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Nutritional Requirements of Shigellae for Growth in a Minimal Medium

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Most (about 81%) of the clinical isolates of shigellae that were tested failed to grow in a minimal medium. Of the auxotrophic isolates belonging to the four *Shigella* species, 98% grew in a minimal medium containing methionine, nicotinic acid, and tryptophan. The combination of methionine and tryptophan appears to be an obligatory requirement for *Shigella dysenteriae* serotype 1 strains, while the combination of nicotinic acid and tryptophan appears to be obligatory for serotype 2. Requirements which varied in other isolates were, however, genetically stable, indicating that the auxotypes may be useful as epidemiological markers. Cultures of shigellae in liquid minimal medium containing the above three supplements showed rapid growth and gave reasonably high cell yields.

Reports on the nutritional requirements of shigellae are few. In the case of some *Shigella flexneri* isolates, a requirement for nicotinic acid and methionine has been reported (1, 2, 5–7). We tried without success to grow a small number of fresh clinical isolates of *S. flexneri*, *S. dysenteriae*, *S. sonnei*, and *S. boydii* in a minimal medium containing glucose, ammonium sulfate, and inorganic salts. The addition of nicotinic acid or methionine or both supported the growth of all but the *S. dysenteriae* isolates. We thus thought it of interest to examine the range of nutritional requirements in shigellae.

The minimal medium used in the study was MA medium (3) solidified with 1.5% agar (GIBCO Laboratories, Inc.) and supplemented with 1% glucose. We detected growth by using sterile tooth picks to patch colonies from the MacConkey agar plate onto the minimal agar plate and by visually examining the plate after 18 to 24 h of incubation at 37°C. All isolates were identified by standard biochemical and serological methods. Serotypes of the *S. flexneri* isolates were determined by monoclonal antibodies (N. I. A. Carlin, Ph.D. dissertation, Karolinska Institute, Stockholm, Sweden, 1986) in a slide agglutination test.

Initially, 375 fresh clinical isolates obtained from the Dhaka Treatment Centre of the International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh, were tested for their ability to grow in the minimal medium. Seventy-two isolates were prototrophic; their species distribution was as follows: 10 of 98 *S. dysenteriae* isolates, 14 of 140 *S. flexneri* isolates, 14 of 59 *S. sonnei* isolates, and 34 of 80 *S. boydii* isolates. Of the remaining 303 isolates, all but 4 *S. flexneri* isolates and 4 *S. boydii* isolates grew in MA medium supplemented with methionine (45 μg ml^{-1}), nicotinic acid (12.5 μg ml^{-1}), and tryptophan (20 μg ml^{-1}).

The minimum number of supplements necesssary for growth of different shigellae in a minimal medium was then determined as an indication of the existence of various auxotypes. For this study, a relatively random assortment of auxotrophic isolates was obtained to represent outbreaks apparently independent of time and place of occurrence and thus to minimize the possibility of close clonal relatedness among most of the isolates tested. Some isolates were obtained fresh from the Dhaka Treatment Centre. A larger number, composed of relatively old isolates (isolations of 1976 and 1980), was obtained from the International Center for Diarrhoeal Disease Research, Bangladesh, Culture Collection, which mainly includes isolates from its two field stations, Matlab in the mideastern part of the country and Teknaf in the southernmost tip, adjacent to the border of Bangladesh and Burma, in addition to isolates from its central facilities at Dhaka. Organisms used in the study represent both epidemic and endemic isolates.

Among the isolates of *S. dysenteriae*, those belonging to serotype 1 had an obligatory requirement for methionine and tryptophan, while those belonging to serotype 2 required nicotinic acid and tryptophan. Twenty isolates of *S. dysenteriae* serotype 1 obtained from Costa Rica, India, Saudi Arabia, and Thailand (five from each source) were all auxotrophic and required methionine and tryptophan for growth in MA medium (data not shown). Isolates of *S. dysenteriae* belonging to serotypes 3 through 10 appeared to have more exacting nutritional requirements. Of the 10 isolates tested, 8 required all three supplements.

