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Improved Enrichment and Isolation Procedures for Obtaining Pure Cultures of *Beggiatoa*

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Scoring agar surfaces with alginate swabs before placing washed filaments of *Beggiatoa* on the agar has greatly increased the rate at which single filaments move from contaminated areas. Numerous morphological types of pure cultures have been grown in organic media supplemented with either catalase or reducing agents. Aerated sewage was used as the enrichment source.

Despite the widespread occurrence of *Beggiatoa*, the difficulty in obtaining pure cultures has prevented the accumulation of sufficiently precise data to draw significant conclusions as to the organism's metabolic capabilities or even classification.

The techniques previously used to obtain pure cultures depend on the gliding motility of *Beggiatoa* filaments to separate them from other microorganisms. These procedures, however, require considerable skill and tend to isolate one predominant type of organism (3). The addition of catalase to cultural media (1, 2) has improved the isolation procedure; however, isolation procedures based solely on random gliding motility are often very difficult when highly motile contaminants are present.

Enrichment cultures of *Beggiatoa* were obtained by filling 1-gallon (ca. 3.8-liter) glass jars with raw municipal sewage and aerating for 72 to 86 h. Bundles of filaments (Fig. 1) were removed from the surface of the sludge and washed in either sterile tap water or sterile tap water plus 0.1% sodium azide. The azide wash worked best when large numbers of motile contaminants were present. Washed bundles of filaments (Fig. 2) were then placed on the surface of dry agar plates, and the water associated with

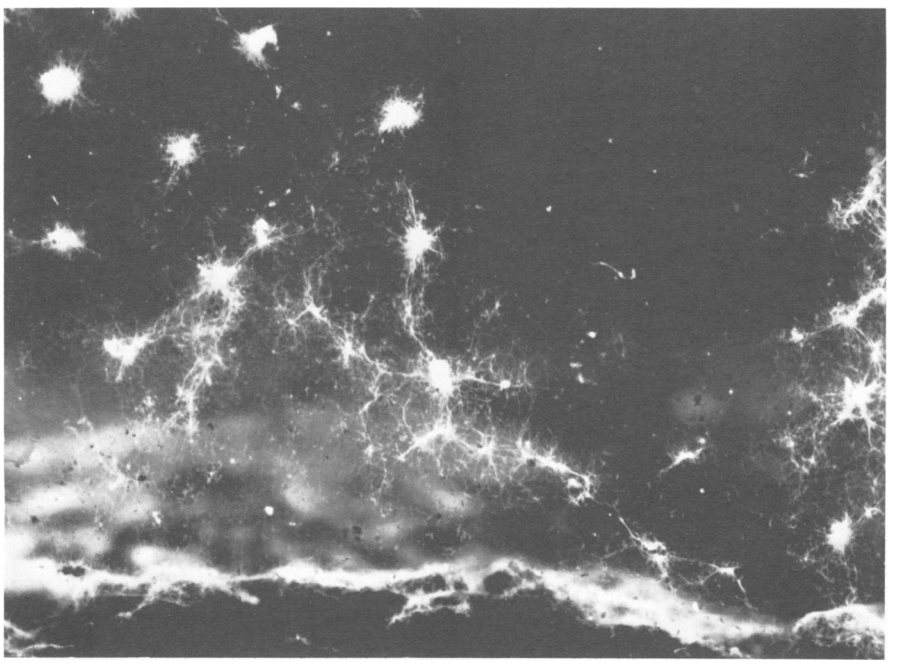

FIG. 1. *Bundles of Beggiatoa filaments growing on the surface of sewage sludge.*

