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Enhancement of *Ureaplasma urealyticum* Growth on a Differential Agar Medium (A7B) by a Polyamine, Putrescine

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Growth of *Ureaplasma urealyticum* isolates from clinical exudates and urine specimens was significantly improved by supplementation of a *Ureaplasma* differential agar medium by putrescine. The improved medium was designated medium A7B.

The most sensitive and specific methods for detection and identification of *Ureaplasma urealyticum* (11) in primary cultures of clinical exudates and urine specimens are those based upon demonstration of *Ureaplasma* urease (6-8). Previously, identification of *U. urealyticum* in primary, standard agar cultures was simplified by the application of a direct spot test for urease in colonies of the organism (7). In this procedure the application of a urea-manganese reagent results in precipitation of a metallic oxide reaction product (MnO_2) within and on the surface of *Ureaplasma* colonies. In the usual low-power (×100) microscopic examination by transmitted light from the microscope substage condenser, such treated *Ureaplasma* colonies are visualized as deep brown (or gray-black) manganese accretion colonies.

The simplicity and success of the direct spot test for urease in colonies of ureaplasmas (7) motivated the development of a differential agar medium (M. C. Shepard and C. D. Lunceford, Bacteriol. Proc. **70**:83, 1970). An improved version of this differential (A6) agar medium subsequently was reported by Shepard and Lunceford (9) and was designated differential agar medium A7. The latter medium featured a 50% reduction in manganese salt concentration. The reduction in manganese resulted in notably improved performance in supporting growth of ureaplasmas in primary cultures of clinical specimens. The A7 differential agar subsequently was used by Shepard and Lunceford (10) in an agar growth inhibition method for the serological typing of *U. urealyticum* isolates from urethritis patients.

The polyamine putrescine (1,4-diaminobutane) was first incorporated in fluid culture media for the cultivation of *U. urealyticum* by Masover and Hayflick (3) and by Masover et al. (4). Razin et al. (5) reported the inclusion of putrescine in agar media containing dialyzed calf serum and dialyzed horse serum. Putrescine by itself, however, did not support growth of ureaplasmas, but the combination of added urea and putrescine (0.01 M each) supported good growth. The beneficial effect of putrescine was also seen in agar media containing nondialyzed normal horse serum, where the addition of putrescine together with urea improved growth considerably over that observed with urea alone. The possible usefulness of putrescine supplementation of differential agar medium A7 (9) was investigated, and the results are reported in this note.

A total of 251 clinical specimens was used in this comparative study, consisting of centrifuged deposits from freshly voided urine specimens from male patients with *U. urealyticum*-associated nongonococcal urethritis. Culture plates of differential agar medium A7 were prepared as previously described (9). A companion set of plates of the same A7 medium was prepared containing a final concentration of 0.01 M putrescine dihydrochloride (Sigma no. P7505), (the same concentration found effective in standard agars by Razin et al. [5]). The polyamine was added as a weighed dry powder to the A7 basal medium (0.33 g in the published formula [9]). Both the A7 (control) plates and the putrescine-supplemented plates were inoculated in identical manner with 0.03-ml drops of sediment from centrifuged urine specimens, generally employing six to eight specimens per plate. After the inocula were absorbed, plates were inverted and incubated for 48 h at 36°C in a GasPak anaerobic system container with the hydrogen-carbon dioxide generator envelope (BBL Microbiology Systems, Cockeysville, Md.). After incubation, the control and putrescine-supplemented plates were compared for performance in supporting growth of *U. urealyticum* by visual inspection and examination under the low power (×100) of the microscope. Measurements were made of 20

randomly selected *Ureaplasma* colonies from paired plantings on each medium from 20 different urine specimens that were positive for ureaplasmas (400 total colonies measured on each type of medium). Measurements were made under the low power with a calibrated ocular micrometer.

The results showed that of the 251 clinical isolates of *U. urealyticum* cultured on putrescine-supplemented A7 differential agar medium, 157 (62.5%) exhibited definitely improved growth consisting of larger colonies or increased numbers of colonies (Table 1). No essential differences were observed in *Ureaplasma* growth on either medium among 85 (33.9%) of the isolates. Nine isolates (3.6%) were reduced in numbers but large in size on putrescine-supplemented medium. The reduced numbers probably reflected sampling error due to inherently low numbers of organisms in these specimens, since the observed differences were small (less than 10 colonies).

