Caprylate-Thallous Agar Medium for Selectively Isolating *Serratia* and Its Utility in the Clinical Laboratory

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A defined agar medium (hereinafter designated caprylate-thallous [CT] agar) containing 0.01% yeast extract, 0.1% caprylic (*n*-octanoic) acid, and 0.025% thallous sulfate is highly selective for all *Serratia* species and effectively discriminates against most non-*Serratia* strains likely to be in the same habitats. The selectivity of CT agar is demonstrated by the very high efficiency of colony formation (mean, 80.7% of that on a nonselective complex medium) on CT agar by known *Serratia* strains and the very low efficiency of colony formation (close to zero) on CT agar by bacterial strains known not to be *Serratia*. The utility of this medium in actual clinical laboratory practice is demonstrated by the more rapid and higher recovery of *Serratia* on this selective medium as compared to conventional procedures of in-tandem runs of 513 consecutive urine, feces, and sputum specimens. Pigmented and nonpigmented *Serratia* strains deliberately added to fecal specimens can be selectively and quantitatively recovered on CT agar. CT agar compares favorably with, or in some cases is an improvement over, other selective media which have been recommended for isolating *Serratia*. This selective CT agar medium could be quite useful in ecological surveys, especially those related to hospital-acquired infections.

Members of the bacterial genus *Serratia* are being encountered with increasing frequency as opportunistic pathogens of human patients in hospitals. In most instances, these patients have been compromised by a variety of other medical conditions and procedures: intravenous, intratracheal, and urological catheterization (5, 14, 24, 26); indiscriminate antibiotic therapy and "prophylaxis" (2, 5, 14); treatment with immunosuppressive, steroid, and antimetabolic drugs (3, 7, 27, 28); massive physical trauma; heroic surgery (25), including organ transplantation; malignant diseases (3, 27); premature birth (18); and very advanced age (4, 27). This ecological niche, the compromised hospital patient, is colonized by an assortment of different "opportunistic" bacteria, many of which cause fatal or at least life-threatening infections that often are extremely difficult to manage medically. This therapeutic difficulty results from two major factors: (i) the bacteria involved often are multiply drug resistant; and (ii) these opportunistic pathogens are "exotics" as far as the routine in the clinical laboratory is concerned, and additional time is usually needed for making a definitive laboratory diagnosis. *Serratia* is one of the more common noso-

comial pathogens, and the difficulty in identifying it promptly is compounded by the fact that the majority of hospital strains do not form the characteristic red pigmentation usually associated with this bacterial genus (10, 27).

Prompt recognition of *Serratia* in the clinical laboratory is essential and might be facilitated by the availability of a primary selective medium. Moreover, exploration of the ecology of *Serratia*, within the hospital as well as extra-hospital habitats and vectors into the hospital environment, also requires a suitable selective medium. We report here a highly selective plating medium, caprylate-thallous-mineral salts agar, on which practically all tested strains belonging to all species and most biotypes of the genus *Serratia* (13) can grow well and on which very few other bacteria likely to be in the same habitats can grow. The utility of this medium in clinical and ecological laboratory practice is demonstrated (i) by the very high efficiency (relative to a rich nonselective medium) of colony formation on the selective medium by known *Serratia* strains and the very low efficiency of colony formation on the selective medium by bacterial strains known not to be *Serratia*; (ii) by the improved detectability of *Serratia* on the selective medium as shown by in-tandem runs (both by conventional procedures

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and with the selective medium) of 513 consecutive urine, feces, and sputum specimens; and (iii) by the sensitive detection and quantitative recovery by the selective medium of both pigmented and nonpigmented *Serratia* strains that were deliberately added to fecal specimens.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in the present study are listed in Table 1 in three groups: *Serratia* spp. (13), enterobacteria other than *Serratia*, and bacteria other than enterobacteria. These cultures include practically all of the *Serratia* strains used in a recent computer taxonomic analysis of this genus (13) as well as additional human clinical strains. The bacterial strains outside the genus *Serratia* were chosen to provide a representative collection of bacteria likely to be in the habitats under study.