In contrast to the relatively invariant and apparently serotype-specific requirements seen with isolates of *S. dysenteriae*, isolates of the other three *Shigella* species, showed considerable nutritional diversity. Thus, among the isolates of *S. flexneri* belonging to five serotypes, no indication of any serotype-specific requirement is apparent. Among the isolates of *S. sonnei* and *S. boydii*, representing two outbreaks, there also is considerable variability in auxotypes.

The invariant and serotype-specific requirements of *S. dysenteriae* isolates may have a diagnostic value in clinical and laboratory situations. The auxotypes seen in the other shigellae may, however, be useful as markers in epidemiological investigations, provided that the requirements are stable. Stability in the gut may be related to both the intrinsic stability of the genes mediating these requirements and a lack of genetic exchange between closely related bacteria that simultaneously inhabit the intestine.

The frequency with which auxotrophs reverted to prototrophy in vitro was determined for a selected number of isolates by plating washed cells from an overnight Trypticase

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soy broth culture on MA agar plates. No revertants were detected in 10^8 cells of each of the following representative isolates: *S. dysenteriae* serotype 1 (Met and Trp requiring); *S. dysenteriae* serotype 2 (nicotinic acid and Trp requiring); the nicotinic acid-requiring isolates of *S. flexneri* serotype 2a, *S. sonnei*, and *S. boydii*; and a Trp-requiring isolate of *S. boydii*.

Growth factor requirements of a sample of *Escherichia coli* and *Salmonella typhi* isolates were examined. Ten *E. coli* and seven *Salmonella typhi* isolates that were concurrently isolated with shigellae at the Dhaka Treatment Centre and 10 enteropathogenic *E. coli* isolates, and 40 enterotoxigenic *E. coli* isolates from a surveillance population at the Matlab center were tested. All the *E. coli* isolates were prototrophic and grew in MA agar. None of the *Salmonella typhi* isolates grew in any of the supplement combinations used (Table 1). The fact that all *E. coli* isolates were prototrophic while most shigellae, particularly *S. dysenteriae* and *S. flexneri*, were auxotrophic may, however, indicate that genetic exchange between these two groups of organisms involving genes mediating these nutritional requirements is not widespread. Exchange between *Salmonella* and *Shigella* species, if it occurs, is not likely to alter the profile of the nutritional requirements of the latter.

The rate and extent of growth of four representative test strains in liquid MA medium containing methionine, nicotinic acid, and tryptophan were determined in shaken liquid cultures. Test strains were grown overnight in Trypticase soy broth. About 10^7 cells, washed in phosphate-buffered saline (pH 7.4), were inoculated in 50 ml of MA medium supplemented with methionine, nicotinic acid, and tryptophan and shaken at 37°C. The number of CFU per milliliter of the culture was determined at different times by plating dilutions of the cultures in Trypticase soy agar and counting the number of colonies that developed after overnight incubation (Fig. 1). For comparison, a culture of *S. dysenteriae* GS01 serotype 1 shaken identically in Luria-Bertani broth was also included. It is clear that growth of *S. flexneri*, *S. sonnei*, and *S. boydii* strains is comparable to that of a culture grown in complex (Luria-Bertani) medium. The *S. dysenteriae* serotype 1 strain was relatively slow growing, but cell density of the stationary-phase culture was similar to that obtained for the other three strains.

