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REVIEW

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The peptidolytic capacity of the spirochete system

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Abstract Relatively scant chemical information has been available on the proteinases and peptidases of spirochetes in spite of the association of spirochetes with several serious infections known to plague humans and other animal species. This situation has partly resulted from difficulties in growing some spirochetes under laboratory conditions. The cells of *Treponema denticola*, a spirochete suggested to be associated with periodontal infections, have turned out to be a good source of new chemical information on those enzymes. Latest studies suggest that the outer cell envelope or the periplasmic space of *T. denticola* contains several novel proteinases and peptidases (hence called "ectoenzymes") which may contribute to the chronicity of periodontal infections. Some of the oligopeptidases discovered are specific for proline-containing host tissue peptides such as substance P, bradykinin, neurotensin, etc., and possibly small collagen fragments. The only spirochetal peptidases purified to give a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis have been obtained from *T. denticola*. One particular peptidase, suggested to be similar to the oligopeptidase B (EC 3.4.21.83) of *Escherichia coli* seems to be present in the cell envelope or in the periplasmic space at quite large concentrations. The presence of this and several other peptidases in the outer cell structures of the treponemes suggests that such enzymes are important for the nutrition of these highly motile and invasive organisms. The biological role of these enzymes can thus be envisaged in the peptidolytic processing of host tissue proteins and peptides to gradually smaller molecules to fulfill the nutritional requirements of these organisms. Although the genetic similarity between *T. denticola* and some other treponemes and spirochetes can be hotly debated, it is nevertheless now possible to use *T. denticula* enzymes as suitable objects for comparison when the chemistry of other spirochetes is studied.

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Introduction

The spirochetes (order *Spirochaetales*) are helical, highly motile and invasive bacteria which are associated with a large number of eukaryotic cells. Based on ecological, morphological and physiological criteria, five spirochete genera have been classified: *Leptospira*, *Spirochaeta*, *Christispira*, *Treponema*, and *Borrelia* [19]. Some spirochetes are pathogenic to humans causing syphilis, bejel, pinta, yaws, intestinal disorders, relapsing fever, Lyme disease, leptospirosis, destructive periodontitis, ulcerative gingivitis, avian spirochetosis, swine dysentery, and other diseases [5, 19, 27, 59]. The human and animal gastrointestinal tract and the urogenital areas of humans and other animals harbor several different spirochetes, most of which have been cultivated [19]. Treponemal infections are ancient diseases which have plagued humans and other animals from times immemorial [58].

In no case is the detailed mechanism of the chemical aggressiveness of a pathogenic spirochete known. Proteolytic enzymes present in the outer cell wall or in the periplasmic space of the cells, among other factors, have been suggested to play a role [22]. In general, bacterial cell surface products or particles such as endotoxins, lipoteichoic acid, capsules, vesicles, mucopeptides, peptidoglycans and other factors, may contribute to inflammation in addition to true enzyme proteins. Several inflammation-associated bacterial factors have been given dubious "-lysin"-ending names. Quite frequently such factors have turned out to be hydrolytic enzymes. Although the molecular-level pathogenesis of spirochetal infections is poorly known, significant advances have been made in the treatment of some infections, such as Lyme disease. Venereal spirochetoses have long been treated by antibiotic therapy [59].

The molecular mechanism of spirochetal infections is poorly known largely because most spirochetes are ex-

tremely difficult to grow outside the mammalian host, not to mention mass-culturing spirochetes for large-scale enzyme purification and characterization. The cells of *T. denticola* can be mass cultured [37].

It has been suggested that all treponematoses are caused by the same organism [21]. Although this idea has not received much support, there may be similarities between the oral disease-associated *T. denticola* and the syphilis-causing *T. pallidum* [21, 22, 56], and between intestinal spirochetes from human and other animal species [26]. Riviere et al. [56] identified the organisms associated with acute ulcerative gingivitis as *T. pallidum*-related spirochetes. Provided that such a bacterial cognation can be vindicated, it is possible that the easily cultivable *T. denticola* can indeed be exploited as a model for *T. pallidum* and a number of other spirochetes, until the latter can be grown outside the mammalian host for in-depth chemical characterization. However, this issue is subject to a hot debate at this stage, since most spirochete experts have not been willing to accept any cognation (other than "genus-based") between the treponemes. The present authors predict a gradual softening in this stand. *T. denticola* has anyway received considerable attention as a producer of peptidases and proteinases. In fact, the cells of *T. denticola* can be figuratively regarded as "motile enzyme packages or hydrolytic work benches equipped with a powerful and versatile proteolytic and peptidolytic armamentarium immobilized in the outer cell envelope" [42].

The aim of this review is to consider the peptidolytic profile of spirochetes, and was prompted by recent advances made in the field: it is likely that the "ectopeptidases" associated with the outer cells envelope of the spirochetes attack host tissues directly by proteolytic digestion. Such enzymatic processes can lead to serious clinical ramifications. Another rationale behind this review can be seen in the increasing interest in Lyme disease, the oral treponemal infections, and venereal and non-venereal treponematoses in general. More emphasis will be placed on *T. denticola* than other spirochetes, owing to the comparatively large number of studies carried out on this organism. *De facto*, the only spirochete peptidases purified to homogeneity and characterized, have all been obtained from *T. denticola*. This review will hopefully stimulate enzyme studies in other spirochetes as well. The role of proteinases and peptidases in spirochetal infections should not be overestimated, either. Some spirochetes seem to lack effective ectopeptidases, being predominantly involved in other hydrolytic enzyme catalyses. *B. burgdorferi* (which causes Lyme disease) seems to produce no endogenous proteinases for the digestion of extracellular matrix proteins [23]. All microbial cells do contain several peptidolytic enzymes which are needed in the cellular intermediate metabolism, cellular protein turnover, cell division, and other events, but such enzymes may not be directly involved in pathogenicity.

Evidence of the tissue-destructive capacity of spirochetes

Exploiting the terminology used by authors of reports published on the pathogenic potential of some spirochetes (Table 1), one can conclude that these organisms are indeed associated with quite diverse pathological phenomena and that the cell wall of some spirochetes is well armed with regard to factors that can directly elicit inflammatory tissue responses. Although peptidolytic processes may not be directly involved in all reactions listed in Table 1, it is obvious, however, that they may contribute indirectly. Most peptidolytic activities discovered in spirochetes are listed in Table 2, while Table 3 lists those spirochetal peptidases and proteinases that have been purified to an SDS-PAGE pure form and chemically characterized.

Arginyl/lysyl oligopeptidase

This enzyme has been more thoroughly studied in *T. denticola* ATCC 35405 and *T. denticola* ASLM (a clinical strain isolated from the subgingival plaque of a periodontitis patient). The enzyme was previously called "trypsin-like" and "BAPNA-peptidas" or "BANA-peptidase." The activity levels of this enzyme in dental plaque, along with similar activities from *Porphyromonas gingivalis*, have been measured in numerous studies, and have been used in a diagnostic test for periodontal infections [28].