Selective CT agar medium. Numerous media were constructed using as a base the M70 inorganic mixture of Véron (22) and testing an assortment of carbon sources and other selective conditions that were suggested by the computer taxonomic analysis (13). The final medium, a caprylate-thallos (hereinafter, CT) agar, has the following composition and mode of preparation. First, a trace elements solution (22) containing the following was prepared: distilled water, 1,000 ml; H_3PO_4 , 1.96 g; $FeSO_4 \cdot 7H_2O$, 0.0556 g; $ZnSO_4 \cdot 7H_2O$, 0.0287 g; $MnSO_4 \cdot 4H_2O$, 0.0223 g; $CuSO_4 \cdot 5H_2O$, 0.0025 g; $Co(NO_3)_2 \cdot 6H_2O$, 0.003 g; and H_3BO_3 , 0.0062 g; this solution was stored unsterilized at 4°C. Then, two solutions designated A and B were prepared, autoclaved separately, and mixed aseptically to yield a liter of the final medium. Solution A contained: $CaCl_2 \cdot 2H_2O$, 0.0147 g; $MgSO_4 \cdot 7H_2O$, 0.123 g; KH_2PO_4 , 0.680 g; K_2HPO_4 , 2.610 g; trace elements solution, 10 ml; caprylic (*n*-octanoic) acid, 1.1 ml; yeast extract (Difco Laboratories, Detroit, Mich.), 2 ml of a 5% (wt/vol) solution; thallos sulfate (E. Merck AG, Darmstadt, Germany), 0.25 g; and distilled water up to 500 ml. The pH was adjusted to 7.2 with NaOH using a pH meter. This solution A was autoclaved at 110°C for 20 min. Solution B contained: NaCl, 7 g; $(NH_4)_2SO_4$, 1 g; agar (Difco), 15 g; distilled water, 500 ml. The pH was adjusted to pH 7.2, and this agar base (solution B) was autoclaved at the same time and under the same conditions as solution A. After autoclaving, solutions A and B were mixed aseptically, and the resulting CT agar medium was poured into sterile plastic petri dishes (25 ml when the diameter of the dish was 9 cm and 80 ml when the diameter was 14 cm). The dishes were stored at 4°C until used; for some as yet undetermined reasons, remelted medium was not satisfactory. Plates of CT agar were dried before use by placing them in a 30 to 37°C incubator for a few hours.

Other culture media. Trypticase soy broth, Trypticase soy agar, and bromocresol purple lactose agar were from Bio-Mérieux (under BBL license); API 20E strips were from API System (La Balme-les-

grottes, France) and were used for identification of enterobacteria. The erythritol medium of Slotnick and Dougherty (21) and the deoxyribonuclease (DNase)-toluidine blue-cephalothin (DTC) agar of Farmer et al. (11) were made according to the instructions published by these authors. A medium based on DTC, but without cephalothin (DNase-toluidine blue agar), was used for the DNase test; DNase-positive strains grow and form a red halo on it.

Inoculation methods. In the survey of various media leading to formulation of CT agar, we used the Denley automatic multipoint inoculator, with the inoculum consisting either of young Trypticase soy broth cultures or (to reduce carry-over of nutrients in the inoculum) young Trypticase soy broth cultures diluted 100-fold with sterile distilled water. In determining efficiency of colony formation, suitable dilutions (usually 10^{-7}) of the Trypticase soy broth cultures were spread, with the aid of a turntable, on the surfaces of dried CT agar and Trypticase soy agar plates.

Clinical samples. A set of 513 consecutive clinical samples (urine, feces, sputum), as received in the bacteriological laboratory of the Pellegrin Hospital (Bordeaux), were run through the routine, conventional procedures of this laboratory (9, 16) as well as on CT agar. These conventional procedures are as follows. The medium used for isolating enterobacteria from urine and sputum samples was bromocresol purple lactose agar. Feces samples usually were streaked onto both bromocresol purple lactose agar and Difco SS agar to recover pathogenic enterobacteria. Media for selective isolation of pseudomonads, gram-positive cocci, and other non-enterobacteria also were used routinely but are of no interest in the present context. After overnight incubation at 35°C, the plates were examined for colonies. Colonies of different aspects and size were suspended in a few drops of saline and examined microscopically before and after Gram staining. Identification of gram-negative rods not readily recognized as *Pseudomonas* spp. (by colonial morphology and oxidase test) was made by means of the API 20E system. Enumeration of the microbes in urine was performed by using the D.G.U. system (Institut Pasteur Production, Paris).

