A Medium for the Isolation of *Edwardsiella tarda*

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ABSTRACT

A new selective-differential medium for the isolation of *Edwardsiella tarda* is described. By the use of this medium ("ET Agar") and membrane filtration, 30% of 116 environmental water samples were found to contain *E. tarda*. A sample from Lake Superior yielded an isolate identified as *E. tarda* Biogroup 1, an extremely rare organism.

INTRODUCTION

Since its recognition as a taxonomic entity (4, 8, 11), *Edwardsiella tarda* has been shown to cause disease in humans and a variety of warm and cold-blooded vertebrates (5). As indicated by Ewing (3), *E. tarda* is easy to identify. Several features which help to differentiate *E. tarda* from other members of the Family *Enterobacteriaceae* can be derived from examination of comprehensive tables of biochemical reactions. Typical *E. tarda* isolates are negative for phenylalanine deamination and fermentation of most sugars (including lactose, sucrose, mannitol and xylose) and positive for lysine decarboxylation and hydrogen sulfide production (1, 6). The need for a dedicated selective-differential isolation medium was indicated by Farmer (5), and suggestions included hydrogen sulfide detection, the use of the antibiotic colistin, and non-fermented sugars added to an appropriate base medium. Within the Family *Enterobacteriaceae*, **the inclusion of colistin would restrict growth to mainly *Edwardsiella*, *Proteus*, *Morganella*, *Providencia*, *Serratia* and *Cedecea*** (5).

MATERIALS AND METHODS

In the present study, the subject of an American Society for Microbiology poster session (10), a plating medium was formulated which utilized MacConkey Agar Base (Difco) and incorporated colistin, the hydrogen sulfide detection system used for XLD Agar (2), lysine, and three of the sugars (sucrose, mannitol and xylose) not fermented by typical strains of *E. tarda* (1,6). The formulation as used in this study is given in Table 1.

To enhance the differentiation of *E. tarda* from the other colistin-resistant genera, a small amount of glucose was included in the medium along with the lysine. Thus, for typical *E. tarda* strains, decarboxylation of the lysine, production of a small amount of acid from the glucose, and non-fermentation of the three major sugars would result in colonies with a **net alkaline** reaction. Other colistin-resistant enterobacteria would tend to produce **net acidic** colonies; for *Morganella* this would be expected to happen with glucose fermentation alone (1, 6).

Besides *E. tarda* and *Proteus*, the other colistin-resistant organisms do not produce hydrogen sulfide. Excessive acid production from xylose fermentation by *Proteus* would be expected to negatively affect hydrogen sulfide detection ( ), thus leaving *E. tarda* effectively differentiated from *Proteus* by its H_2S detection in the projected medium formulation. The aforementioned ability of *E. tarda* to decarboxylate lysine with its consequent alkaline effect would enhance the difference (1, 6).

This projected medium is therefore similar to XLD Agar upon which *Salmonella*-like colonies are differentiated from *Citrobacter* and other genera by a combination of reactions involving sugar fermentation and lysine decarboxylation (2).

In preliminary experiments, the formulation of the medium was given minor adjustments with the testing of laboratory strains which were used singly and in mixtures. The addition of agar (4.5 g per liter) to that portion to be autoclaved was used initially for some isolation work, but it was eventually determined to have no physical effect nor any value (9).

Triple Sugar Iron Agar (Difco) with 1% added mannitol ("TSIM") and Phenylalanine Agar (Difco) were examined as appropriate screening media for isolates. (9)

RESULTS AND DISCUSSION

One hundred and sixteen samples of lake, stream and swamp water (usually containing 5-10% sand or mud) were collected from 79 sites in Wisconsin, Iowa and Illinois during

warm weather periods from 1988 through 1991. The samples were usually refrigerated if they could not be processed within several hours. In the laboratory, each sample was filtered through successive, sterile membrane filters (0.45um pore size); amounts filtered were usually 1, 5, 10 and 25 ml. Each filter was immediately plated on ET Agar and incubated at 37°C. After one day of incubation, the filter plates were examined for isolated, black-centered colonies. On some occasions, the plates were reincubated and examined after an additional day. Black-centered colonies and others of interest were streaked for further isolation on ET Agar.

Each isolate was inoculated into slants of TSIM and Phenylalanine Agar. After one day at 37°C, presumptive identification as *E. tarda* was made if positive reactions were seen only for glucose fermentation and hydrogen sulfide production. Conventional biochemical tests (3) and/or the API-20E identification system (Sherwood Medical, Plainview, New York) were employed as deemed appropriate for confirmed identification.

E. tarda was isolated and identified from 35 (30%) of the samples. Twenty five (32%) of the sites yielded the organism at least once. On the filters, all of the well-separated, black-centered colonies approximately 1 mm in diameter which arose by one day of incubation were identified as *E. tarda*. Smaller such colonies were identified either as *E. tarda* or *Pseudomonas putrefaciens*-like organisms [later given the name Shewanella putrefaciens]. The latter usually formed yellowish colonies on the filters with barely-discernable, pinpoint, black centers which appeared larger when these colonies grew near other types of colonies, as if hydrogen sulfide production were being enhanced. The "other types" were, however, acidic colonies and one (isolate no. F115) was identified as *Vibrio cholerae* by the API-20E system and confirmed as a non-O1 strain by Marjorie Hamacher of the Bacteriology Laboratory of the Wisconsin State Laboratory of Hygiene (Madison, Wisconsin).

Because of the very low number of *E. tarda* colonies generally observed, quantitation of the organism could not be clearly made. For the positive samples, usually no more than a few *E. tarda* colonies were recovered from 10 or more ml of water. These low numbers would not

seem to indicate colonization by *E. tarda* of the various sites but could represent organisms shed by host animals.

After one day at 37°C on ET Agar streak plates, the majority of *E. tarda* isolates produced alkaline (whitish-rimmed) colonies up to 1 mm in diameter with large black centers. Four samples yielded *E. tarda* isolates producing smaller colonies (less than 0.5 mm) with relatively small black centers; these organisms tested positive for hydrogen sulfide in TSIM but negative in the API-20E system.

An organism producing a typical-appearing colony on ET Agar except for an acidic (red) rim around the black center was isolated from Lake Superior at Ashland, Wisconsin in August, 1988. Hydrogen sulfide was indicated in the API-20E system but not in TSIM. This isolate, no. F63, was confirmed by the Enteric Bacteriology Section of the Centers for Disease Control (Atlanta, Georgia) as a representative of *E. tarda* Biogroup 1, a rarely-isolated organism known for its ability to ferment sucrose and mannitol (5). F63 was subsequently included with several other strains from this study in an examination of *E. tarda* virulence factors (7).

ET Agar awaits extensive testing with directly plated samples (other than from membrane filters) and inoculations from broth enrichments. It is hoped that this medium may become valuable as a supplementary isolation medium where the presence of *E. tarda* in clinical, veterinary or environmental samples needs to be determined.

TABLE 1. FORMULA OF ET AGAR

AUTOCLAVED MIXTURE:

MacConkey Agar Base (Difco)	40.0 g
Yeast extract	1.0 g
Distilled water	900.0 ml

FILTER-STERILIZED SOLUTION #1 (added to autoclaved mixture):

Glucose	2.0 g
Sucrose	5.0 g
Mannitol	5.0 g
Xylose	5.0 g
L-lysine	10.0 g
Sodium thiosulfate (pentahydrate)	6.8 g
Ferric ammonium citrate (brown form)	0.8 g
Distilled water	100.0 ml

FILTER-STERILIZED SOLUTION #2 (added to autoclaved mixture):

Colistin (1 mg/ml)	10 ml

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