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Nutritional Requirements for Growth of Uropathogenic *Escherichia coli* in Human Urine

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Enrichment with D-cycloserine was used to identify *Escherichia coli* auxotrophic mutants that exhibited limited growth in human urine. Bacterial synthesis of guanine, arginine, and glutamine was found to be required for optimal growth in urine. Mutants that required leucine, methionine, serine, phenylalanine, or proline also exhibited reduced growth in urine. Several other nutritional mutants, including nicotinamide auxotrophs, which are found frequently among cystitis isolates, exhibited normal growth in urine.

Escherichia coli urinary tract infections (UTIs) are among the most common infectious diseases and account for over 80% of acute community-acquired UTIs and a majority of hospital-acquired UTIs. In addition to possessing unique properties, such as adherence to urinary tract tissue, which enhance their virulence, all uropathogens are able to use urine as a growth medium. Bacterial genera associated with UTIs grow well in human urine, whereas nonuropathogenic genera do not (10). In one study, Gordon and Riley (5) reported that rapid growth of some *E. coli* uropathogens in urine may even permit colonization in the absence of specific adherence organelles. They concluded that the ability of uropathogenic *E. coli* strains to survive and efficiently use resources available in urine is an important adaptation for inhabiting the urinary tract. In addition, Russo et al. (8) have shown that de novo growth factor synthesis may be required for *E. coli* urovirulence.

While it is well known that human urine will support bacterial growth, it is not currently known whether urine contains sufficient levels of all growth factors to support growth in the absence of de novo growth factor synthesis. The present study was undertaken to test the hypothesis that urine is an incomplete growth medium and that synthesis of one or more nutritional factors by uropathogenic *E. coli* is required for bacterial growth.

Cycloserine enrichment. *E. coli* HU1542 is a *lacZ rfe* derivative of *E. coli* P28, a pyelonephritogenic isolate provided by Catharina Svanborg from the Göteborg Collection, Göteborg, Sweden. *E. coli* HU1542 was transduced with Mu *d1lac* as described by Silhavy et al. (9) and grown static overnight in L broth containing ampicillin and 20 μg (each) of guanine, adenine, thymine, cytosine, and uracil per ml (L+ApGATCU). Bacteria were washed once with minimal salts medium, diluted 50-fold into 10 ml of urine supplemented with 0.4% glucose or minimal salts medium, and incubated for 2 h with aeration. Cycloserine was added (200 μg/ml), and incubation was continued for 3 h (2). The surviving bacteria were collected by filtration, washed twice with minimal salts medium, transferred to 10 ml of fresh L+ApGATCU broth, and incubated overnight with aeration. The enrichment procedure was repeated once, and after overnight incubation, survivors were spread on L+ApGATCU agar plates. Single colonies (3,000) were replicated onto urine agar or Davis-Mingioli minimal agar (3). Colonies that failed to grow on urine or minimal agar were selected for further study. Davis-Mingioli medium was supplemented with 0.2% glycerol. Urine studies were done with the first morning urine samples that were collected from volunteers, which were pooled, filter sterilized, and stored at −70°C prior to use. Results were confirmed with fresh unsterilized urine (not pooled) collected from three individuals and from one individual at two different times. No significant differences were observed with the different urine reagents. Media were solidified with 1.2% Bacto agar (Difco Laboratories, Detroit, Mich).

Characterization of auxotrophs. Survivors of cycloserine enrichment that grew on L agar containing added purines and pyrimidines but that did not grow on minimal glycerol were selected for further study. The growth factor requirement of each mutant was identified with the nutrient pools as described by Davis et al. (4). Mutants defective in synthesis of 14 different growth factors were identified. Each mutant was tested for growth in urine. For growth determination, bacteria were inoculated into urine containing 0.4% glucose and grown overnight. The absorbance (optical density at 600 nm) was measured and compared with that of the wild type, HU1542. Mutants defective in synthesis of guanine, arginine, arginine plus uracil, or glutamate exhibited reduced growth in 100% urine compared with that of the parent strain, HU1542 (Table 1). The growth deficiencies in urine could be rescued by addition of the relevant growth factor. These results confirm those of previous studies that suggest that arginine and guanine synthesis is required for growth in urine (8).