Urine samples were spotted onto CT agar, whereas sputum and stool samples were streaked onto CT agar with a loop; the plates were incubated at 30°C for up to 7 days. All colonies that grew on CT agar were checked by spot inoculations onto DNase-toluidine blue agar (most *Serratia* strains are DNase positive; 13). Occasionally, the following replica method was used to disclose *Pseudomonas* colonies (which mainly are oxidase positive) by the oxidase test. A round patch of sterile filter paper was carefully layered onto the colonies to get a print of the composite culture (on the paper). The filter paper was then carefully removed and deposited into an empty petri dish where it was flooded with a freshly prepared 1% (wt/vol) aqueous dimethyl-*p*-phenylenediamine chloride solution. The paper was examined immediately for violet spots, the locations of the oxidase-positive colonies on the plate

TABLE 1. Cultures used and their ability to grow on CT agar

Species name ^a	Biotype ^b	Strains tested (no.)	No. of strains growing on CT agar ^c			Species name ^a	Biotype ^b	Strains tested (no.)	No. of strains growing on CT agar ^c		
			+	(+)	-				+	(+)	-
<i>Serratia liquefaciens</i>	C1a	7	7			<i>Erwinia rhapontici</i>		2			2
	C1b	10	9	1				4			4
	C1c	2	1		1			2			2
	C1d	2	1	1				9			9
	Not yet typed	4	4					2			2
<i>Serratia marcescens</i>	A1	6	6			<i>Levinea mallo-natica</i> ^f		8	7 ^g	1 ^g	
	A2	5	5			<i>Proteus inconstans</i>		3			3
	A3	7	7			<i>Proteus mirabilis</i>		3			3
	A4	14	13	1		<i>Proteus morgani</i>		3	3		
	A5	10	10			<i>Proteus rettgeri</i>		3			3
	A6	2	2			<i>Proteus vulgaris</i>		8			8
	A8	9	7	2		<i>Acinetobacter cal-coaceticus</i>		4			4
	TCT	10	10			<i>Aeromonas hy-drophila</i>		2			2
	NN	4	4			<i>Pseudomonas aeruginosa</i>		43 ^h			43
	Not yet typed	26 ⁱ	22	2	2	<i>Pseudomonas al-caligenes</i>		3			3
<i>Serratia marino-rubra</i>	B1	12	10	2		<i>Pseudomonas fluo-rescens</i>		1			1
	B2	3	3			<i>Pseudomonas pu-tida</i>		1			1
	B3	3	3			<i>Pseudomonas tes-tosteroni</i>		1			1
	Not yet typed	3	3			<i>Staphylococcus aureus</i>		2			2
<i>Serratia plymu-thica</i>	C2a	4	4			<i>Streptococcus fae-cal</i>		2			2
	C2b	11	1	9	1	<i>Vibrio cholerae</i>		2			2
	C2c	5		1	4	<i>Vibrio sp.</i>					
<i>Serratia sp.</i>		1	1								
<i>Citrobacter freun-dii</i>		4			4						
<i>Citrobacter sp.</i> ^e		2			2						
<i>Enterobacter cloa-cae</i>		4		2	2						
<i>Enterobacter aer-ogenes</i>		4		2	2						
<i>Erwinia caroto-vora</i>		4			4						
<i>Erwinia chrysan-themi</i>		3			3						
<i>Erwinia herbicola</i>		5			5						

^a Name as received or (for *Serratia*) as determined by Grimont et al. (13).

^b *Serratia* biotypes according to Grimont et al. (13). "TCT" and "NN" are new *Serratia* biotypes (P. A. D. Grimont and F. Grimont, unpublished observation).

^c Symbols: +, Good growth within 3 days; (+), growth within 4 to 7 days; -, no growth within 7 days.

^d A set of *Serratia* serotypes provided by L. Le Minor.

^e These are the lysine-decarboxylase-positive "*Citrobacter*-like" strains studied by Leclerc and Buttiaux (15).

^f Also known as *Citrobacter diversus*.

^g The seven slow-growing strains are also known as *Providencia stuartii* and the nongrowing strain as *Providencia alcalifaciens*.

^h Including all serotypes of Habs.

were deduced from that of the spots, and then such colonies were ignored (all *Serratia* strains are oxidase negative; 13). The API 20E or Enterotube (Roche Diagnostica) systems were used to complete identification.