Although the enzyme resembles trypsin with regard to some specificity and inhibition characteristics, its cognation with trypsin is more remote. The enzyme has, instead, been proposed to belong to the prolyl oligopeptidase (EC 3.4.21.26; POP) superfamily, and may be similar to *E. coli* oligopeptidase B (EC 3.4.21.83: OPB). Consequently, this enzyme is a true oligopeptidase instead of a proteinase such as trypsin [38]. Further proof on the close similarity of the OPB and the POP superfamily with the *T. denticola* enzyme can be obtained by comparing the available sequence homology data (Figs. 1, 2). Table 4 shows that there is considerable homology between the *T. denticola* OPB-like enzyme and several other proteins. It is obvious that several other bacterial enzymes which hydrolyse BAPNA or BANA, should in reality be regarded as OPB-like. For example, "Enzyme II" described by Shibata et al. [63] in *Capnocytophaga gingivalis* may be an OPB.

The enzyme is specific for substrates which have an Arg or a Lys residue with a blocked amino terminus in the oligopeptide structure. The enzyme hydrolyses true peptides such as angiotensin I and angiotensin II (which are hydrolyzed at Arg^2-Val^3). Other substrates include Met-Lys-bradykinin and neurotensin [38]. The enzyme has been conveniently assayed using BAPNA as substrate which is optimally hydrolyzed in the presence of 1.0 mmol/l Ca^{2+}. OPB is not a metallopeptidase. Its activity is suggested to depend on an active COOH group, on an active seryl res-

Table 1 Examples of pathological effects of spirochetes. Such effects are frequently caused by factors present in the outer cell envelope of the cells, and may also involve direct or indirect peptidolytic reactions catalyzed by spirochete enzymes. The statements and effects (in **bold**) on the left represent those made by authors. Some hemolytic and hemagglutinating effects, reported in several spirochetes, may also involve at least indirect peptidolytic reactions catalyzed by the pathogen's enzymes [14, 45, 60, 70, 77]

Effect or reaction	Remarks and references
1. **Attachment** of the pathogen to human and rat epithelial cells (EC), and to monolayers of ECs of different origin (*Treponema denticola*)	Rounded rat palatal ECs were more receptive for attachment than flattened ECs. Human ECs were quite receptive immediately after mitosis [24, 25, 52]
2. **Inhibition** of fibroblast proliferation, lymphocyte blastogenesis, neutrophil degranulation, and free radical production (oral treponemal extracts)	Observed in vitro, but suggests that the treponemes can inhibit host reparative processes [3, 4, 61, 62]
3. **Distortion** of cell morphology; **adhesion** to fibronectin (FN); **fragmentation** of gingival fibroblasts (GF): **detachment** of GF; **degradation** of FN (*T. denticola*)	These and other cytophathic processes may follow after the contact of GF with the cells of *T. denticola* [8]
4. **Activation** of human vascular endothelial cells (*T. pallidum*)	Spirochete membrane lipoproteins may be involved [55]
5. **Ability to evade** host immune responses (*T. pallidum; T. denticola*)	Many of the unusual properties of *T. pallidum* are ultimately related to its protein content [50, 76]
6. **Adherence** of the pathogen to human gingival fibroblasts, fibronectin, laminin, fibrinogen, gelatin, type I and IV collagens (*T. denticola*)	*T. pallidum* causes similar reactions while the nonpathogenic *T. phagedenis* (Reiter) does not [6, 11, 18, 76]
7. **Invasion** of the pathogen into healthy tissue (*T. denticola*)	*T. pallidum* causes similar reactions [12, 17, 20, 47, 48, 71, 73]
8. **Blockage** of attachment of IgG (*T. pallidum*) [11]	May involve a peptidolytic process
9. **Migration** of the pathogen into healthy tissue (*T. denticola*)	Migration may be associated with the activity of a protease present outside of cell envelope [17]
10. **Interaction** between the pathogen and human gingival fibroblasts (*T. denticola*)	Specific binding proteins are involved [22, 76]
11. **Microulceration** of the sulcular epithelium (*T. denticola*)	*T. pallidum* causes similar reactions [29, 46, 59, 71]
12. **Suppression** of fibroblast proliferation	The inhibitory factor elutes as a 50-kDa substance and is not an endotoxin [3]
13. Mutual symbiotic **growth enhancement** of *T. denticola* and *Porphyromonas gingivalis*	*P. gingivalis* is another potential periodontal pathogen [15, 16]
14. **Activation** of host latent procollagenase (*T. denticola*, oral spirochetes)	Suggests that spirochaetal enzymes participate directly in tissue destruction [65, 72, 74]
15. Direct **degradation** of basement membrane collagen (*T. denticola*)	As above [74]
16. **Keratinolysis** (*T. denticola*)	The activity was cell bound and heat sensitive [43]
17. **Bone resorption** (*T. denticola*)	A lipopolysaccharide-like material in the outer cell membrane may be responsible [13]
18. **Actin rearrangement** and **detachment** of human gingival fibroblasts (*T. denticola*)	The processes might be stimulated by a bacterially associated protease [2]
19. **Stimulation** of growth by tissue proteins (*T. denticola*)	Ceruloplasmin supports growth [66]
20. **Termination** of the biological action of host bioactive peptides (*T. denticola*)	Neurotransmitters, inflammatory mediators and other bioactive peptides of the host may serve as substrates of spirochetal peptidases involved [37, 41, 42]
21. **Cytopathy** (*T. denticola*)	The CTLP may play a role, affecting migrating and stratified epithelial cells [75]

idue, and perhaps on an active tyrosyl group; the latter one may not participate in substrate binding. The enzyme is relatively loosely bound to the outer cell wall structures where it is present at a relatively high concentration, and can be readily extracted in a highly active and stable form with 0.05% Triton X-100.

T. denticola endopeptidase ("FALGPA-peptidase")

The endopeptidase of *T. denticola* ATCC 35405, active on 2-furylacryloyl-L-Leu-Gly-L-Pro-L-Ala (FALGPA) and bradykinin, is most likely associated with the outer cell envelope. It is a true endopeptidase, and does not hydrolyze proteins [37]. The shortest hydrolyzable substrate seems to be FALGPA, the hydrolysis of which at the Leu-Gly bond is strongly and competitively inhibited by bradykinin ($K_i = 5.0$ μM). The enzyme does not hydrolyze typical synthetic collagenase substrates (other than FALPGA), nor does it hydrolyze azocoll, azocasein, and type I and type IV collagen. However, a proposal was made that since a substantial portion of the amino acid sequence of FALGPA is present in collagen (and additionally acknowledging the close structural resemblance between proline and the FA moiety of FALGPA), the natural substrates of this enzyme

Table 2 Peptidase- and proteinase-related enzyme activities reported in spirochetes (*CTLP* chymotrypsin-like proteinase, *PIP* proline iminopeptidase, *PZ-PLGPA* phenylazobenzyloxycarbonyl- L-prolyl- L-leucylglycyl- L-prolyl- D-arginine, *FALGPA* 2-furylacryloyl- L-leucylglycyl- L-prolyl- L-alanine, *POP* prolyl oligopeptidase, *SAAPFNA* *N*-succinyl- L-alanyl- L-alanyl- L-prolyl- L-phenylalanyl-*p*-nitroaniline, *Pro-pNA* prolyl-*p*-nitroaniline, *Pro-2NA* prolyl-2-naphthylamine)