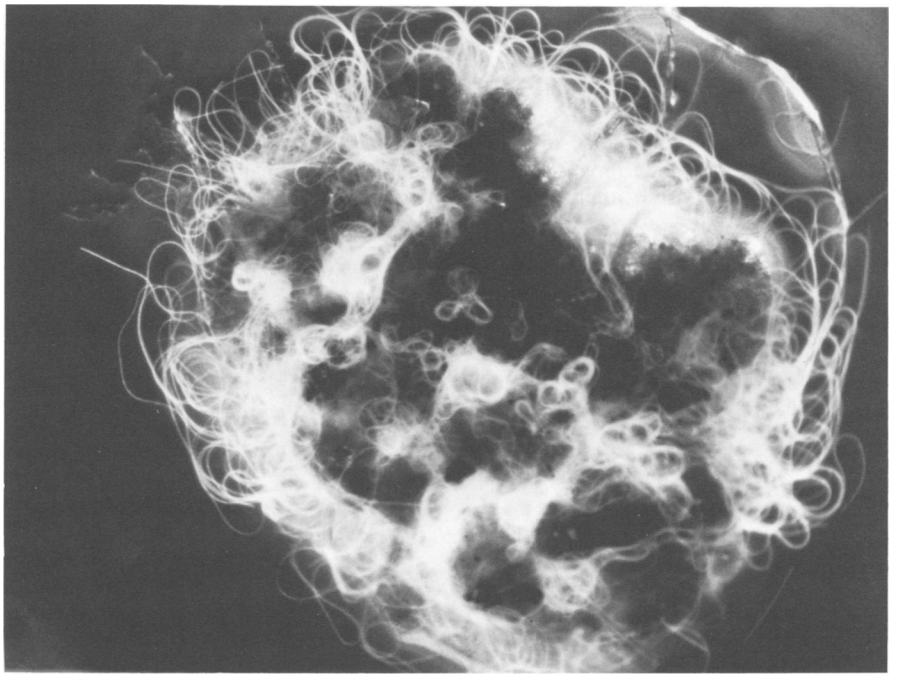

FIG. 2. *Bundles of Beggiatoa filaments on agar surface.*

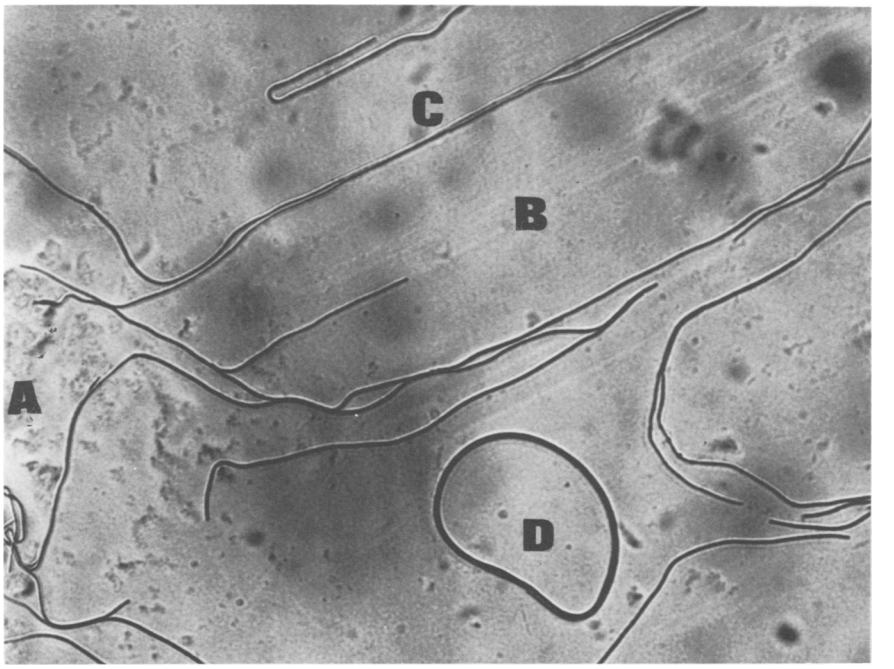

FIG. 3. *Beggiatoa filaments beginning to follow parallel lines prepared on agar surface. (A) Origin of filaments from bundle placed on agar. (B) Parallel lines. (C) Beggiatoa filaments following scored lines. (D) Filaments gliding in a random direction.*

the filaments was gently removed by briefly touching the filaments with sterile filter paper. (The medium contained 10.0 g of agar, 0.1 g of yeast extract, 0.2 g of sodium acetate, 100 ml of sewage that had been aerated for 72 h, 1,000 Sigma units of bovine liver catalase, and tap water to make 1 liter. This medium was poured at 50°C into petri plates and left uncovered for 4 to 6 h until small drops of water, when added to the surface, were absorbed within 10 min. These agar plates were then stored in airtight containers at room temperature and used within 72 h.) The surface of these agar plates had been scored with parallel lines by using a dry calcium alginate swab (Wilson Diagnostics, Inc., Glenwood, Ill.). These lines provided a path and direction for the motility of the *Beggiatoa* filaments (Fig. 3). Without these lines the motility

