violet, and PG⁺ β-gal⁻ bacterial colonies are red. After inoculation on Rambach agar, the colonies were examined after 24 and 48 h of growth at 36°C.

For the C8E test, which was conducted on 309 strains, including 190 *Salmonella* isolates, methylumbelliferyl caprylate (Research Organics Inc., Cleveland, Ohio) was dissolved in ethanol at a concentration of 1 mg/ml, and a drop was deposited onto the colony to be assayed under long-wave UV light (366 nm). When it was stored at 4°C in the dark, the solution keeps for up to 1 week. A fluorescence of the colony or of its surroundings in less than 1 min was scored as positive. All the *Salmonella* colonies were C8E positive on Rambach agar after 24 h of incubation. The test was negative for all the other β-gal⁻ members of the family *Enterobacteriaceae* and was positive for *Pseudomonas* and *Acinetobacter* spp. (see Table 2).

Table 1 shows the results obtained with the 190 *Salmonella* strains. They include *S. typhi*, which does not metabolize PG and whose colonies are colorless or beige. Among the other *Salmonella* isolates belonging to 27 serotypes that were tested, 92% (157 of 170) yielded bright red colonies after 24 h of incubation, and 97% (165 of 170) yielded bright red colonies after 48 h of incubation. There was no decrease in pigmentation after 48 h.

Table 2 summarizes the results obtained with 168 non-*Salmonella* strains. A total of 17 of the 51 *Pseudomonas* strains and all 5 *Acinetobacter* strains yielded red colonies within 24 h. No members of the *Enterobacteriaceae* other than the *Salmonella* strains yielded red colonies: β-gal⁺ colonies were blue or green, and β-gal⁻ colonies were colorless or beige.

Our results on the metabolization of PG by *Salmonella* strains corroborates the earlier study of Rambach (4); apart from the negative *S. typhi* strain, a positive frequency of 92 to 97% was obtained in our study with various *Salmonella* serotypes.

The *Salmonella* colonies were particularly easy to detect because of their bright red color. Apart from *Salmonella paratyphi* A, which is exceptional in coprocultures, the rate of detection of *Salmonella* isolates that were detectable directly by the bright red color of their colonies rose to 94 to 99%.

Our C8E results were positive for all *Salmonella* strains as well as for the *Pseudomonas* and *Acinetobacter* strains that may be present in feces. No positive reaction occurred when 63 β-gal⁻ non-*Salmonella* members of the family *Enterobacteriaceae* were grown on Rambach agar. Conversely, other investigators (1) found 5 and 12% false-positive strains among the lactose-negative bacteria when they used MacConkey and SS agars, respectively. However, we do not know which caprilate solvent they used.

The bright red pigmentation obtained with Rambach agar allows for the easy detection of most *Salmonella* isolates in coprocultures and an important decrease in both the work load and the cost usually required for the detection of these strains. The few false-positive *Pseudomonas* and *Acinetobacter* strains that we revealed could be distinguished further. The additional C8E test on colorless or beige colonies could also be used to detect the rare PG⁻ salmonellae.

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