Enzyme activities detected	Source or cellular location of activity	Organisms and references
Fibrinolytic (extracellular)	Cultures and spent media	*T. denticola* (several oral strains; 2 from spinal fluid) [49]
Aminopeptidase	Extracts of sonicated cells	*T. phagedenis* (Reiter) [69]
Proteinase (trypsin like), peptidase (cell bound; APIZYM) [a]	Media and cells	Small-sized oral spirochetes [10]
Proteinase (against type IV collagen, gelatin, etc.), elastase, trypsin like	Neutral salt extracts of cells	Intermediate-size oral spirochetes [72]
Trypsin like [b]	Extracts of sonicated cells	*T. denticola* ASLM [51]
PIP (hydrolyzes Pro- *p*NA and Pro-2NA)	Triton X-100 extracts; sonicated cells extracts	*T. denticola* ATCC 35405 and several clinical isolates [32, 33, 39]
Keratinolytic	Cells	*T. denticola* L12D [44]
Proteinases; collagenase like, arginine aminopeptidase	Extracts of sonicated cells	*T. vincentii* ATCC 35580 [34]
Aminopeptidases (RapID-ANA) [a]	Washed cells	72 Isolates of *T. denticola* and 4 reference strains (ATCC 35404, 35405, 33520, 33521), *T. socranskii* (ATCC 35534, 35536), *T. vincentii* (ATCC 35580) [67]
Proteinases (against type IV collagen, gelatin, elastin, fibronectin, casein, synthetic substrates)	Extracts of sonicated cells	*T. denticola* ATCC 35405 [73]
CTLP[c] (hydrolyzes SAAPFNA)	Extracts of sonicated cells; Triton X-100 extracts; cells	*T. denticola* ATCC 35405 [8, 42, 73, 75]
Collagenase like (substrates: PZ-PLGPA, FALGPA)	Extracts of sonicated cells	*T. denticola* ATCC 35405 and several clinical isolates; *T. vincentii* ATCC 35580; *T. socranskii* [36]
Peptidases (APIZYM) [a]	Cells	*T. denticola* ATCC 33520 and several isolates; *T. vincentii* ATCC 35580 (and isolates); *T. pectinovorum* ATCC 337768; *T. hyodysenteriae* ATCC 271664, 31212 (and isolates); *T. phagedenis* (Reiter) [43]
Endopeptidase	Extracts of sonicated cells	*T. denticola* ATCC 35405 [37]
Peptidases, proteinases (APIZYM) [a]	Cells	*T. denticola* ATCC 33520 [48]
CTLP (hydrolyzes SAAPFNA)	Extracts of sonicated cells	*T. denticola* ATCC 29522 [65]
Proteinases, PIP, trypsin-like (RapID ANA) [a]	Extracts of sonicated cells; cells	*T. denticola* ATCC 35405 [68]
Trypsin-like	Cells	*T. denticola* ATCC 33520, 33521, 35405 and other isolates [53]
Proteinases, collagenase-like	Extracts of sonicated cells	*T. denticola* ATCC 35405; *T. vincentii* ATCC 35580; *T. socranskii* ATCC 35536 [64]
Fibrinolytic	Triton X-114 extracts, cells, crude outer sheats	*T. denticola* ATCC 35404, 33520 [57]
POP (proline specific)	Triton X-100 extracts	*T. denticola* ATCC 35405 [41]
Oligopeptidase B [b]	Triton X-100 extracts	*T. denticola* ATCC 35405 [38]
Leucine arylamidase, etc. (APIZYM) [a]	Cells	58 Spirochetal isolates (*B. burgdorferi* , *B. hermsii* , leptospires, serpulinas, and a treponeme) [7]
γ-Glutamyl (trans)peptidase	Triton X-100 extracts	*T. denticola* ATCC 35405 [40]

[a] APIZYM, RapID-ANA refer to so-called “rapid enzyme profile systems” which utilize microwell plates for testing a large number of enzyme activities simultaneously. Such methods are suitable for screening purposes

[b] The *T. denticola* “trypsin-like” enzyme may be an oligopeptidase B (EC 3.4.21.83)

[c] Que and Kuramitsu [54] and Arakawa and Kuramitsu [1] have reported isolation and characterization of genes that code for CTLP and an enzyme active on PZ-PLGPA

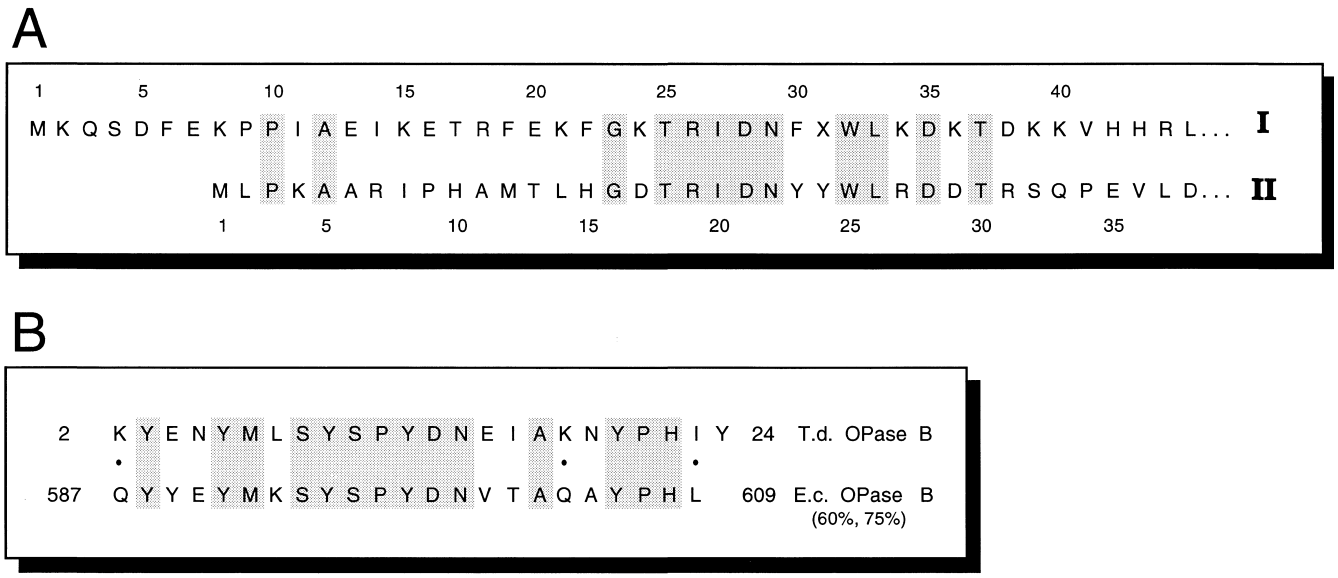

Fig. 1 **A** Alignment of amino acids present in the N-terminal (undigested) end of the *Treponema denticola* oligopeptidase (I) and the *Escherichia coli* oligopeptidase B (OPB) (II). Identical amino acid residues have been *shaded*. **B** Alignment of amino acids present in *E. coli* OPB and in a peptide fragment of the *T. denticola* enzyme, obtained by means of CNBr cleavage. Identical amino acid residues have been *shaded* (60%) while those residues that are chemically related (sum of identical and related = 75%) are indicated with a *dot*. The *T. denticola* oligopeptidase is most likely an OPB

