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# Swarming motility in *Bradyrhizobium japonicum*

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**Abstract**

Flagellar-driven bacterial motility is an important trait for colonization of natural environments. *Bradyrhizobium japonicum* is a soil species that possesses two different flagellar systems: one subpolar and the other lateral, each with a filament formed by a different set of flagellins. While synthesis of subpolar flagellins is constitutive, translation of lateral flagellins was detected in rhizobia grown with L-arabinose, but not with D-mannitol as sole carbon source, independently of whether bacteria were in liquid or semisolid medium. We characterized swarming of *B. japonicum* in semisolid medium and found that this motility was faster with L-arabinose than with D-mannitol. By using mutants with deletions in each flagellin set, we evaluated the contribution of each flagellum system to swarming in semisolid culture media, and in soil. Mutants devoid of either of the flagella were affected in swarming in culture media, with this impairment being stronger for mutants without lateral flagella. In sterile soil at 100% or 80% field capacity, flagellar-driven motility of mutants able to swim but impaired in swarming was similar to wild type, indicating that swimming was the predominant movement here.


*Keywords:* *Bradyrhizobium*; Swimming; Swarming; Flagellum; Soil; Rhizosphere

## 1. Introduction

Bacterial motility is a key trait affecting fitness and survival in natural environments. Almost all species in the bacterial realm have evolved different devices for locomotion, the construction and propulsion of which consume a considerable fraction of the metabolic energy produced by the cell. Flagella are among the best characterized of these locomotion devices. Some bacteria, among them *Aeromonas* spp., *Azospirillum* spp., *Plesiomonas shigelloides*, *Rhodospirillum centenum*, *Vibrio alginolyticus*, and *Vibrio parahaemolyticus* possess two different flagellar systems in the same cell: a polar (or subpolar) flagellum and lateral flagella (Inoue et al., 1991; Jiang et al., 1998; Kawagishi et al., 1995; McClain et al., 2002; Shimada et al., 1985; Shinoda and Okamoto, 1977; Tarrand et al., 1978). It was observed that these flagellar systems are propelled by $H^+$ or $Na^+$ gradients respectively (Jiang et al., 1998; Kawagishi et al., 1995; McClain et al., 2002; Shinoda and Okamoto, 1977). Furthermore, lateral flagellar expression is upregulated by contact with surfaces or in response to impairment of polar flagellar motion (Kirov, 2003; Merino et al., 2006; Merino and Tomás, 2009).

*Bradyrhizobium japonicum*, the diazotrophic symbiont of soybean plants, has two different flagellar systems: a thick subpolar flagellum and thinner peritrichous flagella, which are often sinusoidal in shape (Althabegoiti et al., 2008; Kanbe et al., 2007). Morphologically, these flagella are similar to the respective polar and lateral flagella of *P. shigelloides* (Inoue et al., 1991), *R. centenum* (McClain et al., 2002) and *V. parahaemolyticus*, one of the best-characterized dual-flagella systems (Bardy et al., 2003). However, in *B. japonicum*, both

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flagella are powered by an $H^+$ proton gradient and are expressed in agitated liquid AG medium (Althabegoiti et al., 2008; Kanbe et al., 2007).

The flagellins that constitute the filament of the *B. japonicum* subpolar flagellum, which is constitutively expressed in liquid medium, are encoded in four genes, named *fliC1*, *fliC2*, *fliC3*, and *fliC4* (locus tags bll5843, bll5844, bll5845, and bll5846, respectively). In addition, two genes named *fliCI* and *fliCII* (locus tags bll6866, and bll6865, respectively) encode the structural flagellin of the peritrichous flagellum, which is produced in liquid AG complex medium but not in Götz minimal medium (Althabegoiti et al., 2008, 2011; Kanbe et al., 2007). Due to their homology with other *fliC* genes, Kanbe et al. (2007) termed the latter two genes subpolar flagellins, according to the *Salmonella* model, which has only one flagellum type. However, we agree with other authors that these flagellins are part of a lateral flagellum (Merino and Tomás, 2009) in view of the morphology of the *B. japonicum* peritrichous flagellum and the response of its flagellin synthesis to different culture conditions. Thus, in this work, we will refer to bll6866 and bll6865 as *lafA1* and *lafA2*, respectively.