Sensitivity of CT agar for detecting *Serratia* in feces. Known numbers of *Serratia* cells were added

to replicate portions of a *Serratia*-free, pooled fecal specimen. This pooled fecal specimen was rendered fluid by dilution with an equal volume of sterile distilled water. The additions ranged from 10^3 to 10^7 *Serratia* cells/ml of the pooled, diluted feces. Samples (0.05 ml) of each of the resulting artificially contaminated specimens were streaked onto CT

TABLE 2. Comparison of CT agar with DTC agar and erythritol agar for growth of *Serratia* and other bacteria

CT medium growth ^a	DTC medium ^b		Erythritol medium ^d	Strains (no.)		
	Growth ^a	DNase ^c	Growth ^a	<i>Serratia</i>	<i>Pseudomonas</i>	Others
+	+	+	+	41	0	0
+	+	W	+	2	0	0
(+)	+	+	+	1	0	0
+	+	+	-	30	0	0
+	+	W	-	5	0	0
(+)	+	+	-	4	0	0
(+)	+	W	-	1	0	0
+	+	-	+	2	0	0
+	+	-	-	3	0	0
(+)	+	-	-	0	0	2 ^c
+	-	-	-	1	0	0
(+)	-	-	-	1	0	3 ^f
-	+	+	-	2	4	0
-	+	W	-	1	7	0
-	+	-	+	0	0	1 ^g
-	+	+	(+)	1	0	0
-	+	-	-	0	31	4 ^h
-	(+)	-	-	0	0	1 ⁱ
-	-	-	-	0	2	34 ^j
-	-	-	+	0	0	1 ^k
-	-	-	(+)	0	0	2 ^l

^a Symbols: +, Good growth within 3 days; (+), growth within 4 to 7 days; -, no growth within 7 days.

^b Procedure of Farmer et al. (11).

^c Symbols: +, Distinct red halo within 2 days; -, medium remains blue at 2 days. Abbreviation: W, Weak reddening within 2 days.

^d Procedure of Slotnick and Dougherty (21).

^e One strain each of *Enterobacter aerogenes* and *Enterobacter cloacae*.

^f One strain each of *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Proteus inconstans* (= *Providencia stuartii*).

^g *Vibrio* sp.

^h *Enterobacter cloacae* (one strain), *Hafnia alvei* (two strains), and *Proteus morgani* (one strain).

ⁱ *Acinetobacter calcoaceticus*.

^j *Acinetobacter calcoaceticus* (one strain), *Aeromonas hydrophila* (four strains), *Bacillus cereus* (two strains), *Citrobacter freundii* (four strains), *Enterobacter aerogenes* (two strains), *E. cloacae* (one strain), *Erwinia carotovora* (one strain), *E. herbicola* (three strains), *Escherichia coli* (two strains), *Klebsiella pneumoniae* (three strains), *Levinia maulonatica* (= *Citrobacter diversus*) (two strains), *Proteus inconstans* (= *Providencia alcalifaciens*) (one strain), *Proteus mirabilis* (one strain), *Proteus vulgaris* (one strain), *Staphylococcus aureus* (one strain), *Streptococcus faecalis* (two strains), *Vibrio cholerae* (two strains), *Vibrio* sp. (one strain).

^k *Acinetobacter calcoaceticus*.

^l One strain each of *Acinetobacter calcoaceticus* and *Vibrio* sp.

agar. The plates were incubated at 30°C, and the colonies were counted after 48 h. Representative colonies that appeared on the CT agar were verified as being *Serratia* by spotting on DNase-toluidine blue agar. The experiment was carried out with two red-pigmented *Serratia marcescens* strains (biotype NN from neonatal enteritis and biotype A2 from sputum) and with four nonpigmented *S. marcescens* strains (biotype A4 from a septicemia, biotype A5 from an insect, biotype A8 from a septicemia, and biotype TCT from soft-tissue infection).

RESULTS

Selectivity of CT agar for *Serratia*. Practically all *Serratia* strains tested, including representatives of all species and most biotypes (13), grew well on CT agar from relatively small inocula (about 10⁵ cells deposited by the Denley automatic multipoint inoculator; Table 1). Very few of the non-*Serratia* cultures tested grew under these conditions. The rare non-*Serratia* cultures that grow on CT agar (occasional *Pseudomonas*, *Proteus*, and *Enterobacter* strains) can easily be distinguished from *Serratia* by other procedures. Eight strains of *Serratia* spp. (out of 160 tested) failed to grow on CT agar; six of these (including four strains belonging to biotype C2c of *Serratia plymuthica*) originated from culture collections rather than from clinical material. Biotype C2c of *S. plymuthica* (13) presently consists only of historical strains that were isolated about a century ago and the inability of these strains to grow on CT agar may not be representative of this taxon. These biotype C2c strains did grow on caprylate media in which the thallosulfate was either omitted or reduced in concentration to 0.012%, but the resulting media allowed the growth of too many *Pseudomonas* strains to provide the selectivity desired for our present purposes.