Table 3 Proteinases and peptidases purified to homogeneity (as judged from SDS-PAGE electrophoretograms) from *T. denticola* ATCC 35405

Enzyme	References
Oligopeptidase B[a]	[38, 51]
CTLP[b]	[42, 73]
FALGPA-Peptidase	[37]
POP	[41]
PIP[c]	[39]
GGTP	[40]

[a] Previously known as BANA-peptidase, BAPNA-peptidase, or "trypsin-like" enzyme

[b] Arakawa and Kuramitsu [1], and Que and Kuramitsu [54] have reported on the isolation and characterization of the gene coding for CTLP from *T. denticola* ATCC 35405, and on sequence analysis of the enzyme

[c] *T. phagedenis* (Reiter) aminopeptidase [69] was obviously also obtained in a relatively pure form

may be small, soluble collagen fragments [37]. This enzyme was initially purified from sonicated cell extracts. However, washed whole cells also hydrolyze FALGPA, and it is thus likely that this enzyme is present in the outer cell structures.

Proline-specific endopeptidase

An endo-acting proline-specific endopeptidase (POP) was purified from mild Triton X-100 extracts of the cells of *T. denticola* ATCC 35405 [41]. The minimum hydrolyzable peptide size was tetrapeptide $P_3P_2P_1P'_1$, while the maximum substrate mass was about 3 kDa. An imino acid residue in position P_1 is absolutely necessary. This *Treponema* enzyme has considerale homology with the *Aeromonas hydrophila* POP and with the POP precursor of *Flavobacterium meningosepticum.* Since the *T. denticola* POP hydrolyzes various proline-containing human bioactive peptides as a high rate, it was suggested that the enzyme contributes to the chronicity of infections by participating in the peptidolytic processing (inactivation) of such peptides [41].

Chymotrypsin-like proteinases

Two research groups initially reported on chymotrypsin-like proteinase (CTLP)-like activity in the cells of *T. denticola.* One enzyme was partially purified from the ATCC strain 35405 [74, 75] and was suggested to be attached to the outside of the cell envelope [17]. Another group reported the isolation and characterization of a CTLP from the same ATCC strain, after cloning the proteinase in *E. coli* [1, 54]. The molecular mass of the latter enzyme was reported to be about 30 kDa, while the former CTLP has a mass of about 95 kDa. Based on the reported enzyme characteristics, it appears that the proteinase described by Uitto et al. [17, 73–75] in the sonicated cell extract, is identical to the CTLP purified by conventional fast protein liquid chromatography (FPLC) techniques from a mild Triton extract of *T. denticola* ATCC 35405 [42], but differs from the enzyme described by Kuramitsu's group [54]. The activity of both enzymes has been determined using SAAPFNA as substrate (see Table 2).

In studies designed to elucidate the peptidolytic inactivation of substance P by washed whole cells of *T. denticola* ATCC 35405, it appeared that the Phe^8-Gly^9 bond of this peptide was hydrolyzed at a fast rate by a membrane-

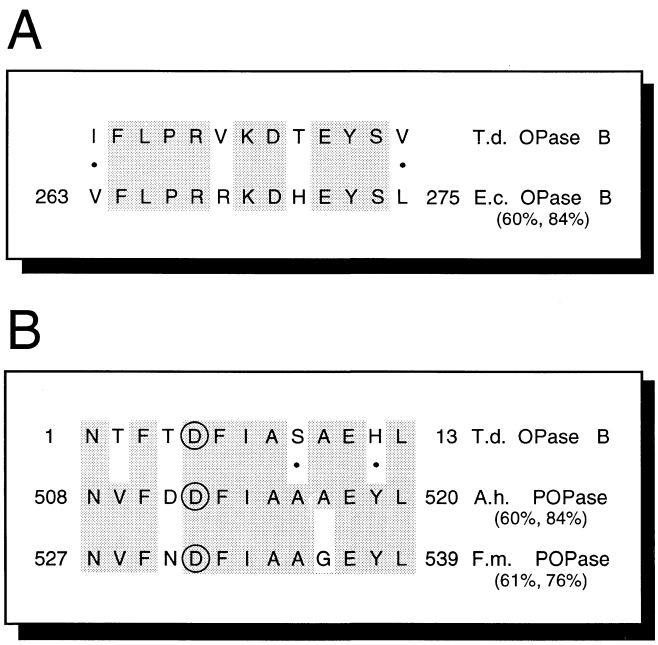

Fig. 2 **A** Alignment of amino acids present in *E. coli* OPB and in a peptide fragment of the *T. denticola* oligopeptidase, obtained by means of Glu-C digestion. Identical amino acid residues have been *shaded* (60%) while those with the same polarity (sum of identical and related = 84%) are indicated with a *dot*. **B** Alignment of amino acids present in *Aeromonas hydrophila prolyl oligopeptidase* (POP), *Flavobacterium meningosepticum* POP, and in a peptide fragment of *T. denticola* oligopeptidase, obtained by means of Lys-C cleavage. Identical amino acid residues have been *shaded* while those with the same polarity are indicated with a *dot*. The *percentage figures* indicate the relative amount of identical residues and the sum of identical and chemically related residues, respectively. The putative active site aspartic acid residue has been *circled*. The *T. denticola* oligopeptidase is most likely an OPB

bound enzyme. Subsequent purification and characterization [42] proved this enzyme to correspond to the CTLP discovered by Uitto et al. and Grenier et al. [17, 73]. The purification of the enzymes is hampered by its association with large membrane structures, but can be accomplished using polymyxin treatment. Without this treatment, the Triton-extracted enzyme appears on FPLC on a Superose 12 column in the void volume, giving a false molecular mass of 500–600 kDa for the enzyme itself (owing to association of the proteinase with membrane structures). After polymyxin treatment the enzyme gives a molecular mass of 95 kDa. The enzyme shows some strict specificity requirements, hydrolyzing the P_1P_1' bond in the sequence $-P_2P_1P_1'P_2'-$, where P_1 is preferably a Phe residue, while P_2 if preferably a Phe or a Pro residue (Leu, for example, can occupy position P_2, but leads to a much slower rate of hydrolysis). Val in position P_1 does not result in any hydrolysis. This enzyme hydrolyzes proteins (fibrinogen, gelatin, histone, casein, etc.) and peptides that meet the above specificity requirements. There is no Pro-Phe sequence in insulin β-chain, but this peptide is still hydrolyzed at a fast rate between Leu and Tyr in sequence $P_2P_1P_1'$ = Ala^{14}-Leu^{15}-Tyr^{16}. Much slower hydrolysis occurs at Tyr^{16}-Leu^{17} and at Phe^{25}-Tyr^{26}. These specificity features suggest that the CTLP has a relatively extended active site. The enzyme activity depends on an active seryl residue, but not on SH groups or metal cations. Chymostatin is a potent inhibitor of the enzyme. In standard SDS-PAGE (i.e. including treatment at 100 °C) this protein shows three different molecular forms, all with a mass <95 kDa [42, 73]. The CTLP molecule was suggested to contain proline-rich regions [42] characteristic of some proteins involved in binding interactions [78]. A role for the CTLP in the invasion and destruction of basement membrane was proposed [17, 73].

