Growth stimulation of Treponema denticola by periodontal microorganisms

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Received 10 February 1989; accepted 29 June 1989

Keywords: Bacteroides intermedius, Eubacterium nodatum, Fusobacterium nucleatum, serum, Treponema denticola, Veillonella parvula

Abstract

Previous experiments have indicated that enrichment of subgingival plaque in human serum can lead to the accumulation of Treponema denticola. T. denticola depends on bacterial interactions for its growth in serum. Aim of the present study was to identify specific microorganisms involved in the growth stimulation of T. denticola. To this end, strains isolated from previous plaque enrichment cultures were tested for growth stimulation in co-cultures with T. denticola. In addition, growth of T. denticola was tested in culture filtrates of the same strains. Bacteroides intermedius, Eubacterium nodatum, Veillonella parvula and Fusobacterium nucleatum were found to enhance growth of T. denticola in co-cultures. A continuous co-culture of T. denticola, F. nucleatum and B. intermedius in human serum gave very high levels of T. denticola, up to $3 \times 10^9 \cdot ml^{-1}$. Mechanisms involved in growth stimulation may include the ability of B. intermedius and E. nodatum to cleave the protein-core of serum (glyco-)proteins, making these molecules accessible for degradation by T. denticola. In addition, E. nodatum was found to produce a low-molecular weight growth-factor for T. denticola, that was heat-stable and acid as well as alkaline resistant. V. parvula may provide peptidase activities complementary to those of T. denticola. The nature of the growth enhancing activity of F. nucleatum is yet unknown. The data support the dependency of T. denticola on other bacterial species for growth in the periodontal pocket.

Introduction

Spirochetes were first noted in periodontal disease by Antonie van Leeuwenhoek. In recent years evidence has accumulated that the species Treponema denticola is generally present in high proportions in severely diseased sites (Loesche et al. 1987; Simonson et al. 1988). As a consequence, this organism is suggested to be an etiologic agent in periodontitis (Moore et al. 1983).

A major ecological determinant of the microflora in periodontitis seems to be the increased availability of serum components from gingival exudate. This condition favours serum (glyco-)protein degrading consortia of microorganisms. Earlier work has demonstrated that T. denticola can be isolated as a predominant species after enrichment of subgingival plaque in chemostats using human serum as culture medium (Ter Steeg et al. 1988). A dependency of T. denticola on other microorganisms was noted by Hampp and Mergenhagen (1961) and Macdonald et al. (1963). The nature of the in vivo relevant interactions between T. denticola and the above species however, is still not identified. Recent findings suggested that bacterial interactions, originating from host-derived sub-
strate utilization, were major ecological factors of the microenvironments in the oral cavity (Beighton et al. 1986; De Jong & Van der Hoeven 1987; Ter Steeg et al. 1987, 1988, 1989a, 1989b).

The aim of the present study was to test periodontal organisms, originally isolated from serum degrading consortia (Ter Steeg et al. 1988), for their ability to stimulate the growth of *T. denticola*. The identity of these organisms, and the mechanisms of growth stimulation, may elucidate how bacterial interactions can explain the development of pathogenic microflora.

**Material and methods**

**Summary of experimental strategy**

The search for periodontal micro-organisms capable of growth enhancement of *T. denticola* in human serum, was restricted to a limited number of strains isolated from enrichment cultures of periodontal plaque in serum (Ter Steeg et al. 1988, 1989a). Criteria for selection were:

- occurrence with *T. denticola* in previous enrichment cultures;
- association with stages of (glyco-)protein degradation;
- other potentially interesting characteristics (Table 1) (Ter Steeg et al. 1988, 1989a, 1989b).

The search included the following steps:

- Screening of the growth of *T. denticola* in batch-wise co-cultures with single and multiple (1–8) species. For practical reasons, the number of test combinations had to be limited.
- Furthermore, culture supernatants of the same strains or combinations of strains were used as medium for *T. denticola*.
- A combination of strains found to be successful in stimulating the growth of *T. denticola* was finally tested in continuous culture.

**Bacterial strains**

The following strains from previous enrichments of subgingival microflora on human serum (Ter Steeg et al. 1988, 1989a) were selected:

- *Treponema denticola* Ny375
- *Bacteroides intermedius* Ny365
- *Bifidobacterium adolescentis* Ny369
- *Eubacterium alactolyticum* PS406
- *Eubacterium brachy* Ny402
- *Eubacterium lentum* PS538
- *Eubacterium nodatum* Ny393
- *Eubacterium saburreum* Ny396
- *Fusobacterium nucleatum* Ny373
- *Lactobacillus catenaforme* Ny374
- *Peptostreptococcus anaerobius* Ny407
- *Peptostreptococcus micros* Ny370
- *Propionibacterium acnes* Ny371
- *Streptococcus mitis* PS301
- *Veillonella parvula* Ny368

**Conservation and cultivation**

Bacterial isolates were stored in skim milk (−80°C). Cultivation was carried out in an anaerobic chamber (Braun, Garching, FRG; 91%, N₂, 5% CO₂, 4% H₂, 0.2–0.4 ppm O₂, 37°C). *T. denticola* was maintained in pre-reduced HPY-SC broth (Holdeman et al. 1977). Thiamine pyrophosphate (25 μg·ml⁻¹ final concentration), volatile fatty acids solution (Holdeman et al. 1977), Na₂CO₃ (0.05% w/v) and 10% newborn bovine serum were added aseptically after autoclavation. In our hands, newborn bovine serum gave better reproducible growth of *T. denticola* than regular bovine serum. All other strains were cultivated on enriched cysteine blood agar (ECBA) (Ter Steeg et al. 1988) or in PY-medium (Holdeman et al. 1977) + 10% heat-inactivated (30’, 56°C) human serum (HS). All co-cultures were grown in human serum. The serum was provided by the local blood donor service and was pooled from at least 3 donors. Inoculation of co-cultures was directly from agar plates or from the liquid pre-cultures using at least 100-fold dilution. Co-culture studies were carried out batch-wise except in one case where a 5 ml chemostat as described previously (Ter Steeg et al. 1988) was used. Growth stimulation of *T. denticola*