Efficiency of colony formation on CT agar. Parallel plates of CT agar and Trypticase soy agar were streaked with the same dilutions of various *Serratia* and non-*Serratia* cultures. The colonies were counted after 3 days at 30°C. After normalizing the Trypticase soy agar colony counts to 100, the relative numbers of colonies formed on CT agar ranged from 41 to 117 (mean, 80.7) for the *Serratia* strains, and they were close to 0 for the non-*Serratia* strains so tested.

Sensitivity of CT agar for *Serratia* deliberately added to a pooled fecal specimen. Practically quantitative recovery was achieved of the 10³ to 10⁷ *Serratia* cells/ml added to the pooled fecal specimen. Pigmented and nonpigmented *Serratia* strains were recovered with equally high sensitivity. Interference from the fecal bacteria was negligible, amounting at the most to a few pinpoint colonies.

TABLE 3. Results of in-tandem runs of 513 consecutive clinical samples on CT agar and by conventional procedures

Kind of samples (no. of samples)	Results by conventional procedures ^a (no. of samples)	Results on CT agar (no. of samples yielding):		
		Isolation of <i>Serratia</i>	Isolation of non- <i>Serratia</i>	No growth
Urine (362)	No bacteria (123)	0	0	123
	No <i>Serratia</i> (224)	7	6 ^b	211
	<i>Serratia</i> present (15)	15	0	0
Feces (65)	No <i>Serratia</i> (65)	4	0	61
	<i>Serratia</i> present (0)	0	0	0
Sputum (86)	No <i>Serratia</i> (85)	1	5 ^c	79
	<i>Serratia</i> present (1)	1	0	0

^a The conventional procedures used at the Pellegrin Hospital are detailed in the Materials and Methods section.

^b These corresponded to four isolates of *Providencia* sp. (= *Proteus inconstans*) and two isolates of *Klebsiella* sp.

^c All of these were *Pseudomonas aeruginosa*, easily recognized by their pigmentation on CT agar, and confirmed by oxidase positivity and other procedures. These sputum samples contained very large numbers of *P. aeruginosa*; the appearance on CT agar of *P. aeruginosa* colonies may have resulted from the very large inoculum, the carry-over of nutrients or thallate-counteracting materials from the sputum, and/or other factors.

Comparison of CT agar with other media recommended for isolating *Serratia*. CT agar generally detected *Serratia* and discriminated against non-*Serratia* somewhat more accurately than did the DTC medium (11), although there are a few instances in which the DTC medium was superior to the CT agar in these respects (Table 2). CT agar was substantially more accurate in discriminating *Serratia* from non-*Serratia* than was the erythritol medium (21).

Comparison of CT agar with conventional procedures for isolating *Serratia* directly from clinical specimens. The use of CT agar on 513 consecutive clinical specimens permitted direct isolation (and prompt tentative identification) of *Serratia* from clinical specimens (Table 3). In each one of the 16 specimens (3.1% of the series) in which *Serratia* was detectable by the conventional procedures, *Serratia* was also detected by means of CT agar. More importantly, *Serratia* was detected in 12 additional specimens (2.3% of the series) in which *Serratia* was not detectable by the conventional procedures—a substantial (74.3%) improvement in detectability of *Serratia* in actual clinical laboratory practice.

DISCUSSION

The rationale in devising this selective medium was to test various substances and/or conditions uniquely favoring *Serratia* and counterselecting non-*Serratia* strains likely to be present in the same environments. The task of finding suitable selective conditions for *Serratia*

was facilitated by the availability of a recent computer taxonomic survey of that genus (13). The results of that survey (13) indicate that there are four phenons in *Serratia*, each of which corresponds to a discrete species of that genus: *S. marcescens*, *S. liquefaciens*, *S. plymuthica*, and *S. marinoruba* (the latter species includes most strains previously placed in *S. rubideae*). From the results of the computer taxonomic survey (13) and other indications in the literature (23), caprylate appeared to be a suitable and quite selective carbon source for practically all strains of *Serratia* and for only rare strains of other enterobacteria. However, in our first trials with a caprylate medium, almost all *Pseudomonas aeruginosa* strains tested showed an ability to grow on this medium. This species, common in the hospital environment, had to be selectively inhibited. After trial and error, thallous salts were found to inhibit *Pseudomonas* spp. and still allow practically all *Serratia* strains to grow. Moreover, the efficiency of colony formation by *Serratia* spp. on CT agar was close to that on a rich, nonselective medium (Trypticase soy agar).

