



Changes in *Azospirillum brasilense* motility and the effect of wheat seedling exudates

Igor V. Borisov, Andrei V. Schelud'ko, Lilia P. Petrova, Elena I. Katsy*

Laboratory of Microbial Genetics, Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, 13 Prospekt Entuziastov, Saratov 410049, Russian Federation

Received 22 January 2007; received in revised form 5 July 2007; accepted 16 July 2007

KEYWORDS

Azospirillum brasiliense;
Wheat seedling exudates;
Motility;
Mutation

Summary

The rhizobacterium *Azospirillum brasilense* Sp245 swims, swarms (Swa⁺ phenotype) or, very rarely, migrates with the formation of granular macrocolonies (Gri⁺ phenotype). Our aims were (i) to identify Sp245 mutants that swarm faster than the parent strain or differ from it in the mode of spreading and (ii) to compare the mutants' responses to wheat seedling exudates. In isotropic liquid media, the swimming speeds of all motile *A. brasilense* strains were not influenced by the exudates. However, the exudates significantly stimulated the swarming of Sp245. In several Sp245 mutants, the superswarming phenotype was insensitive to local colonial density and to the presence of wheat seedling exudates. An *A. brasilense* polar-flagellum-defective Gri⁺ mutant BK759.G gave rise to stable Swa⁺⁺ derivatives with restored flagellum production. This transition was concurrent with plasmid rearrangements and was stimulated in the presence of wheat seedling exudates. The swarming rate of the Swa⁺⁺ derivatives of BK759.G was affected by the local density of their colonies but not by the presence of the exudates.

© 2007 Elsevier GmbH. All rights reserved.

Introduction

Plant-growth-promoting rhizobacteria of the genus *Azospirillum* have been widely used as models for studying the mechanisms of the associative plant–bacterial interactions (Steenhoudt and Vanderleyden, 2000). All *Azospirillum* species

are motile because of the presence of a single polar flagellum (Fla), and several of them have a mixed type of flagellation. For example, in *A. brasilense* and *A. lipoferum*, Fla is produced in a liquid environment, and numerous lateral flagella (Laf) are induced in addition to Fla on viscous and solid media (Tarrand et al., 1978). Fla is responsible for swimming (Mot⁺ phenotype), whereas Laf are used for swarming (Swa⁺ phenotype) (Tarrand et al., 1978; Hall and Krieg, 1983). After being stab-inoculated into media containing 0.2–0.5% Bacto

*Corresponding author. Tel.: +7 8452 970403;
fax: +7 8452 970383.

E-mail address: katsy@ibppm.sgu.ru (E.I. Katsy).

agar, wild-type *A. brasilense* spreads with the formation of ring structures (Moens et al., 1995; Scheludko et al., 1998). As in the closely related α -purple photosynthetic bacterium *Rhodospirillum centenum* (Jiang et al., 1998), not only Laf but also a functional polar flagellum seems to be required for *A. brasilense* swarming (Scheludko et al., 1998).

Recently, spreading with the formation of microcolonies (Swa⁻ Gri⁺ phenotype) was revealed in *A. brasilense* strain Sp245. Under standard laboratory conditions, the culture of Sp245 consisted of Swa⁺ Gri⁻ (95–91% of the clones), Swa⁻ Gri⁻ (4.7–8% of the clones) and Swa⁻ Gri⁺ (0.3–1% of the clones) subpopulations (Shelud'ko and Katsy, 2001). As compared with the aerated cultures, precultivation of Sp245 in liquid media under non-aerated, anoxic conditions before sowing them into semisolid media slightly increased the frequencies of occurrence of the Swa⁻ Gri⁻ and Swa⁻ Gri⁺ clones. The Swa⁻ Gri⁻ and Swa⁻ Gri⁺ clones of Sp245 gave rise to Swa⁺ Gri⁻ descendants. In the non-swarming Omegon-Km mutants of Sp245, the quantity of clones with a stable Swa⁻ Gri⁺ phenotype approximated to 70–90%, and the rest were Swa⁻ Gri⁻ (Shelud'ko and Katsy, 2001). In all the mutants with the predominant Swa⁻ Gri⁺ phenotype, the polar flagellum was not produced or was paralysed. The diameter of the granular dispersal zones did not depend on the number of lateral flagella on the *A. brasilense* cells. For instance, the Fla⁻, Fla⁻ Laf⁻ and Fla⁻ leaky Laf⁻ mutants of Sp245 formed Gri⁺ colonies of approximately the same diameter. No effects of the nutrients on the dispersal of *A. brasilense* cells in semiliquid media were revealed. For example, irrespective of the presence of NH₄Cl, malate, leucine, proline, tryptophan, arabinose and rhamnose in semisolid media (0.2–0.6% agar), the non-swarming Omegon-Km mutants formed colonies with either the Swa⁻ Gri⁺ or the Swa⁻ Gri⁻ phenotype (Shelud'ko and Katsy, 2001).