Ureaplasma colonies on agar media are characteristically small in size and often difficult to recognize. Any modification of the agar medium that results in larger colonies is therefore a needed and distinct improvement, provided that colony numbers are not adversely affected. The incorporation of putrescine in the A7 differential agar medium resulted in a significant increase in mean colony size of *Ureaplasma* isolates of from 70.3-μm diameter on the control medium to 95.8-μm on putrescine-supplemented medium, a 36.3% increase in size (Table 2). In addition, visualization of ammonia diffusion in the agar surrounding single *Ureaplasma* colonies or masses of colonies (indicated by zone-gradient manganese reaction product) was intensified in putrescine-supplemented medium, and weak or absent in the control medium. A related polyamine, 1,7-diaminoheptane (2) (Aldrich no. D1740-8) was also examined in addition to putrescine and found to produce enhancement of

TABLE 1. *Effect of putrescine supplementation of differential agar medium A7 on growth of Ureaplasma urealyticum isolates in primary cultures*[a]

U. urealyticum colonies	No.	%
Improved growth[b]	157	62.5
No essential differences	85	33.9
Reduced colony numbers	9	3.6

[a] Putrescine, 0.01 M.

[b] Compared with control medium without putrescine, 157 clinical specimens yielded improved growth of *U. urealyticum* as indicated by development of larger colonies (N = 132) or by larger numbers (N = 25).

TABLE 2. *Effect of putrescine on colony size of Ureaplasma urealyticum isolates in primary cultures on differential agar medium A7*

Supplementation	Mean colony diam (μm)[a]	Size increase (%)
None	70.3 ± 14.3	
Putrescine[b]	95.8 ± 22.8	36.3

[a] Measurements were made of 20 colonies from each of 20 different *Ureaplasma*-positive specimens on each of the two media (400 colonies measured on each type of medium).

[b] Putrescine, 0.01 M.

Ureaplasma growth in A7 medium which was essentially identical to that provided by putrescine. Polyamines are stimulatory agents for a variety of bacteria, mammalian tissue cell lines, viruses, and plants and are involved in the stabilization of cell membranes, preventing cell lysis (1). Different species of *Candida*, for example, metabolize putrescine as a sole source of nitrogen and produce gamma-aminobutyric acid through the action of putrescine oxidase (M. Gunasekaran, T. K. Narayanan, and S. Feldman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, K21, p. 149). The explanation of the growth-enhancing property of putrescine for *U. urealyticum* is presently unknown. The superior performance of putrescine-supplemented A7 differential agar medium has been utilized in our laboratory for over a year and a half for the primary isolation of ureaplasmas from male patients with nongonococcal urethritis and infertility problems and from females with reproductive failure. The modified differential agar medium has been designated medium A7B.

LITERATURE CITED

1. **Bachrach, U.** 1973. Function of naturally occurring polyamines, p. 56–59, Academic Press, Inc., New York.
2. **Brock, M. L.** 1965. The effects of polyamines on the replication of T4rII mutants in *Escherichia coli* K12(λ). Virology **26**:221–227.
3. **Masover, G. K., and L. Hayflick.** 1973. The growth of T-strain mycoplasmas in media without added urea. Ann. N.Y. Acad. Sci. **225**:118–130.
4. **Masover, G. K., J. R. Benson, and L. Hayflick.** 1974. Growth of T-strain mycoplasmas in medium without added urea: effect of trace amounts of urea and of a urease inhibitor. J. Bacteriol. **117**:765–774.
5. **Razin, S., G. K. Masover, M. Palant, and L. Hayflick.** 1977. Morphology of *Ureaplasma urealyticum* (T-mycoplasma) organisms and colonies. J. Bacteriol. **130**: 464–471.
6. **Shepard, M. C.** 1973. Differential methods for identification of T-mycoplasmas based on demonstration of urease. J. Infect. Dis. **27**(Suppl):S22–S25.
7. **Shepard, M. C., and D. R. Howard.** 1970. Identification of "T" mycoplasmas in primary agar cultures by means of a direct test for urease. Ann. N.Y. Acad. Sci. **174**: 809–819.

8. **Shepard, M. C., and C. D. Lunceford.** 1970. Urease color test medium U-9 for the detection and identification of "T" mycoplasmas in clinical material. Appl. Microbiol. **20**:539–543.
9. **Shepard, M. C., and C. D. Lunceford.** 1976. Differential agar medium (A7) for identification of *Ureaplasma urealyticum* (human T mycoplasmas) in primary cultures of clinical material. J. Clin. Microbiol. **3**:613–625.
10. **Shepard, M. C., and C. D. Lunceford.** 1978. Serological typing of *Ureaplasma urealyticum* isolates from urethritis patients by an agar growth inhibition method. J. Clin. Microbiol. **8**:566–574.
11. **Shepard, M. C., C. D. Lunceford, D. K. Ford, R. H. Purcell, D. Taylor-Robinson, S. Razin, and F. T. Black.** 1974. *Ureaplasma urealyticum* gen.nov., sp.nov.: proposed nomenclature for the human T (T-strain) mycoplasma. Int. J. Syst. Bacteriol. **24**:160–171.