The use of thallium (thallous) salts in selective media was probably introduced by Edwards (8) for isolating *Mycoplasma* spp. Wilson and Salvin (29) used thallium salts for isolating streptococci. Because of its relatively high solubility, thallous acetate is the most commonly used thallium salt. Resistance of *Serratia* strains to thallous acetate had been noted in the numerical taxonomic studies (12, 13). In the present study, introduction of acetate ions into

the defined medium was avoided by using 0.025% (wt/vol) thallos sulfate (molecular weight of Ti_2SO_4 , 504.85; hence, 1.0 mM Ti^-) as the thallium salt. Thallos sulfate should be handled with care (Poisonous! mean lethal dose: 25 mg/kg, orally in rats). It is sufficiently soluble in water for our purpose (4.87 g in 100 ml of water at 20°C).

CT agar allows growth of practically all tested strains of *S. marcescens*, *S. marinorubra*, *S. liquefaciens*, and of two out of the three biotypes (13) of *S. plymuthica* (a species not of clinical significance). Biotype C2c of *S. plymuthica*, most strains of which did not grow on CT medium, is probably of minor taxonomic significance as it presently (13) consists only of old historical strains. We want to emphasize that the species *S. plymuthica* itself has never been associated with any infection of humans; however, if some other study requires better recovery of this species, the thallos sulfate concentration should be reduced to 0.012%; in our experience, all *S. plymuthica* strains do grow in the presence of 0.012% Ti_2SO_4 .

CT agar is highly selective for *Serratia* and compares well with, or is a definite improvement over, other media recommended for that purpose. Goldin, Shaffer, and Brown (Bacteriol. Proc., p. 96, 1969) described a medium that was made selective by the use of cephalothin (100 $\mu\text{g}/\text{ml}$) and differential by the use of DNase test agar supplemented with toluidine blue and egg yolk. Cate (Proc. Int. Congr. Chemother., 7th, p. 763-764, 1971) used DNase test agar plus toluidine blue rendered selective for *S. marcescens* by addition of 30 μg each of cephalothin and colistimethate per ml. Farmer et al. (11) described the DTC agar that is evaluated in this paper. However, in the recent computer taxonomic study (13), 7% of *S. marcescens* strains were found to be DNase negative, as were also 8% of *S. liquefaciens* strains and 5% of *S. plymuthica* strains. Such DNase-negative strains are unlikely to be recovered as *Serratia* by means of any of the preceding DNase-based media. In the same computer taxonomic study (13), resistance to colistimethate was found irregularly distributed among the four species of *Serratia*: although no *S. marcescens* strains were inhibited by colistimethate at 10 $\mu\text{g}/\text{ml}$ or higher concentrations, as little as 10 μg of colistimethate per ml (one-third the concentration recommended by Cate) still inhibited 41% of *S. marinorubra* strains, 15% of *S. plymuthica* strains, and 4% of *S. liquefaciens* strains. Media rendered very selective with antibiotics can be devised to isolate a given sort of nosocomial *Serratia* strain (6), but such media cannot provide the general utility

that we desire in our overall program on *Serratia* ecology.

Slotnick and Dougherty (21) and Lynch and Kenealy (17) described, for isolation of *Serratia*, minimal media with erythritol as the sole carbon source. These are certainly very selective media for those *Serratia* strains that can grow on this carbon source. However, strains belonging to two species of *Serratia* (*S. liquefaciens* and *S. plymuthica*) and several biotypes of *S. marcescens* do not grow on erythritol (13).

Apart from the unsatisfactory growth of some biotype C2c *S. plymuthica* strains, a minor disadvantage of CT agar, as presently formulated, is that, when complete in one flask, it cannot be remelted; otherwise, it does not, for an unknown reason(s), allow growth of *Serratia*. However, poured plates of CT agar keep well at 4°C for several weeks, and the efficiency of colony formation by *Serratia* on such stored plates is the same as on freshly prepared medium.

CT agar could be very useful in the clinical laboratory for the more rapid and certain detection of *Serratia* from urine specimens, and for the much more sensitive recovery of *Serratia* from fecal samples, than any procedure hitherto described. We have already found in preliminary probes that CT agar is quite useful in ecological surveys, especially those related to hospital-acquired *Serratia* infections. Ecology of *Serratia* is not now soundly documented, and studies of this subject, certain aspects of which (intrahospital and extrahospital) are currently being investigated by us, should now be facilitated by the use of CT agar.

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