Swarming is a kind of flagellar-driven movement that, in contrast to swimming into a liquid, can be observed on the surface of medium (0.5–0.8%) to hard (1.0–2.0%) agar. It looks like a colony expanding with branches or tendrils that radiate from the colony center toward the border, where cells are often enlarged and multiflagellated (Butler et al., 2010; Harshey, 2003). This social movement was reported in some rhizobial species, namely *Ensifer meliloti* (Nogales et al., 2010, 2012; Soto et al., 2002), *Rhizobium etli* (Braeken et al., 2008; Daniels et al., 2006) and *Rhizobium leguminosarum* bv. *viciae* (Tambalo et al., 2010), which are evolutionarily distant from *B. japonicum* and have only one flagellar system. Furthermore, it is believed that swarming might be required for bacterial motility in soils or in the rhizosphere. Because the role of *B. japonicum* motility in soil and in relation to competitiveness for soybean root infection and nodulation is not clear (Althabegoiti et al., 2008, 2011; Liu et al., 1989; López-García et al., 2002; McDermott and Graham, 1989), it is important to study the possible contributions of the two flagella to this type of motility and how it might impact *B. japonicum*'s ability to thrive in its natural environment. Kanbe et al. (2007) suggested that, as in other species with dual flagella systems, the *B. japonicum* subpolar flagellum is used for swimming and the lateral flagellum is used for swarming. However, studies of swarming in this species have not yet been addressed. Therefore, in this paper, we characterize *B. japonicum* swarming and evaluate the expression of the lateral flagellin and the role of each flagellum in swarming. We further use a laboratory approach to estimate the contributions of swimming and swarming to bacterial dispersal in soil.

## 2. Materials and methods

### 2.1. Bacterial strains and culture conditions

*B. japonicum* strains used here are listed in Table 1 and were described by López-García et al. (2002) and Althabegoiti et al. (2008, 2011). These strains are LP 3004 (a spontaneous streptomycin-resistant derivative from USDA 110), LP 3008 (selected for higher swimming motility from LP 3004) and flagellin deletion mutants derived from these backgrounds, as follows. From LP 3004 we obtained LP 5843 (Δ*fliC1234*), LP 6865 (Δ*lafA12*) and LP 6543 (Δ*fliC1234*Δ*lafA12*). From LP 3008 we obtained LP 5844 (Δ*fliC1234*), LP 6866 (Δ*lafA12*) and LP 6644 (Δ*fliC1234*Δ*lafA12*). The rhizobia were grown in Götz minimal medium (Götz et al., 1982) with 5.0 g $l^{-1}$ carbon source or in AG medium (Sadowsky et al., 1987) with appropriate antibiotics (Althabegoiti et al., 2011). Conditions for growth in liquid medium were 28 °C and 180 rpm. Antibiotics were used at the following concentrations (μg $ml^{-1}$): streptomycin, 400; spectinomycin, 200; kanamycin, 150; gentamicin, 100; and cycloheximide, 100.

### 2.2. Reverse transcription PCR

Rhizobial suspensions cultured in Götz medium with the different carbon sources, as described above, were centrifuged at 11,000 × *g* at 4 °C for 40 min and washed twice with 1 M NaCl. Then the cells were disrupted with lysozyme in TE buffer, pH 8.0. Total RNA was obtained with Trizol (Invitrogen, Buenos Aires, Argentina) following the manufacturer's instructions and then treated with DNase I at 37 °C for 15 min. Complementary DNA (cDNA) was synthesized using random primers with M-MLV reverse transcriptase (Invitrogen, Buenos Aires, Argentina) following the manufacturer's instructions. After reverse transcription, PCR was performed as described by Quelas et al. (2010) using the following primers: for *fliC1*, fliC1Fw: 5′-CGATGGCACCACCGTACTGT-3′ and fliC1Rv: 5′-ACCGCGGTTCCCTCATAGA-3′; for *fliC2(34)*, fliC2(34)Fw: 5′-CGGTCCTTTGCAAGCACT-3′ and fliC2(34)Rv: 5′-TTCACGGTCAGCGTATCGC-3′; for *lafA1*, lafA1Fw: 5′-CCTCACCAACTCGTCTGCAA-3′ and lafA1Rv: 5′-CCGTGTTCAGAGCGGTGTATT-3′; for *lafA2*, lafA2Fw: 5′-GGTTACATCGCGCAGGTCA-3′ and lafA2Rv: 5′-GGGTGGACTCCTGGTTCATGT-3′, and for *sigA* (internal control, Hauser et al., 2006): sigAFw: 5′-CTGATCCAGGAAGGCAACATC-3′ and sigARv: 5′-TGGCGTAGGTCGAGAACTTGT-3′. The following reaction controls were employed: 1) reaction without template to test for contamination in the reagents, 2) reaction with a product obtained by omitting the retrotranscription step to test for DNA remaining after DNAse treatment, and 3) reaction with genomic DNA as

Table 1
Strains used in this study (Althabegoiti et al., 2011).

| Strain | Parental strain | Flagellin genes deleted | Flagellar filaments produced |
|---|---|---|---|
| LP 3004 | USDA 110 | None | Subpolar and lateral |
| LP 3008 | LP 3004 | None | Subpolar and lateral |
| LP 5843 | LP 3004 | *fliC1234* | Lateral |
| LP 5844 | LP 3008 | *fliC1234* | Lateral |
| LP 6865 | LP 3004 | *lafA12* | Subpolar |
| LP 6866 | LP 3008 | *lafA12* | Subpolar |
| LP 6543 | LP 6865 | *fliC1234*; *lafA12* | None |
| LP 6644 | LP 6866 | *fliC1234*; *lafA12* | None |