It was demonstrated that adsorption of the vital sulphonated azodye Congo Red confers on wild-type *A. brasilense* the ability to consistently spread in semiliquid agar with the formation of microcolonies. Rapidly swarming spontaneous derivatives of *A. brasilense* Sp245 and derivatives that swarmed in the presence of Congo Red were identified (Shelud'ko et al., 2006).

Seed and root exudates are a major source of nutrients and signals for rhizobacteria (Lynch and Whipps, 1990; Zhu et al., 1997; Fan et al., 2001; Bacilio-Jiménes et al., 2003). Tactic responses of *Azospirillum* and other plant-associated bacteria towards plant exudates are important for successful bacterial establishment on plant roots (Vande

Broek et al., 1998; Bacilio-Jiménes et al., 2003). On and inside the roots, the appearance of non-motile variants of *A. lipoferum* was much more frequent (~40% of the clones) than it was outside the rhizosphere and in a laboratory culture (~5% of the clones) (Alexandre et al., 1996). When colonizing plant roots, azospirilla frequently form microcolonies; however, the underlying mechanisms are not well understood (Burdman et al., 2000). The relationship between the Gri⁺ *Azospirillum* spreading under laboratory conditions and the rhizosphere microcolonies has not been investigated.

The aim of this work was to identify *A. brasilense* Sp245 mutants that swarm faster than the parent strain or differ from it in the mode of spreading. We also intended to analyse whether the complex of substances exuded by the seedlings of wheat, an associative partner of *A. brasilense*, could affect bacterial swimming and swarming speeds and the phenotypic differences in bacterial behaviour in soft media (transitions between Swa⁺ Gri⁻, Swa⁻ Gri⁻ and Swa⁻ Gri⁺ phenotypes).

Materials and methods

Bacterial strains, plasmids and growth conditions

The bacteria and plasmids used are listed in Table 1. The mutants of Sp245 were obtained by Katzy et al. (1998) by mating Sp245 with *Escherichia coli* S17-1, harbouring a vector for Omegon-Km mutagenesis, pJFF350 (Fellay et al., 1989). *Azospirilla* were routinely grown in malate-salt medium (Döbereiner and Day, 1976) (MSM; pH 7.0) at 30 °C. Semisolid media contained Bacto agar at 4 or 5 g l⁻¹. Adsorption of Congo Red by the *A. brasilense* colonies was determined on a solid medium containing 37.5 µg ml⁻¹ of the dye (Bastarrachea et al., 1988). Calcofluor white (Fluorescent Brightener 28, Aldrich) was added to tryptic soya agar medium (TSA, Serva) to a final concentration of 1 mg ml⁻¹. When appropriate, kanamycin (Km) and NH₄Cl were added to the media to final concentrations of 30 µg ml⁻¹ and 0.5 g l⁻¹, respectively.

To examine the effect of wheat seedling exudates (SE) on bacterial spreading, we added 10 ml of SE (pH 5.9), preheated to 50 °C, to an equal volume of melted MSM containing 10 g l⁻¹ of Bacto agar (SE+MSM; pH after mixing, 6.3). In control experiments, SE were replaced with plant medium (PM) (PM+MSM; pH after mixing, 6.2). Overnight liquid cultures of azospirilla (at equal OD₅₄₀ 0.1) were serially diluted with sterile MSM.

Download English Version:

<https://daneshyari.com/en/article/2092610>

Download Persian Version:

<https://daneshyari.com/article/2092610>

[Daneshyari.com](https://daneshyari.com)