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Improved Enrichment and Isolation Procedures for Obtaining Pure Cultures of Beggiatoa

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 agentsè Aerated sewage was used as the enrichment source.

Despite the widespread occurrence of Beggia- toa, 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 Beg- giatoa filaments to separate them from other microorganisms. These procedures, however, re- quite 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 re-moved from the surface of the sludge and washed in either sterile tap water or sterile tap water plus

Cải thiện quá trình làm giàu và phân lập để thu được các chủng Beggiatoa thuần.

Khắc rãnh các bề mặt agar bằng miếng gạc alginit trước khi đặt các sợi Beggiatoa đã làm sạch lên sẽ làm tăng tốc độ di chuyển của các sợi từ vùng nhiễm bẩn. Các loại chủng thuần đa dạng về mặt hình thái học đã tăng trưởng trong môi trường hữu cơ được bổ sung catalase hoặc các tác nhân khử. Nước thải ngậm khí được sử dụng như một nguồn làm giàu.

Mặc dù Beggiatoa đã rất phổ biến thu

chuyển trượt các sợi

cấy
phân lập

chuyển động trượt
có sự hiện diện của

nước thải đô thị bùn máy Natri 0.1% sodium azide. The azide wash worked best when large numbers of motile con-taminants were present. Washed bundles of fil-aments (FigÉ 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. Beggwtoa filaments begũmùig to follow parallel lutes prepared on agar surface. (A) Origm of filaments from bundle placed on agar. (B) Parallel lùies. (C) Beggiatoa filaments followuig scored lùies. (D) Filaments gliding m a random direction.

the filaments was gently removed by briefly touching the filaments with sterile filter paperă (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 minê 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., Glen- wood, 111.). These lines provided a path and direction for the motility of the Beggiatoa filaments (Fig. 3). Without these lines the motility is random in direction (Fig. 4),

tấm thạch
dùng giấy lọc vô trùng chạm nhẹ
vào các sợi
bùn thải
agar
thạch agar
được đặt trên thạch agar

chiết xuất
máy

phút Sau đó

này

the filaments often and return 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 cat- alase or powdered FeS, have been successfully

TABLE 1. Composition of basal medium employed for growth of Beggiatoa employed in growing a rather large variety of Beggiatoa filaments.

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 isola-tion. All isolates produced sulfur granules when exposed to H2S.

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.

Chúng tôi thấy rằng Thành phần của môi trường cơ bải tấm thạch agar tính chất