The poor growth of guanine mutants on urine was consistent with the observation that guanine is present in urine at reduced levels, 0.1% of the concentration required for luxurious *E. coli* growth (4).

The requirement for arginine and glutamine biosynthesis for growth in urine may also be attributed to low arginine and glutamine concentrations, since the amino acids are present at about 5 and 2% of the optimal concentration, respectively (11). Auxotrophic mutants exhibited the same growth rate as the wild type at low cell density ($<10^4$/ml) (data not shown) but grew to a lower final density as urine arginine and glutamine pools were depleted. In addition, uptake of arginine from the environment requires expression of the *argT* gene product, which is subject to nitrogen regulation (7). Transcription of the *argT* gene was reported to be reduced in the presence of high concentrations of NH_4 (10 mM), at a level approximating the ammonia concentration in normal urine (15 mM). Transport of glutamine is also subject to nitrogen regulation (7). As a consequence, reduced transport efficiency of arginine and glu-

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TABLE 1. Properties of auxotrophic mutants of *E. coli* HU1542

Strain	Growth factor requirement	Growth in urine[a]	P^b
HU1776	Guanine	−	<0.001
HU1785	Arginine	+	0.001
HU1790	Uracil + arginine	+	<0.01
HU1794	Glutamine (20 mM)[c]	+	<0.01
HU1779	Serine	++	0.05
HU1782	Proline	++	<0.01
HU1784	Leucine	++	<0.01
HU1786	Phenylalanine	++	0.05
HU1788	Methionine	++	<0.01
P28	Prototrophic	+++	

[a] Bacteria were inoculated into urine containing 0.4% glucose and grown overnight. The absorbance (optical density at 600 nm) was measured and compared with that of the wild type, HU1542. −, <1% of wild-type growth; +, 1 to 5% of wild-type growth; ++, 5 to 50% of wild-type growth.

[b] Significance of growth rate differences compared with growth of *E. coli* P28 was determined with the Excel analysis of variance statistics program.

[c] Elevated levels of glutamine were required for growth (4).

tamine may restrict their availability to bacteria growing in urine.

Mutant strain HU1790 required both arginine and uracil for growth in minimal media. This phenotype is typical of mutations in the *pyrA* gene. The *pyrA* gene product, carbamylphosphate synthetase, catalyzes the synthesis of carbamylphosphate from glutamine or free ammonia (1). Since carbamylphosphate is required for synthesis of both arginine and uracil, *pyrA* mutants require both growth factors. It is likely that the urine growth defect attributed to *pyrA* is due primarily to the arginine requirement. No mutants that required only uracil to grow were identified after enrichment in urine. Moreover, addition of arginine alone but not uracil alone restored wild-type growth in urine.

Additional auxotrophic mutants were isolated after selection in minimal salts medium. Mutants that required leucine, methionine, serine, phenylalanine, or proline grew in urine to a significantly lower density than did the wild type (Table 1); these amino acids are present in urine at 5 to 50% of the optimal concentration for bacterial growth. Mutants that required nicotinamide, histidine, tryptophan, cysteine, or adenine grew to as high a density in urine as the wild-type strain, a result consistent with the reported high concentration of these growth factors in urine. Nicotinamide-requiring mutants are also common among human cystitis isolates (6).

It is clear from the present and other studies that urine is an adequate medium for growth of uropathogenic *E. coli* strains (10). However the data presented here show that several factors required for bacterial growth are limiting in urine and must be provided by de novo bacterial synthesis. Agents that specifically inhibit synthesis of one or more of these factors may have therapeutic value for prevention or treatment of UTIs.

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