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Filamentous *Escherichia coli* cells swimming in tapered microcapillaries

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Abstract

This study analyzed the swimming characteristics of filamentous *Escherichia coli* cells inside tapered capillaries with a diameter decreasing from 700 μm to 4 μm and a mean body length of 27.8 μm ± 11.9 μm. Cells that were pre-oriented towards the narrower diameter section of the tapered capillary swam with high directional persistence, following conical-helix trajectories along the capillary wall. The confinement of the tapered capillary significantly diminished the mean swimming speed of filamentous cells when compared to their unrestricted mean swimming speed. The cell body rotation of individual filamentous bacteria decreased along the tapered direction, likely due to increased steric interactions with the capillary wall. Filamentous cells that swam under imposed flow rates ranging from 0.2 μl/min to 0.8 μl/min showed positive rheotaxis inside the 150 μm–350 μm diameter region of the tapered capillary. Depending on the imposed flow rate, none of the bacteria could advance beyond a critical diameter in the tapered capillary. This critical diameter is likely to be the position of the maximum shear rate they can tolerate without being flushed away. This work showed experimental evidence of how a simple flow constriction such as a tapered tube forms a hydrodynamic barrier that can deter the advance of bacterial rheotaxis.

Keywords: Filamentous cells; Restrictive geometry; Swimming; Motility; Rheotaxis

1. Introduction

Bacterial filamentation occurs when growth continues in the absence of cell division, resulting in the formation of elongated organisms that have multiple chromosomal copies. Filamentation has been found to be a crucial survival strategy of bacteria during exposure to environmental stresses including host effectors, protist predators, increased hydrostatic pressure and antimicrobial therapies [20,43]. Although filamentous cells are typically 10–50 times longer compared to their bacillary counterparts, they not only remain motile, but also retain cellular activity such as chemotaxis. Filamentous bacteria demonstrate a swimming pattern that involves "running" and "stopping" sequences in solution, whereas normal size cells exhibit the characteristic "running" and "tumbling" swimming sequences [27]. Filamentous cells can be produced by exposure to antibiotics such as cephalexin, which have been shown to inhibit cell division while allowing cell growth at a uniform exponential rate until growing is abruptly terminated by cell lysis [38].

Bacterial filaments are known to play important roles in diverse scenarios, as in the case of wastewater plants, where they serve as the backbone for floc formation [5,40]. Flocs cannot be formed properly if there are too few filaments; and conversely, they cannot settle properly if the filaments are too numerous [40]. Additionally, the presence of filamentous cells in biofilms has been attributed to energy loss in both high- and low-velocity tubular flow systems, including heat exchangers, secondary oil recovery systems and wastewater management systems. Without filamentous cells in these biofilms, there is little frictional resistance in the system because the film is relatively thin; however, as filaments begin to grow, both energy loss and frictional resistance increase [35]. Filamentous bacteria are also of concern in the medical field; for example,

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they are present in the late stages of urinary tract infections (UTIs), where filaments of up to 70 μm in length are formed in response to a Toll-like-receptor-4-mediated innate defense mechanism [19], and in other cases, filamentation undermines antibiotics that target actively dividing cells, avoids neutrophil extracellular traps and resists antimicrobial peptides such as cathelicidin [41].

Microbial life accounts for up to 50% of the total pore volume in soil, with more than 80% preferentially located in the region termed the 'inner part,' which hosts micropores with diameters from 2 to 6 μm. Comparatively, the "outer part" of the soil is formed by pores with diameters greater than 6 μm [37]. Bacteria found in geological formations are known to have a variety of shapes, including filamentous forms [15,35,46], which play diverse roles in the soil's bacterial community. For example, it has been found that long filaments of *Desulfobulbaceae* bacteria are able to transport electrons in upper layers of marine sediments, transmitting electric currents across centimeter distances from the oxygen reduction zone at the surface to the sulfide oxidation zone at the subsurface; they have accordingly been termed 'living micro-cables' [35].