CTLP-like cell-bound activity was reported in the cells of *T. denticola* ATCC 33520 [48]. The characteristics reported for this enzyme do not, at this stage, entitle its identification with the CTLP of strain ATCC 35405.

Proline iminopeptidase

There is limited chemical information on bacterial proline iminopeptidases (PIPs). The only studies on a spirochetal PIP have dealt with the *T. denticola* ATCC 35405 enzyme which was regarded as a "dominant" aminopeptidase in ultrasonically prepared cell extracts [33]. The high PIP activity, determined with Pro-2NA, was indeed striking, since other aminoacyl 2NAs were hydrolyzed at a much lower rate, suggesting that this activity may be quite vital for the organism. The biological role of the enzyme in the metabolism of the spirochete has not been established, but the enzyme may contribute to the completion of the peptidolytic processing of proline-containing peptides. Whether such peptides are derived from the host's collagen or proline-containing bioactive peptides, such as hormones, kinins, neurotransmitters, or salivary proline-containing peptides, remains an intriguing question. It is also possible that the high proline content of some treponemal cell surface proteins and the high PIP activity of the cell envelope, are interrelated properties. The PIP itself contains a relatively large number of proline residues [39].

The enzyme is activated by NaCl at physiological salt concentration, hydrolyzes Pro-*p*NA optimally at pH 7.5–8.0, and does not require metal cations for full activity. The purification of the enzyme from Triton X-100 extracts of *T. denticola* was complicated by the cofractionation of a large component with a mass approximately 500 kDa [39]. After successful purification, it appears that this PIP has a mass of 120–144 kDa and represents a tetramer, the minimum molecular mass of the monomer being approximately 32 kDa. The activity levels of the PIP vary greatly from strain to strain, and also depend on growth conditions [68].

PZ-PLGPA peptidase

The systematic classification of the enzyme trivially called PZ-PLGPA peptidase is not clear. The enzyme has been

Table 4 Homology of various *T. denticola* OPase peptide sequences with those of known proteins (the length of the latter, in number of amino acid residues, is shown in parentheses). Identical amino acids are indicated by a vertical line. The two figures at the end give the percentages of identical amino acid residues and of those with the same polarity, respectively. The *T. denticola* OPase sequence in number 6 is preliminary

	Source of peptide	Alignment
1.	*Plasmodium falciparum* P-type cation translocating ATPase (1984 residues). Fragment 1491–1503. *T. denticola* OPase fragment FKIFLPRVKDTEYSVYPH (Glu-C cleavage). 61,76.	1491 K V E L P R I K D I N Y S 1503 2 K I F L P R V K D T E Y S 14
2.	*Amscata moorei* poxvirus GIL protein (464 residues). Fragment 271–282. *T. denticola* OPase fragment AEGKQAFIFDDYVVSPDNKKAAYFYNETG (CnBr cleavage). 66,91.	271 D K K A A Y F F N S S G 282 18 N K K A A Y F Y N E T G 29
3.	Immunoglobulin VH region (anti-idiotypic; mouse) (212 residues). Fragment 27–35. *T. denticola* OPase fragment (as in 2). 66,66.	27 F S I T D Y V V S 35 7 F I F D D Y V V S 15
4.	*Streptococcus mutans* sugar-binding protein MsME (420 residues). Fragment 106–115. *T. denticola* OPase fragment (as in 2). 90,80	106 K A G Y F Y N M T G 115 20 K A A Y F Y N E T G 29
5.	Lyme disease spirochete lipoprotein (194 residues). Fragment 118–124. *T. denticola* OPase (as in 2). 85,100.	118 G K E A F I F 124 3 G K Q A F I F 9
6.	Synaptonemal complex protein 1 (mouse) (993 residues). Fragment 805–813. *T. denticola* OPase N-terminal end (undigested). 77,88	805 L K D K K D K K I 813 34 L K D K T D K K V 42
7.	Intracellular serine proteinase inhibitor, placental thrombin inhibitor 38 K (human) (376 residues). Fragment 202–214. *T. denticola* OPase N-terminal end (undigested). 61,69.	202 K Q S T F K K T Y I G E I 214 2 K Q S D F E K P P I A E I 14
8.	*Coxiella burnetii* superoxide dismutase (EC 1.15.1.1.) (193 residues). Fragment 48–56. *T. denticola* OPase N-terminal end (undigested). 66,77	48 F E K E P L E E I 56 6 F E K P P I A E I 14
9.	*Bacillus subtilis* protein ComE ORF3 (776 residues). Fragment 25–35. *T. denticola* OPase fragment GFPIIFLFKIFL (Asp-N cleavage). 72,81.	25 F P A I F L F I L F L 35 2 F P I I F L F K I F L 12

demonstrated and partially characterized in *T. denticola* ATCC 35405 and in several of its clinical isolates [32, 36]. The cells of *T. vincentii* ATCC 35580 and *T. socranskii* also display activity on PZ-PLGPA which is hydrolyzed at the Leu-Gly bond by the enzymes present in these organisms [36]. The rationale of using PZ-PLGPA as a substrate in *Treponema* studies lies in the suggestion that this molecule, along with FALGPA, may be used as a substrate by microbial collagenases. Both peptide derivatives can, however, be hydrolyzed by enzymes other than collagenases and it is not known at present whether the hydrolysis of PZ-PLGPA by the treponemes indeed involves "true" collagenase action. However, the concept of "true collagenase" has also changed.

The extracts of ultrasonically treated cells of *T. vincentii* ATCC 35580 contained two enzymes hydrolyzing PZ-PLGPA, one with a molecular mass of approximately 75 kDa, the other with a mass about 23 kDa (based on molecular permeation FPLC) [34]. The larger form hydrolyzed gelatin (the smaller one was not tested), and both forms were sensitive to *p*-chloromercuribenzoate (*p*CMB) and were moderately inhibited by metal chelators (although a metalloenzyme nature was not established with certainty). Both the *T. denticola* and the *T. vincentii* enzymes were similar in their reactions with *p*CMB and metal chelators.

γ-Glutamyl transpeptidase activity

A convenient substrate for the measurement of this enzyme activity is γ-glutamyl-*p*NA. γ-Glutamyl transpeptidase (GGTP) activity was demonstrated in the cells of *T. vincentii* ATCC 35580 and *T. denticola* ATCC 35405 [32, 34]. A similar enzyme was partially purified from *Fusobacterium nucleatum*, another prospective periodontopathogen [35], and was active on γ-glutamylcysteinylglycine (glutathione). The presence of cell-associated GGTP in periodontal pathogens may be significant since these enzymes may contribute to the metabolism of glutathione, or break down γ-glutamyl linkages present in the connective tissue, or act in general in γ-glutamyltransferase reactions. A GGTP was purified to homogeneity from

a mild (0.5%) Triton X-100 extract of the cells of *T. denticola* 35405 [40]. This enzyme gives a molecular mass of approximately 213 kDa in FPLC, but breaks down into 26-kDa forms in SDS-PAGE (to be reported).