The defined medium containing nicotinic acid, methionine, and tryptophan may facilitate screening of isolates for the expression of virulence-related regulated functions in clinical situations, since it supported the growth of about 98% of the shigellae. The growth rate of the relatively slow-growing *S. dysenteriae* serotype 1 strain in MA broth containing methionine, nicotinic acid, and tryptophan was sufficient to cause ampicillin-induced cell lysis, which thus facilitated enrichment of cultures with auxotrophic

TABLE 1. Growth of auxotrophic shigellae in MA agar

Species, serotype, and place and yr of isolation	No. tested	No. growing in MA agar containing[a]:						
		M	N	T	M + T	M + N	N + T	M + N + T
S. dysenteriae								
Serotype 1								
Dhaka, 1987	40	0	0	0	40	0	0	40
Teknaf epidemic, 1987	6	0	0	0	6	0	0	6
Dhaka, 1980	17	0	0	0	17	0	0	17
Teknaf, 1980	7	0	0	0	7	0	0	7
Serotype 2								
Dhaka, 1987	12	0	0	0	0	0	12	12
Teknaf, 1980	7	0	0	0	0	0	7	7
Dhaka, 1976	6	0	0	0	0	0	6	6
Serotypes 3–10								
Dhaka, 1987	10	0	0	0	0	0	0	8
S. flexneri[b]								
Serotype 1	31	6	7	0	13	29	8	31
Serotype 2	33	5	28	0	7	33	30	33
Serotype 3	7	3	6	0	0	7	6	7
Serotype 4	7	3	3	0	0	7	6	7
Serotype 5	9	4	6	0	0	7	6	9
S. sonnei								
Dhaka, 1987	16	0	0	0	0	8	16	16
Dhaka, 1976	11	0	11	0	0	11	11	11
S. boydii								
Dhaka, 1987	16	0	0	0	0	9	10	16
Dhaka, 1976	11	0	5	8	8	10	8	11
E. coli[c]	10[d]							
Salmonella typhi[c]	7[d]	0	0	0	0	0	0	0
Enterotoxigenic *E. coli*	40[d]							
Enteropathogenic *E. coli*	10[d]							

[a] Abbreviations: M, methionine; N, nicotinic acid; T, tryptophan.
[b] All *S. flexneri* serotypes were isolated at the Matlab center in 1986.
[c] Concurrently isolated with *Shigella* isolates.
[d] All *E. coli*, enterotoxigenic *E. coli*, and enteropathogenic *E. coli* isolates tested grew in unsupplemented MA. None of the *Salmonella typhi* isolates tested grew in MA.

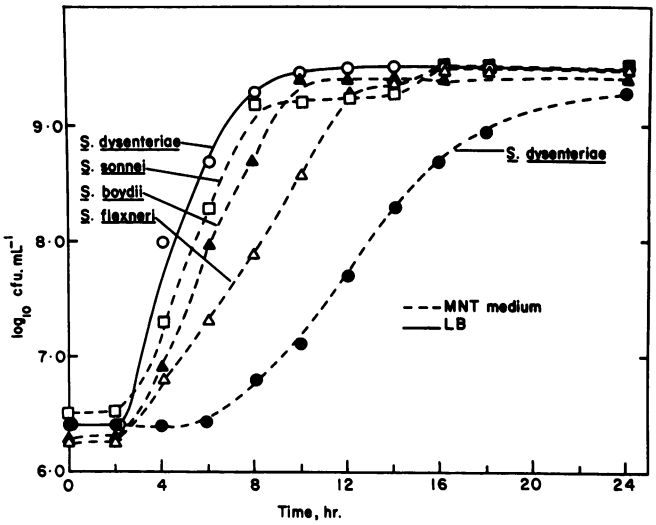

FIG. 1. Growth curves of strains of *Shigella* spp. in liquid MA medium supplemented with methionine, nicotinic acid, and tryptophan (MNT). For comparison, the growth curve of a *S. dysenteriae* serotype 1 strain in Luria-Bertani (LB) broth was also determined.

mutants. The unsupplemented MA medium was successfully used in effective counterselection of *S. dysenteriae* serotype 1 in crosses with *E. coli* to study the transfer of drug-resistant plasmids from shigellae that lacked useful counter-selectable markers, as exemplified by the multiply drug-resistant clinical strains that are currently being isolated (4).

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