Therefore, a detailed comprehension of the swimming behavior of filamentous bacteria in restricted geometries may be of great interest in diverse fields. Presently, most studies in confined spaces concentrate on normal-sized bacteria [3,4,6,9,13,14,24–26,28]. Some studies with model filamentous cells of *Escherichia coli* have explored their swimming and chemotactic behavior [17,27,39]. Maki et al. published one of the most relevant works at the single-cell swimming on a slide [27], where the motility and chemotaxis characteristics of filamentous *E. coli* were used to establish that they exhibit very similar responses to those of normal-sized cells, noting little difference in the motility and chemotactic responses between bacillary and filamentous counterparts. In the only study of filamentous cells in a confined environment, Takeuchi et al. reported how confinement affected growth of *E. coli* in microchambers in the direction of filamental elongation, confirming that filamentous bacteria can bend during growth and conform to the shapes of the microfabricated structures [41].

Considering that filamentous cells are actively present in a variety of scenarios where flow may be involved, exploring their swimming characteristics under such conditions would also be beneficial. It has been found that normal-sized *E. coli* exposed to shear flow near a surface are able to align parallel to the flow and exhibit preferential upstream swimming [16,21]. This directional movement is known as positive rheotaxis [29]. It has been suggested that the motility of bacteria in the presence of flow may even hinder chemotaxis in the case where they are oriented away from the nutrient source [29]. Interestingly, demonstrations show that bacteria entering a region with flow can rapidly migrate upstream much faster than a gradually advancing biofilm [21]. Although several contributions on the characterization of shear-driven bacterial motility have been made [16,21,29,30], none of these studies have addressed filamentous bacteria. Given that narrow spaces are suitable habitats for filamentous cells, one concern is how the migration of these longer cells can be mitigated as a means of avoiding their proliferation in situations where they are not desired. We hypothesized that a simple flow constriction, such as a tapered microtube, can create a hydrodynamic barrier to filamentous cells, that may in turn deter the advance of bacterial rheotaxis, as has been previously suggested by Kaya and Koser for normal-size bacteria [21].

In this paper, we first focused on characterizing bacterial motility of filamentous *E. coli* cells in restrictive geometries (tapered capillaries), since currently there are no studies of how these long bacteria move and penetrate confined spaces. Subsequently, the motility of filamentous cells inside tapered capillaries was studied for the purpose of observing how this geometry could affect bacterial migration in the presence of convective flow.

2. Materials and methods

2.1. Bacterial preparation

This paper describes the behavior of *E. coli* filamentous swimming cells, the chemotactic strain RP437 [34] and the non-chemotactic strain UU2612 [22,44], which were originally cultured from cryopreservative beads onto an LB-agar culture plate and allowed to grow overnight at 37 °C. Three colonies per strain were used to inoculate 5 ml of LB medium for 4 h and incubated on a rotatory shaker at 180 rpm at 37 °C. Cells were harvested by centrifugation at 7000 g for 3 min at 25 °C, then removed from LB medium and re-suspended in LB medium containing 20 μg/ml of cephalexin at a concentration of $\sim 10^6$ cells per ml, which were incubated for 2 h on previously mentioned shaker at identical conditions.

2.2. Scanning electron microscopy (SEM) of E. coli cells

E. coli cells were prepared for SEM imaging by following a similar procedure as the one described by Fischer et al. [10]. Briefly, the cells were captured on 13-mm polycarbonate filters (Isopore™, EMD Millipore Corporation, Billerica, MA, USA) where they were held during preparatory steps. The filter-attached bacteria were fixed with 2.5% glutaraldehyde in 0.1 M potassium phosphate buffer pH 7.0 for 2 h. The fixative was replaced every 30 min. The fixing process was followed by rinsing the microstructures in motility buffer (11.2 g of K_2HPO_4, 4.8 g of KH_2PO_4 and 0.029 g of EDTA per liter of water) three times, each time for 10 min. The rinsed microstructures were then dehydrated with a graded series of ethanol (50%, 70%, 90%, 95%, 100% [3×]) for 10 min each. Samples were then prepared for drying by treating the microstructures with a series of baths of HMDS. The first bath consisted of two parts ethanol/one part HMDS, followed by a bath of one part ethanol/two parts HMDS and finally two baths of 100% HMDS. Samples were incubated in each bath for 15 min. The HMDS-treated samples were then allowed to air-dry and mounted onto SEM stubs with adhesive tabs and were then sputter-coated with gold at 10 mA for 75 s and imaged