Selective effect of the gaseous environment

The gaseous environment understandably effects the growth of the spirochetes. There is preliminary information on the effects of the growth atmosphere on the activity levels of cell-associated peptidases [68]. The activity levels of the OBP-like enzyme were not appreciably affected by the gaseous atmosphere, wherease the PIP activity varied significantly depending on the composition of the gaseous environment, anaerobic growth conditions being associated with higher specific activity than aerobic conditions.

Selective effect of Cl^-

It is not surprising that Cl^- exerts quite diverse chemical effects on enzyme proteins. The activity of treponemal peptidases seems to depend selectively on Cl^-. This property can perhaps be utilized to differentiate some of those peptidases with overlapping specificities. For example, among the *T. denticola* cell-associated enzymes, FALGPA peptidase is inhibited by Cl^-, while the activities of the POP, PIP and GGTP are strongly increased by Cl^- [30, 32, 34, 35]. However, these effects depend on the buffer used. Very low, physiological levels of Cl^- may cause significant increase of PIP activity [39], while higher salt concentrations may be slightly inhibitory [41].

Such chloride effects may be partly unspecific. However, they may reflect an evolutionary dependency of some of the pathological peptidolytic processes on the specific [Cl^-] provided by the host. Even the gingival crevicular fluid may contain sufficiently high Cl^- levels to be utilized by peptidases present in periodontopathic treponemes [9]. A mammalian enzyme, aminopeptidase B (EC 3.4.11.6), suggested to play a role in the extracellular inflammatory processes, is specifically activated by physiological [Cl^-] [30, 31]. The above Cl^- effects can be used as an additional identification procedure of spirochetal peptidases.

Peptidolysis caused by whole cells

From the pathological point of view it is important to observe that several peptidolytic enzymes described in this review – perhaps most of them – seem to be located in the outer cell envelope or in the periplasmic space. Such information has by and large been derived from studies on *T. denticola*, but offers interesting points of comparison for studies with other spirochetes as well. In fact, for enzyme purification and localization studies, it is advisable to first use mild detergent extracts of washed whole cells rather than ultrasonic treatment or other disruptive procedures. Ultrasonic treatment can be used to disintegrate more tightly bound enzyme-organel complexes. At this laboratory, virtually all peptidolytic reactions, first described with sonicated cell extracts, have also been observed to be caused by extracts of washed whole cells which frequently catalyze such reactions much faster than the former preparations. Microscopic examination of whole cells after those reactions have shown the cells to remain morphologically intact. It is important to observe that small substrate molecules may enter the periplasmic space of the cells, to be hydrolyzed by peptidases located within that cell compartment, while larger molecules can be hydrolyzed by enzymes located on the outer cell surface. The ready hydrolysis of various tissue proteins [74, 75] and human bioactive peptides [41, 42] by the ectoenzymes of *T. denticola* exemplify reactions that may be vital to the propagation of the spirochetes, and important in human spirochetal infections.

Proline specificity of treponemal peptidases

Proline-specific peptidases (PSPs) were designated as those enzymes that prefer the hydrolysis of peptide bonds that involve an imino acid residues at or near the scissile bond (in the treponemes, proline – not hydroxyproline – has been involved in most of such reactions). Attention was paid to the existence of such enzymes in the cell extracts of *T. denticola* owing to a rapid hydrolysis of peptides that meet the above structural requirements. Enzymes that were thus regarded as PSPs include the FALGPA peptidase [37], the POP [41], the enzyme that hydrolyzes PZ-PLGPA [36], and the PIP [39]. It is possible that such specificity requirements are coincidental, or that the natural substrates of these enzymes are indeed proline-containing peptides (for the PIP this requirement is obvious). It is possible that the true in vivo substrates of those PSPs include human bioactive proline-containing peptides, small collagen fragments, and proline-rich salivary peptides.

Trypsin-like enzymes

The peptidase and proteinase literature is replete with information on trypsin-like enzymes whose identity with trypsin (EC 3.4.2.4) is, however, in many cases equivocal. It may not always be appropriate to call an enzyme trypsin like, even if the enzymes is active on synthetic trypsin substrates (such as BANA and BAPNA), or is inhibited by some trypsin inhibitors. As pointed out above, the evolutionary relationship between the treponemal "trpysin-like" proteinases and trypsin may be more remote, the former being often more closely related to enzymes of the POP superfamily. The *T. denticola* trypsin-like enzyme [38, 51] is most likely an OPB (an enzyme first described in *E. coli*).

The proteolytic profile of spirochetes other than treponemes

Regrettably scant information is available about the proteinases and peptidases of spirochetes other than *T. denticola*. The cells of *B. burgdorferi*, *B. hermsii*, and those of leptospires and serpulinas do exhibit arylamidase activities [7], but none of these enzymes has been characterized, and no firm conclusions on their biological role has been presented. The present authors predict that several of the enzymes present in the cells of *T. denticola* will also be demonstrated in *T. pallidum*.