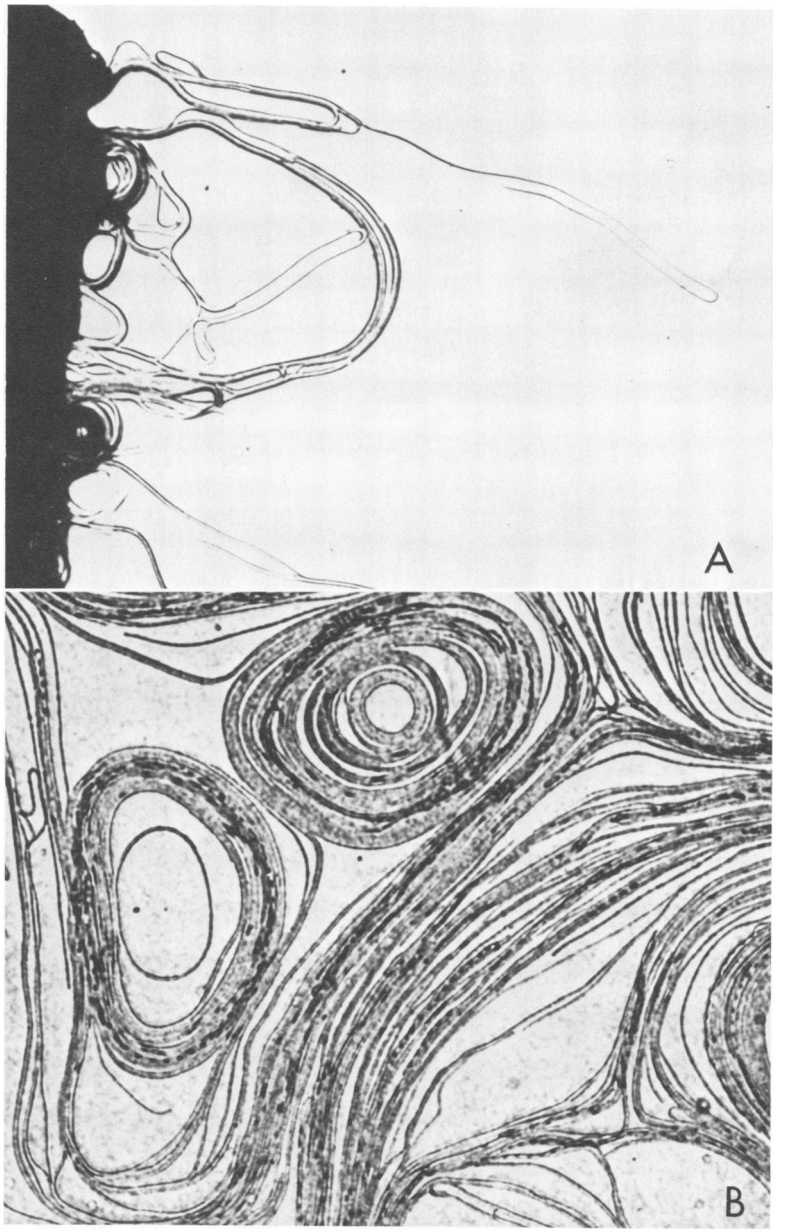

FIG. 4. *(A) Random motility of Beggiatoa filaments leaving point of origin without parallel lines scored on agar. (B) Pure culture of Beggiatoa showing typical circular motility patterns when not on scored agar.*

is random in direction (Fig. 4), and the filaments often return to contaminated areas. Once the filaments have entered the pathways on the surface of the agar, they are rapidly separated and can be picked up with a capillary pipette and placed in growth medium. No single medium has been found that will permit the growth of all the various isolates obtained; however, AC medium (Difco Laboratories, Detroit, Mich.; 0316-17-0) diluted 1:10, thioglycolate medium (Difco; 0430-17-1) diluted 1:10, and the basal medium listed in Table 1, containing either catalase or powdered FeS, have been successfully employed in growing a rather large variety of *Beggiatoa* filaments.

TABLE 1. *Composition of basal medium employed for growth of Beggiatoa*

Ingredient	%
Nutrient broth (Difco)	0.1
Yeast extract	0.05
Sodium acetate	0.01
Casamino Acids (Difco)	0.01
Calcium chloride	0.01
Agar	0.1

All of the various isolates were microaerophilic, and some required reducing agents (1.0 g of powdered FeS per liter, 0.05 g of sodium thioglycolate per liter, or 0.02 g of ascorbic acid per liter) in the growth medium on initial isolation. All isolates produced sulfur granules when exposed to H_2S.

The use of scored agar plates should also be useful in the isolation of other microorganisms that exhibit gliding characteristics similar to *Beggiatoa*.

Investigations on the ultrastructure and biochemistry of some of these isolates are now being pursued.

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