Development of a Broth Medium for *Bacteroides forsythus*

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***Bacteroides forsythus* has been implicated as a possible pathogen in certain destructive periodontal diseases. This species has been extremely difficult to grow in broth, thus hampering its characterization and identification. A broth medium was developed consisting of 30 g of phytone, 10 g of NaCl, and 1 g of filter-sterilized L-cysteine per liter. The medium supported the growth of 23 fresh isolates of *B. forsythus* from six subjects to optical densities averaging 0.71 ± 0.20 (standard deviation) at 550 nm after 48 to 72 h of anaerobic incubation.**

Bacteroides forsythus (formerly known as the fusiform *Bacteroides* sp.; 3) has been implicated as a pathogen in certain forms of destructive periodontal disease (1, 2; S. Sasaki, S. S. Socransky, M. Lescord, and E. A. Sweeney, J. Dent. Res. 56:157, abstr. no. 422, 1977). The organism was found, on average, to be eight times as numerous in "actively breaking down" periodontal lesions as compared with inactive (control) sites (J. L. Dzink, unpublished data). This species was rarely detected in sites in which *Fusobacterium nucleatum* was absent. When both organisms were detected in periodontal pockets, the lesions were found to be in an active stage of disease.

Fresh isolates of *B. forsythus* have been successfully maintained on agar plates only as satellite colonies of *F. nucleatum*. *B. forsythus* has been almost impossible to grow in broth (3), although biphasic culture permitted marginally successful growth (A. C. R. Tanner, S. S. Socransky, and C. M. Smith, J. Dent. Res. 60:46, abstr. no. 703, 1981). The purpose of the present investigation was to develop an agar-free broth medium for *B. forsythus*.

Development of the broth medium occurred in four stages. Initially, it was necessary to determine whether *B. forsythus* could be supported by *F. nucleatum* in the absence of agar. This was accomplished by growing *F. nucleatum* within a dialysis bag and *B. forsythus* outside the dialysis bag in mycoplasma broth supplemented with 5 mg of hemin and 1 g of glucose per liter. In stage 2, the broth was preconditioned by growing *F. nucleatum* in a dialysis bag for 48 h. The bag containing *F. nucleatum* was removed, and the preconditioned medium was used to successfully support the growth of *B. forsythus*. This demonstrated that *F. nucleatum* did not have to be continuously present to support the growth of *B. forsythus*. Growth in the preconditioned medium experiments was consistent, but at low cell yields (optical density, 0.1 to 0.3 at 550 nm). In stage 3, the preconditioning effect of *F. nucleatum* was replaced by the addition of factors suspected to be produced by this species, as well as other factors. The addition of the sodium salts of acetate, succinate, and sulfite, ammonium chloride, and L-cysteine to supplemented mycoplasma broth permitted low levels of growth of *B. forsythus* (optical density, 0.2 to 0.3 at 550 nm) in the absence of conditioning. Finally, growth was markedly enhanced by systematic substitution of various growth factors. Phytone and sodium chloride were critical in en-

hancing growth. Maximum growth was achieved by raising the phytone and sodium chloride levels to 30 and 10 g/liter, respectively, and adding filter-sterilized L-cysteine to a final concentration of 1 g/liter. Fresh isolates grown in this medium usually formed adherent flocculent clumps at the base of the culture tube, but after several transfers the growth became more dispersed. Optical densities of the broth cultures averaged 0.71 ± 0.20 (standard deviation) at 550 nm after 48 to 72 h of anaerobic incubation at 35°C. All 23 isolates from six subjects were successfully grown in this medium.

In view of the simplicity of the broth medium, it is surprising that *B. forsythus* has been such a difficult organism to grow, both on agar and in broth. The recognition of the dependence of *B. forsythus* on *F. nucleatum* provided the catalyst for medium development. *B. forsythus* previously had not grown in a wide variety of commercially available media or in any of an extended series of bases when supplemented with a wide range of known growth factors and additives (2; Dzink, unpublished observations). Growth in the dialysis bag system provided evidence that *B. forsythus* could be grown in broth. Although meager, growth in the preconditioned medium was consistent and was used as a starting point for the alteration of medium constituents. The successful development of a broth medium for *B. forsythus* facilitates the maintenance, characterization, and identification of strains of this species and permits a more thorough evaluation of its role in destructive periodontal diseases and other human infections.

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