References

1. Arakawa S, Kuramitsu HK (1994) Cloning and sequence analysis of a chymotrypsin-like protease from *Treponema denticola*. Infect Immun 62:3224–3433
2. Baehni PC, Song M, McCulloch CAG, Ellen RP (1992) *Treponema denticola* induces actin rearrangement and detachment of human gingival fibroblasts. Infect Immun 60:3360–3368
3. Boehringer H, Taichman NS, Shenker BJ (1984) Suppression of fibroblast proliferation by oral spirochetes. Infect Immun 45:155–159
4. Boehringer HR, Berthold PH, Taichman NS (1986) Studies on the interaction of human neutrophils with plaque spirochetes. J Periodont Res 21:195–209
5. Canale-Parola E (1977) Physiology and evolution of spirochetes. Bacteriol Rev 41:181–204
6. Dawson JR, Ellen RP (1990) Tip-oriented adherence of *Treponema denticola* to fibronectin. Infect Immun 58:3924–3928
7. Dettori G, Grillo R, Cattani P, Calderaro A, Chezzi C, Milner J, Truelove K, Sellwood R (1995) Comparative study of the enzyme activities of *Borrelia burgdorferi* and other nonintestinal and intestinal spirochaetes. Microbiologica 18:13–26
8. Ellen RP, Song M, McCulloch CA (1994) Degradation of endogenous plasma membrane fibronectin concomitant with *Treponema denticola* 35405 adhesion to gingival fibroblasts. Infect Immun 62:3033–3037
9. Ferris G, Grow TE, Low SB, Ferris RT (1987) Measurement of gingival crevicular fluid conductivity, in vivo. J Periodontol 58:46–50
10. Fiehn N-E (1986) Enzyme activities from eight small-size oral spirochetes. Scand J Dent Res 94:132–140
11. Fitzgerald TJ, Repesh LA, Blanco DR, Miller JN (1984) Attachment of *Treponema pallidum* to fibronectin, laminin, collagen IV, and collagen I, and blockade of attachment of immune rabbit IgG. Br J Vener Dis 60:357–363
12. Goldhaber P, Giddon DB (1964) Present concepts concerning the etiology and treatment of acute necrotizing ulcerative gingivitis. Int J Dent Res 14:468–496
13. Gopalsami C, Yotis W, Corrigan K, Schade S, Keene J, Simonson L (1993) Effect of outer membrane of *Treponema denticola* on bone resorption. Oral Microbiol Immunol 8:121–124
14. Grenier D (1991) Characteristics of hemolytic and hemagglutinating activities of *Treponema denticola*. Oral Microbiol Immunol 6:246–249
15. Grenier D (1992) Nutritional interaction between two suspected periodontopathogens, *Treponema denticola* and *Porphyromonas gingivalis*. Infect Immun 60:5298–5301
16. Grenier D (1992) Demonstration of a bimodal coaggregation reaction between *Porphyromonas gingivalis* and *Treponema denticola*. Oral Microbiol Immunol 7:280–284
17. Grenier D, Uitto V-J, McBride BC (1990) Cellular location of *Treponema denticola* chymotrypsinlike protease and importance of the protease in migration through the basement membrane. Infect Immun 58:347–351
18. Haapasalo M, Singh V, McBride BC, Uitto VJ (1991) Sulphydryl-dependent attachment of *Treponema denticola* to laminin and other proteins. Infect Immun 59:4230–4237
19. Harwood LS, Canale-Parola E (1984) Ecology of spirochetes. Annu Rev Microbiol 38:161–192
20. Heylings RT (1967) Electron microscopy of acute ulcerative gingivitis (Vincent's type). Demonstration of the fusospirochaetal complex of bacteria within prenecrotic gingival epithelium. Br Dent J 122:51–56
21. Hollander DH (1981) Treponematosis from pinta to venereal syphilis revisited – hypothesis for temperature determination of disease patterns (editorial). Sex Transm Dis 8:34–37
22. Holt SC, Bramanti TE (1991) Factors in virulence expression and their role in periodontal disease pathogenesis. Crit Rev Oral Biol Med 2:177–281
23. Hu LT, Perides G, Noring R, Klempner MS (1995) Binding of human plasminogen to *Borrelia burgdorferi*. Infect Immun 63:3491–3496
24. Keulers RAC, Maltha JC, Mikx FHM, Wolterslutgerhorts JML (1993) Attachment of *T. denticola* strains ATCC 33520, ATCC 35405, B11 and Ny541 to a morphologically distinct population of rat palatal epithelial cells. J Periodont Res 28:274–280
25. Keulers RAC, Maltha JC, Mikx FHM, Wolterslutgerhorts JML (1993) Attachment of *Treponema denticola* strains to monolayers of epithelial cells of different origin. Oral Microbiol Immunol 8:84–88
26. Koopman MBH, Kasbohrer A, Beckmann G, Vanderzeijst BAM, Kusters JG (1993) Genetic similarity of intestinal spirochetes from humans and various animal species. J Clin Microb 31: 711–716
27. Loesche WJ (1976) Periodontal disease and the treponemes. In: Johnson RC (ed) The biology of parasitic spirochetes. Academic Press, New York, pp 261–275
28. Loesche WL, Bretz WA, Kerschensteiner D, Stoll J, Socransky SS, Hujoel P, Lopatin DE (1991) Development of a diagnostic test for anaerobic periodontal infections based on plaque hydrolysis of benzoyl-DL-arginine-naphthylamine. J Clin Microbiol 28:1551–1559
29. Maltha JC, Mikx FHM, Kujipers FJ (1985) Necrotizing ulcerative gingivitis in beagle dogs. III. Distribution of spirochetes in interdental gingival tissues. J Periodont Res 20:522–531
30. Mäkinen KK, Mäkinen P-L (1971) Effect of sodium chloride on substrate constant and maximum velocity in the enzyme hydrolysis of *N*-L-aminoacyl-2-naphthylamines and *N*-L-aminoacyl-*p*-nitroanilines. Acta Chem Scand 25:969–975
31. Mäkinen KK, Oksala E (1973) Evidence on the involvement in inflammation of an enzyme resembling aminopeptidase B. Clin Chim Acta 49:301–309
32. Mäkinen KK, Syed SA, Mäkinen P-L, Loesche WJ (986) Benzoylarginine peptidase and iminopeptidase profiles of *Treponema denticola* strains isolated from the human periodontal pocket. Curr Microbiol 14:85–89
33. Mäkinen KK, Syed SA, Mäkinen P-L, Loesche WJ (1987) Dominance of iminopeptidase activity in the human oral bacterium *Treponema denticola* ATCC 35405. Curr Microbiol 14:341–346
34. Mäkinen KK, Syed SA, Loesche WJ, Mäkinen P-L (1988) Proteolytic profile of *Treponema vincentii* ATCC 35580 with special reference to collagenolytic and arginine aminopeptidase activity. Oral Microbiol Immunol 3:121–128
35. Mäkinen KK, Söderling E, Syed SA, Mäkinen P-L (1990) Hydrolysis of γ-glutamyl linkages by *Fusobacterium nucleatum*. Curr Microbiol 20:5–11
36. Mäkinen KK, Syed SA, Salvador SL, Mäkinen P-L (1990) Hydrolysis of the Leu-Gly bond of phenylazobenzyloxycarbonyl-L-Pro-L-Leu-Gly-L-Pro-D-Arg (a substrate of microbial collagenases) by treponemes isolated from the subgingival plaque of periodontitis patients. Curr Microbiol 20:69–74
37. Mäkinen KK, Mäkinen P-L, Syed SA (1992) Purification and substrate specificity of an endopeptidase from the human oral spirochete *Treponema denticola* ATCC 35405, active on furylacryloyl-Leu-Gly-Pro-Ala and bradykinin. J Biol Chem 267: 14 285–14 293

