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Periplasm Organization in *Treponema denticola* as Studied by Cryo-electron Tomography

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As a spirochete, the genus *Treponema* is one of the few major bacterial groups whose natural phylogenic relationships are evident at the level of gross phenotypic characteristics such as their morphology. *Treponema* spp. are highly invasive due to their unique motility in dense media, and their ability to penetrate cell layers [1]. This feature is associated with the helical cell body and the presence of flagellar filaments in the periplasm [2].

Treponema denticola is an oral pathogen involved in endodontic infections and periodontal diseases. The presence and quantity of *T. denticola* in the subgingival biofilm is correlated with the severity of periodontal disease and tissue destruction [3,4]. The organism has also been detected in 75% of severe endodontic abscesses [5]. A better understanding of *Treponema* ultrastructure and motility will aid development of new strategies to control infection. Because of the similarity in ultrastructural organization among spirochetes, knowledge gained from *T. denticola* can be applied to other spirochetes causing diseases in human and animals (syphilis, digital dermatitis, Lyme disease, relapsing fever, leptospirosis, etc.).

Our interest here is to study the ultrastructure of the periplasm of *T. denticola*. The periplasm is limited by the inner and outer membranes, and contains the flagellar filaments and the peptidoglycan layer [2,6]. A crucial question involves the organization of the outer membrane and the peptidoglycan layer so that the rotating flagellar filaments can be accommodated without interfering with protein trafficking.

We are studying the 3-D structure of intact *T. denticola* by plunge-freezing in liquid ethane at liquid nitrogen temperature, followed by cryo-electron tomography. Tilt series (1° increment, 120° angular range) are recorded at -178°C and 400 keV acceleration voltage using a JEOL JEM4000FX with Gatan GIF2002 energy filter operated in zero-loss mode. The ice thickness is typically 0.3 μm, which proves to be sufficient to prevent flattening of the cells. We have confidence that this technique reveals the native structure of *T. denticola*, since artifacts due to chemical fixation, dehydration or staining are avoided.

The flagellar filaments become closely parallel within the first helical turn of the cell, as shown in Fig. 1A. In the absence of flagellar filaments, the periplasmic space has a uniform thickness. The periplasmic space widens at a bundle of flagellar filaments, as well as at a single flagellar filament. The increase in width of the periplasmic space is less than the diameter of a flagellar filament. This suggests that the peptidoglycan layer is locally thinned or stretched (due to contrast matching, the peptidoglycan layer appears simply as a space in this preparation). [7]

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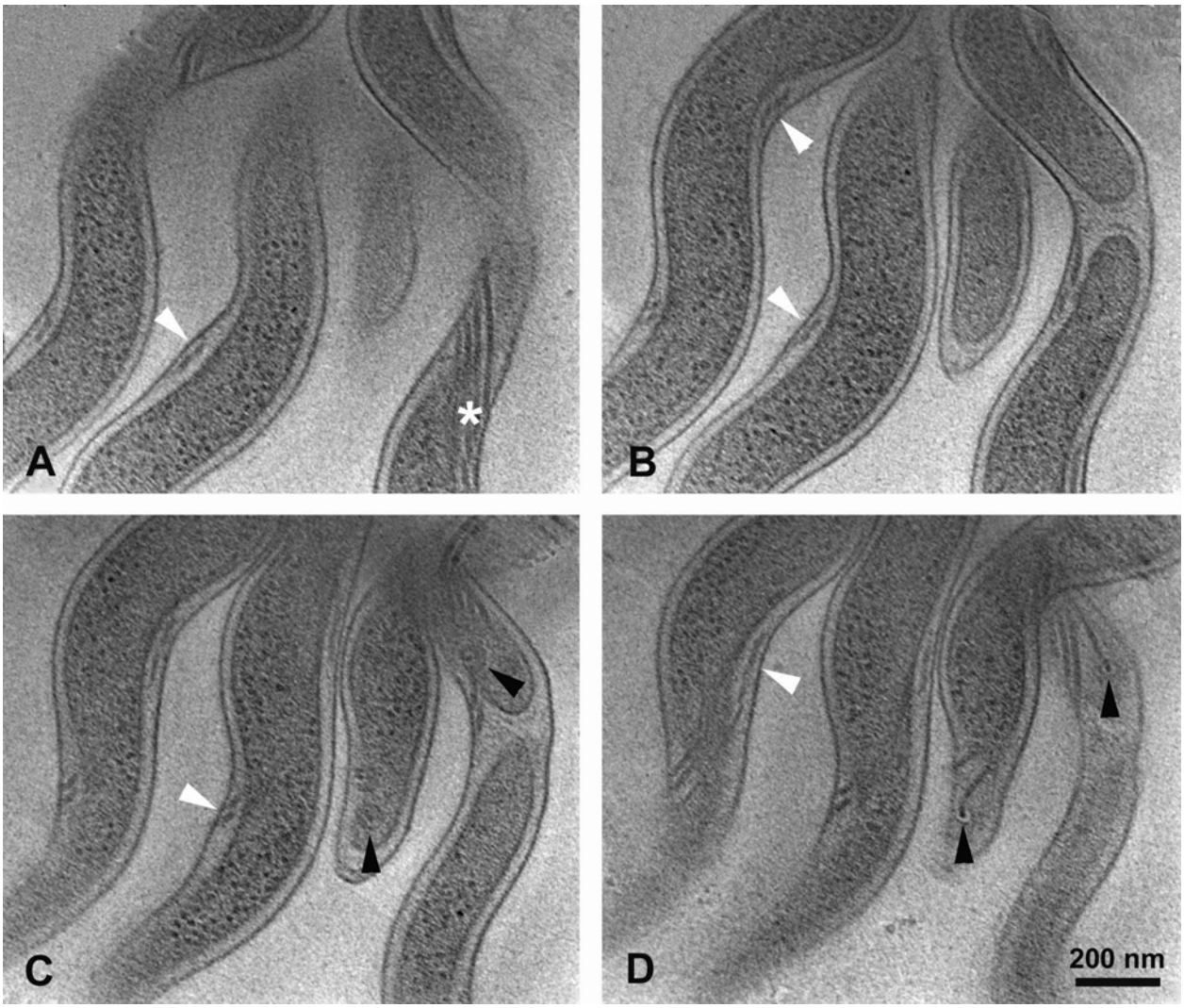

FIG. 1. Z-slices, 1.8 nm thick, from a tomographic reconstruction of frozen-hydrated *Treponema denticola* cells. A bundle of four parallel flagellar filaments is indicated by * in (A). White arrowheads indicate a widening of the periplasmic space at the flagellar filaments. Black arrowheads indicate flagellar basal bodies, with attachment to flagellar filaments seen in (D). Depth from A to B = 33 nm; from B to C = 70 nm; from C to D = 19 nm.

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