Selective Medium for Growth of *Proteus*

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A medium containing heart infusion agar supplemented with bile salts, lithium chloride, sodium thiosulfate, and sodium citrate was developed for the selective growth of *Proteus*.

Proteus grow well on several media, but no selective medium exists for their primary isolation. Because we were interested in the isolation of clinical strains of *Proteus* to be used for antibiotic susceptibility studies, we have developed a selective medium for such isolation.

The medium has the following composition (grams/liter): heart infusion agar (Difco), 40.0; bile salts (Oxoid no. 3), 5.0; LiCl, 5.0; $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 6.0; $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 5.5\text{H}_2\text{O}$, 6.0; water 1,000 ml. The pH of the medium was adjusted to 7.2; it was sterilized in the autoclave at 121 C for 15 min. The formulation of the medium was evaluated by testing a lot of antimicrobial substances, in different concentrations, using a rapid plating technique (3). The supplementation of the medium with bile salts, lithium chloride (1), sodium thiosulfate, and sodium citrate (2) at the concentrations stated above produced the selective growth of *Proteus*.

For qualitative evaluation of the medium, a total of 257 freshly isolated strains of *Proteus* from clinical material, including all the four species (Table 1), were examined. A total of 85 other bacterial cultures was also tested by a surface plating of a standard loopfull (diameter 3 mm) from an overnight broth culture and by incubation at 37 C for 24 h. Three to ten cultures of the following were examined: *Providencia stuartii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter* spp., *Aeromonas* spp., *Citrobacter freundii*, *Bacillus subtilis*, *Bacillus cereus*, *Serratia marcescens*, *Salmonella typhimurium*, *Shigella flexneri*, *Streptococcus faecalis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. All but 12 strains of the *Proteus* tested grew prolifically (Table 1). The same has been observed with the five strains of *P. stuartii* examined (*Providencia alcalifaciens* were not tested). On the contrary, no growth could be observed among the other bacteria tested. Heavy inoculum of *Pseudomonas aeruginosa* gave a fairly sparse growth on the medium.

Colonies of *Proteus* spp. on our medium were

moderate, large, thick, greyish-white, circular disks, dome-shaped and smooth. The opacity and size varied with different strains.

For the quantitative evaluation of the medium, parallel colony counts of *Proteus* were done, by surface plating, on this medium and on MacConkey agar (Oxoid no. 3). Such comparative studies were done in cultures from the exponential and stationary phase of growth. The results (Table 2) clearly demonstrate that the colony-forming ability of *Proteus* cultures from the exponential phase of growth is about the same on MacConkey agar and our medium; however, the size of colonies is somehow smaller. With cultures in the stationary phase, the viable counts on MacConkey agar exceeded those on our medium, whereas a significant diminution in the size of colonies was again noted. The results are in agreement with previous observations, supporting the view that the selectivity of the medium may be related to the size of the colonies of the microorganisms (3, 4), and to the existing difficulty of interpreting counts on selective media when the populations tested are not growing (3).

The usefulness of our medium for the direct isolation of *Proteus* from heavily contaminated inocula was partially assessed by mixing overnight cultures of *Proteus mirabilis* and *E. coli* with sterilized (Seitz filtered) feces and urine. Table 3 gives the numbers of microorganisms recorded after growth on MacConkey agar and our medium. It is evident that no difference

TABLE 1. Growth of *Proteus* strains on the selective medium

No. of <i>Proteus</i> strains	Positive cultures	
	No	%
168 <i>P. mirabilis</i>	159	94.6
32 <i>P. rettgeri</i>	32	100
31 <i>P. vulgaris</i>	30	96.9
26 <i>P. morganii</i>	24	92.3

TABLE 2. Recovery of *Proteus* strains in exponential and stationary phase of growth^a

Strains	Exponential phase ^b (CFU/ml)		% ^c	Stationary phase ^d (CFU/ml)		% ^c
	MacConkey agar	Selective medium		MacConkey agar	Selective medium	
<i>P. mirabilis</i>						
17/74 ^e	1.2×10^8	0.9×10^8	75	1.5×10^9	7.8×10^8	52
21/74	9.5×10^7	1.1×10^8	110	8.4×10^8	7.2×10^8	85.7
28/74	1.5×10^8	1.9×10^8	126.6	7.9×10^8	6.2×10^8	107
23/74	3.0×10^8	3.1×10^8	103	1.8×10^9	9.4×10^8	52.2
24/74	2.8×10^8	2.4×10^8	86.1	5.9×10^8	2.6×10^8	45.2
<i>P. rettgeri</i>						
1/74	1.2×10^8	1.4×10^8	112	1.0×10^9	6.4×10^8	64
132/74	2.6×10^8	2.3×10^8	88.4	2.0×10^8	1.3×10^8	65
134/74	1.7×10^8	1.9×10^8	111.3	5.3×10^9	9.9×10^8	18.6
209/74	2.1×10^8	1.8×10^8	85.7	6.9×10^8	4.8×10^8	69.5
217/74	1.9×10^8	2.3×10^8	121	8.2×10^8	4.0×10^8	48.9
<i>P.morganii</i>						
18/74	6.5×10^7	5.6×10^7	86	1.4×10^8	1.0×10^8	71.5
31/74	8.9×10^8	9.2×10^8	103	1.6×10^9	7.0×10^8	43.7
41/74	1.3×10^8	6.8×10^7	52.3	2.1×10^9	9.1×10^8	43.3
47/74	8.7×10^7	7.3×10^7	84	9.5×10^8	2.6×10^8	27.3
48/74	1.8×10^8	1.6×10^8	86.5	8.5×10^8	6.4×10^8	75.2
<i>P. vulgaris</i>						
16/74	3.2×10^7	3.1×10^7	97.2	5.8×10^8	4.5×10^7	7.8
19/74	1.0×10^8	0.8×10^8	80	7.0×10^8	3.9×10^8	55.7
26/74	1.5×10^7	1.2×10^7	80	7.1×10^7	2.6×10^7	36.8
55/74	3.6×10^7	4.1×10^7	114.2	6.3×10^8	3.9×10^8	61.9
66/74	8.1×10^7	4.2×10^7	52.8	1.2×10^8	5.8×10^8	48.3

^a Incubation at 37 C for 24 h.^b 8-h aged cultures. CFU, Colony-forming units.^c The number of colony-forming units on MacConkey agar is taken as 100%.^d 48-h aged cultures.^e Laboratory numbers.TABLE 3. Recovery of *Proteus* from artificial clinical material^a

Artificial clinical material	No. added microorganisms/ml (MacConkey agar)		No. recovered microorganisms/ml				%
	<i>E. coli</i>	<i>P. mirabilis</i>	MacConkey agar		Selective medium		
			<i>E. coli</i>	<i>P. mirabilis</i>	<i>E. coli</i>	<i>P. mirabilis</i>	
Feces	1.5×10^7	1.2×10^7	1.4×10^7	1.3×10^7	0	1.15×10^7	96
Urine	1.5×10^7	1.2×10^7	1.6×10^7	1.1×10^7	0	1.17×10^7	98

^a The artificial clinical material was prepared by adding 0.1 ml from overnight cultures of *E. coli* and *P. mirabilis* in 1 ml of sterilized (Seitz filtered) urine and feces saline solution.

exists in the recovery of *Proteus* from artificially contaminated feces and urine.

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