38. Mäkinen KK, Mäkinen P-L, Loesche WJ, Syed SA (1995) Purification and general properties of an oligopeptidase from *Treponema denticola* ATCC 35405 – a human oral spirochete. Arch Biochem Biophys 316:689–698
39. Mäkinen KK, Chen C-Y, Mäkinen P-L (1996) The proline iminopeptidase (EC 3.4.11.5) activity of the human oral spirochaete *Treponema denticola* ATCC 35405. Infect Immun (in press)
40. Reference deleted
41. Mäkinen P-L, Mäkinen KK, Syed SA (1994) An endo-acting proline-specific oligopeptidase from *Treponema denticola* ATCC 35405: evidence of hydrolysis of human bioactive peptides. Infect Immun 62:4938–4947
42. Mäkinen P-L, Mäkinen KK, Syed SA (1995) Role of the chymotrypsin-like membrane-associated proteinase from *Treponema denticola* ATCC 35405 in inactivation of bioactive peptides. Infect Immun 63:3567–3575
43. Mikx FHM (1991) Comparison of peptidase, glycosidase and esterase activities of oral and non-oral *Treponema* species. J Gen Microbiol 137:63–68
44. Mikx FHM, DeJong MH (1987) Keratinolytic activity of cutaneous and oral bacterial. Infect Immun 55:621–625
45. Mikx FHM, Keulers RAC (1992) Hemagglutination activity of *Treponema denticola* grown in serum-free medium in continuous culture. Infect Immun 60:1761–1766
46. Mikx FHM, Ngassapa DNB, Reijntjens FMJ, Maltha JC (1984) Effect of splint replacement on black-pigmented *Bacteroides* and spirochetes in the dental plaque of beagle dog. J Dent Res 63:1284–1288
47. Mikx FHM, Maltha JC, Campen GJ (1990) Spirochetes in early lesions of necrotizing ulcerative gingivitis experimentally induced in beagles. Oral Microbiol Immunol 5:86–89
48. Mikx FHM, Jacobs F, Satumalay C (1992) Cell-bound peptidase activities of *Treponema denticola* ATCC 33520 in continuous culture. J Gen Microbiol 138:1837–1842
49. Nitzan D, Sperry JF, Wilkins TD (1978) Fibrinolytic activity of oral anaerobic bacteria. Arch Oral Biol 23:465–470
50. Norris SJ (1993) Polypeptides of *Treponema pallidum* – Progress toward understanding their structural, functional, and immunologic roles. Microbiol Rev 57:750–779
51. Ohta K, Mäkinen KK, Loesche WJ (1986) Purification and characterization of an enzyme produced by *Treponema denticola* capable of hydrolyzing synthetic trypsin substrates. Infect Immun 53:213–220
52. Olsen I (1984) Attachment of *Trepnoema denticola* to cultured human epithelial cells. Scand J Dent Res 92:55–63
53. Pederson ED, Miller JW, Matheson S, Simonson LG, Chadwick DE, Covill PH, Turner DW, Lamberts BL, Morton HE (1994) Trypsin-like activity levels in *Treponema denticola* and *Porphyromonas gingivalis* in adults with periodontitis. J Clin Periodont 21:519–525
54. Que X-C, Kuramitsu HK (1990) Isolation and characterization of the *Treponema denticola* prtA gene coding for chymotrypsin-like protease activity and detection of a closely linked gene encoding PZ-PLGPA-hydrolyzing activity. Infect Immun 58: 4099–4105
55. Riley BS, Oppenheimermarks N, Hansen EJ, Radolf JD, Norgard MV (1992) Virulent *Treponema pallidum* activates human vascular endothelial cells. J Infect Dis 165:484–493
56. Riviere GR, Wagoner MA, Bakerzander SA, Weisz KS, Adams DF, Simonson L, Lukehart SA (1991) Identification of spirochetes related to *Treponema pallidum* in necrotizing ulcerative gingivitis and chronic periodontitis. N Engl J Med 325:8
57. Rosen G, Naor R, Kutner S, Sela MN (1994) Characterization of fibrinolytic activities of *Treponema denticola*. Infect Immun 62:1749–1754
58. Rothschild BM (1989) On the antiquity of treponemal infection. Med Hypotheses 28:181–184
59. Rudolph AH, Duncan WC, Kettler AH (1988) Treponemal infections. J Am Acad Dermatol 18:1121–1129
60. Scott D, Siboo IR, Chan ECS, Klitorinos A, Siboo R (1993) Binding of hemin and Congo Red by oral hemolytic spirochetes. Oral Microbiol Immunol 8:245–250
61. Sela MN, Weinberg A, Borinsky R, Holt SC, Dishon T (1988) Inhibition of superoxide production in human polymorphonuclear leukocytes by oral treponemal factors. Infect Immun 56:589–594
62. Shenker BJ, Listgarten MA, Taichman NS (1984) Suppression of human lymphocyte responses by oral spirochetes. A monocyte-dependent phenomenon. J Immunol 132:2039–2045
63. Shibata Y, Fujimura S, Nakamura T (1992) Isolation and characterization of enzymes hydrolyzing chymotrypsin synthetic substrate (Enzyme I) and trypsin synthetic substrate (Enzyme II) from the envelope of *Capnocytophaga gingivalis*. Med Microbiol Immunol 181:107–115
64. Söderling E, Syed SA, Mäkinen P-L, Mäkinen KK (1994) Proteolytic activity of treponemes from the subgingival plaque of periodontitis patients. Microb Ecol Health Dis 7:71–81
65. Sorsa T, Ingman T, Suomalainen K, Haapasalo M, Konttinen YT, Lindby O, Saari H, Uitta V-J (1992) Identification of proteases from periodontopathogenic bacteria as activators of latent human neutrophil and fibroblast-type intestitital collagenases. Infect Immun 60:4491–4495
66. Suzuki M, Loesche WJ (1989) Ceruloplasmin can substitute for rabbit serum in stimulating the growth of *Treponema denticola*. Infect Immun 57:643–644
67. Syed SA, Salvador SL, Loesche WJ (1988) Enzyme profiles of oral spirochetes in RapID-ANA system. J Clin Microbiol 26:2226–2228
68. Syed SA, Mäkinen KK, Mäkinen P-L, Chen C-Y, Muhammad Z (1993) Proteolytic and oxidoreductase activity of *Treponema denticola* ATCC 35405 grown in an aerobic and anaerobic gaseous environment. Res Microbiol 144:317–326
69. Takahashi T, Asari K, Yamaya S, Sugahara T (1985) Purification and properties of an aminopeptidase from *Treponema phagedenis* (Reiter strain). Curr Microbiol 12:283–288
70. Tanner A, Stillman N (1993) Oral and dental infections with anaerobic bacteria – clinical features, predominant pathogens, and treatment. Clin Infect Dis 16 [Suppl 4]:S304–S309
71. Thomas DD, Navab M, Haake DA, Fogelman AM, Miller JN, Lovett MA (1988) *Treponema pallidum* invades intercellular junctions of endothelial cell monolayers. Proc Natl Acad Sci USA 85:3608–3612
72. Uitto V-J, Chan ECS, Quee TC (1986) Initial characterization of neutral proteinases from oral spirochetes. 21:95–100
73. Uitto V-J, Grenier D, Chan ECS, McBride BC (1988) Isolation of a chymotrypsin-like enzyme from *Treponema denticola*. Infect Immun 56:2717–2722
74. Uitto V-J, Haapasalo M, Laakso T, Salo T (1988) Degradation of basement membrane collagen by proteases from some anearobic oral microorganisms. Oral Microbiol Immunol 3:97–102
75. Uitto V-J, Pan Y-M, Leung WK, Larjava H, Ellen RP, Finlay BB, McBride BC (1995) Cytopathic effects of *Treponema denticola* chymotrypsin-like proteinase on migrating and stratified epithelial cells. Infect Immun 63:3401–3410
76. Weinberg A, Holt SC (1990) Interaction of *Treponoma denticola* TD-4, GM-1, and MS25 with human gingival fibroblasts. Infect Immum 58:1720–1729
77. Williams LR, Austin FE (1992) Hemolytic activity of *Borrelia burgdorferi*. Infect Immun 60:3224–3230
78. Williamson MP (1994) The structure and function of proline-rich regions in proteins. Biochem J 297:249–260