

Isolation of Bacillary and Streptococcal Variants from *Bacterionema matruchotii*

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Bacterionema matruchotii, an oral filamentous organism, dissociated to form unusual flat colonies. Subculture of the flat colonies, composed of diphtheroids, yielded pure cultures of bacillary and streptococcal variants.

Bacterionema matruchotii, a filamentous organism recoverable from human dental plaque (1, 5, 6), is able to form calcium hydroxyapatite, a major mineral of vertebrate calcification (2). Gilmour et al. (4) classified this organism in the family *Actinomycetaceae*. According to Gilmour (3), reproduction occurs by fragmentation of filaments into bacillary forms which germinate to form new filaments. During studies of hydroxyapatite formation by *B. matruchotii* Richardson strain 13, ATCC 14265 and 14266, unusual flat colonies appeared. Subculture of flat colonies yielded pure cultures of bacillary and streptococcal variants. The findings were based on cultures originally purified by the dilution technique. Contamination and the interaction of unrelated species was considered. Subsequent studies, however, using 25 cultures of each strain initiated from single filaments confirmed the morphological dissociation.

The preliminary results obtained with strain 13 are reported. Filaments were isolated with a Cailloux micromanipulator (G. H. Stoelting Co., Chicago, Ill.) from Brain Heart Infusion (BHI) agar (Difco) streaked with sparse inocula of 3-day-old colonies. Filamentous cultures initiated in BHI broth from single filaments were designated F₇. Two milliliters of second-passage cultures in exponential growth phase were inoculated into separate 50-ml volumes of BHI broth. The initial samples were removed at zero time, and subsequent samples were taken at 2- to 4-hr intervals up to 40 hr. One-tenth milliliter of an appropriate 10-fold dilution of each culture in BHI broth was distributed in triplicate on BHI agar with a bent glass rod and incubated at 37°C for 3 days.

Transfer of periodic samples from liquid culture onto BHI agar resulted in the formation of characteristic colonies composed of filaments during the first 36 hr (Fig. 1 and 2). After 36 and

40 hr, unusual flat colonies consisting of diphtheroids (Fig. 1 and 3) were found ranging in frequency from 0.6 to 18%. Bacillary variant, RF₇ (Fig. 4 and 5), and streptococcal variant, S₁ (Fig. 6 and 7), were cultured in BHI broth from flat colonies on different plates. Streptococcal variants S₈ and S₉ were recovered from separate flat colonies on the same plate. S₁, S₈, and S₉ were morphologically similar but produced different fermentation profiles when grown in the presence of 12 carbohydrates. Many of the diphtheroid flat colonies grew poorly or failed to grow when cultured in BHI broth.

The present demonstration that *B. matruchotii* produces morphological variants indicates a more complex growth cycle than previously reported (3). Studies are in progress to characterize the dissociated forms to understand better the mechanism of variant formation.

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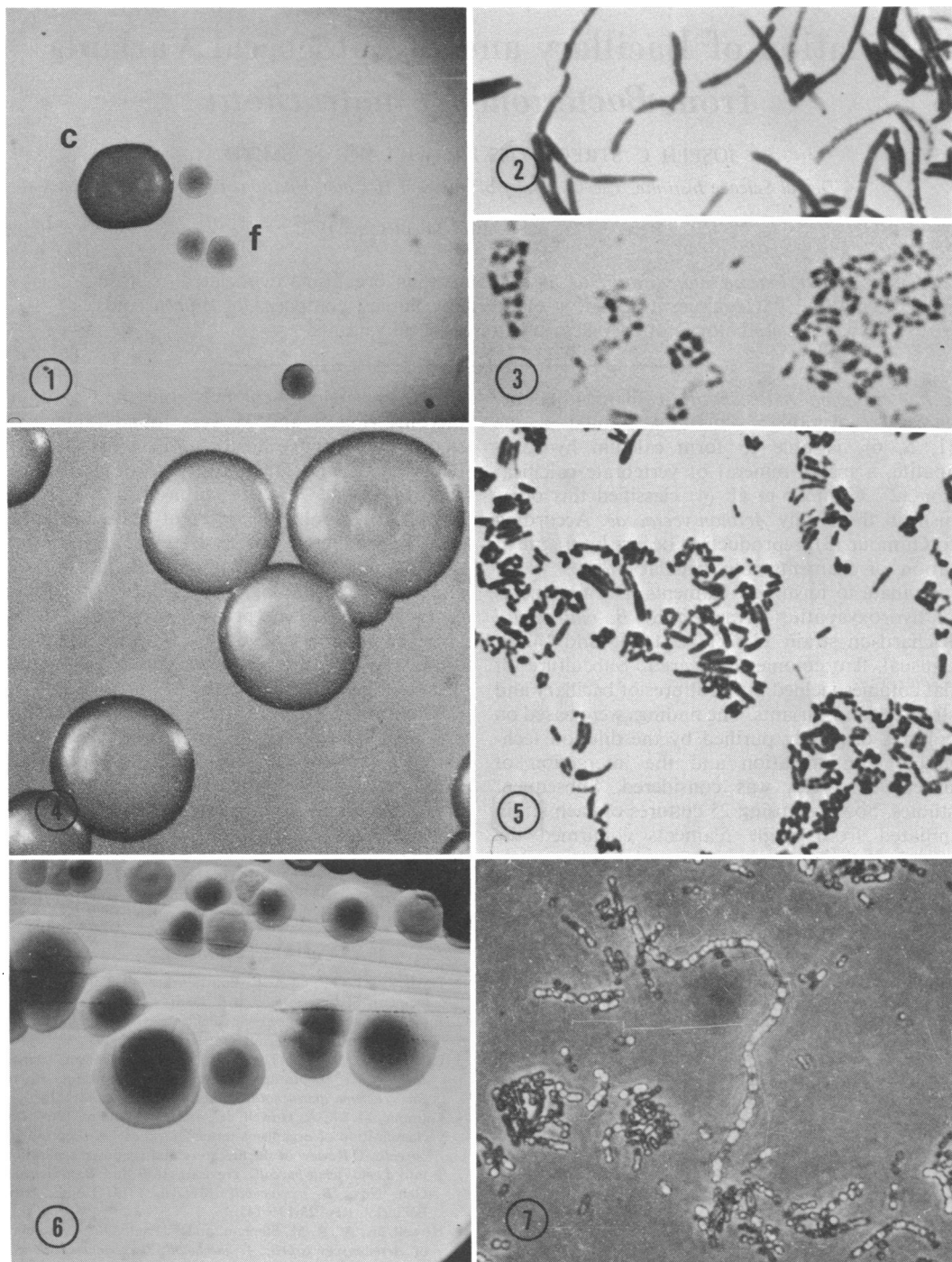


FIG. 1-7. *Bacterionema matruchoti*. Fig. 1. Characteristic colony (c) and flat colonies (f) of F_7 after 3 days of incubation. $\times 40$. Fig. 2. Gram stain of characteristic F_7 colony. $\times 3,800$. Fig. 3. Gram stain of flat colony demonstrating diphtheroid forms. $\times 6,100$. Fig. 4. Characteristic RF_7 colonies after 3 days of incubation. $\times 40$. Fig. 5. Gram stain of RF_7 colony. $\times 3,800$. Fig. 6. Characteristic S_1 colony after 5 days of incubation. $\times 40$. Fig. 7. Phase micrograph of wet preparation of S_1 colony demonstrating chains and clumps of cocci. $\times 3,800$.