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Complex Spatial Organization and Flagellin Composition of Flagellar Propeller from Marine Magnetotactic Ovoid Strain MO-1

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Marine magnetotactic ovoid bacterium MO-1 is capable of swimming along the geomagnetic field lines by means of its two sheathed flagellar bundles at a speed up to 300 μm/s. In this study, by using electron microscopy, we showed that, in each bundle, six individual flagella were organized in hexagon with a seventh in the middle. We identified 12 flagellin paralogs and 2 putative flagellins in the genome of MO-1. Among them, 13 were tandemly located on an ~ 17-kb segment while the 14th was on a separated locus. Using reverse transcription PCR and quantitative PCR, we found that all the 14 flagellin or putative flagellin genes were transcribed and that 2 of them were more abundantly expressed than others. A nLC (*n*ano*l*iquid *c*hromatography)–ESI (*e*lectro*s*pray *i*onization)–MS/MS (*m*ass *s*pectrometry/*m*ass *s*pectrometry) mass spectrometry analysis identified all the 12 flagellin proteins in three glycosylated polypeptide bands resolved by one-dimensional denaturing polyacrylamide gel electrophoresis and 10 of them in 21 spots obtained by means of two-dimensional electrophoresis of flagellar extracts. Most spots contained more than one flagellin, and eight of the ten identified flagellins existed in multiple isoforms. Taken together, these results show unprecedented complexity in the spatial organization and flagellin composition of the flagellar propeller. Such architecture is observed only for ovoid–coccoid, bilophotrichously flagellated magnetotactic bacteria living in marine sediments, suggesting a species and environmental specificity.

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Abbreviations used: RT-PCR, reverse transcription PCR; MTB, magnetotactic bacterium; two 2D, two-dimensional.

Introduction

Bacterial flagella are rigid structures protruding from cell surface, each about 20 nm in diameter and 15–20 μm long. They provide bacteria with a highly efficient means of locomotion and also play a central role in adhesion, biofilm formation and host invasion.[1–4] Despite sharing similarities in structure, bacterial flagella exhibit extensive variation in number and placement between species, which had been used as criteria in bacterial taxonomy in the past. Bacteria may have single flagellum (monotrichous) at one end of the cell (polar flagellum) or a single flagellum at both ends (amphitrichous), as well as numerous flagella as a tuft (lophotrichous) or distributed all over the cell (peritrichous). Interestingly, *Vibrio* spp. possess two flagellar systems: a single sheathed polar flagellum propelling the cell in liquid environments and numerous unsheathed lateral flagella moving the cell over surfaces.[4] Flagellar filaments can be differentiated into plain and complex flagella when analyzed by transmission electron microscopy. Plain filaments have a smooth surface, whereas complex flagellar filaments have a distinct ridging pattern and appear thicker, more rigid and brittle than the plain filaments.[5,6]

A flagellar filament is formed from tens of thousands copies of identical flagellin proteins or several kinds of flagellins in multiple flagellin systems. It has been observed in some cases that different flagellins are segmented in a filament. Whereas in bacteria having both polar and lateral flagella, the utilization of flagellin is believed to be environmentally specific.[4] Besides the variation of intrinsic property determined by the flagellin sequence, posttranslational modification also increases the diversity of flagellar filaments. For example, glycosylation of flagellins has been found to be important for numerous flagellar systems; it plays an integral role either in flagellar assembly or for a number of bacterial pathogens, a role in virulence.[7]

Recently, we reported a peculiar architecture of the flagellar apparatus of marine magnetotactic bacterium (MTB) MO-1 isolated from sediments of the Mediterranean Sea.[8] MTBs are a heterogeneous group of Gram-negative bacteria that can orient and swim along the magnetic field lines, thanks to intracellular magnetic crystals named magnetosomes.[9,10] Such behavior, called magneto-aerotaxis, is believed to facilitate cells finding and maintaining a preferred position at the oxic–anoxic transition zone.[9] MO-1 cells are bilophotrichously flagellated, having two flagellar tufts on one side of the cell.[8] We previously reported that the flagellar bundle is enclosed within a sheath that is assembled from a large (>350 kDa) glycoprotein in a calcium-ion-dependent manner and is required for smooth swimming of MO-1 cells.[11] In this study, we analyzed the ultrastructure of the flagellar bundles and flagellin composition of the flagellar filament. We identified 12 flagellin and 2 putative flagellin genes in this bacterium and found that they were all transcribed. Further biochemical and proteomic analyses revealed that these flagellins were glycosylated and that all 12 flagellins were assembled into flagellar filaments. We report an unprecedented level of complexity of spatial organization and flagellin redundancy and discuss the environment specificity of utilization of such a bacterial propeller.

Results

Spatial organization of MO-1 flagella

Previously, we reported that the marine magnetotactic ovoid strain MO-1 has two bundles of flagella on one side of the cell when examined with transmission electron microscopy.[8] Here, a typical image of cryo-electron microscopy confirms the bilophotrichous feature of MO-1 flagella (Fig. 1a, black arrows). During the binary fission of MO-1 cells, each daughter cell inherited one flagellar bundle (Fig. 1b). Interestingly, magnetosome chain was bent at the center and divided into two parts at the constricting septum (Fig. 1b, white arrow). Electron microscopy showed that the flagellar filaments were about 12 nm in width and had a smooth surface, lacking the recognizable cross-hatched pattern observed in the complex flagellar of *Rhizobium meliloti*[12] and *Rhizobium lupini*[13] (Fig. 1c).

Using transmission electron microscopy, we found that each flagellar bundle had seven conspicuous flagella (Fig. 1d). A representative architecture of the MO-1 flagellar apparatus resembled a 6+1 distribution, with six flagella organized in hexagon array, separated by 55–60 nm, while the seventh located in the middle of the array (Fig. 1d1 and d2). Such spatial organization of flagella has also been observed by cryo-electron microscopy (data not shown).

MO-1 genome contains 14 flagellin genes

The strain most closely related to MO-1 is magnetotactic coccus MC-1, sharing 93.2% identity between their 16S ribosomal RNA genes. Like MO-1, MC-1 cells also have two flagellar bundles.[14] Genomic analysis revealed that MC-1 contains 15 flagellin genes.[15] Interestingly, we found 14 flagellin homologs at two locations in the genome of MO-1. Thirteen of them (named *fliC1* to *fliC13*) were in a tandem array, but the 14th (*fliC14*) was located on another contig (Fig. 2a). Among the first 13 flagellin genes, *fliC13* was the only one located close to the accessory genes involved in flagellar synthesis. It