Microbiology, Vol. 74, No. 4, 2005, pp. 495–497. Translated from Mikrobiologiya, Vol. 74, No. 4, 2005, pp. 572–574.

SHORT COMMUNICATIONS

Plasmid Rearrangements in *Azospirillum brasilense*

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Received December 16, 2004; in final form, February 10, 2005

Bacteria of the genus *Azospirillum* live in various econiches in association with plants. *Azospirillum* cells contain many plasmids. The 90-MDa plasmid (pRhico) of type strain *A. brasilense* Sp7 and the 120- and 85-MDa plasmids (p120 and p85, respectively) of *A. brasilense* Sp245 have been found to be involved in the regulation of motility (Mot phenotype) and swarming (Swa phenotype), as well as in the formation of polar (Fla phenotype) and lateral flagella (Laf phenotype) and the synthesis of lipopolysaccharides (LPS phenotype) and calcofluor-binding exopolysaccharides (Cal phenotype) [1, 2]. The Rhico plasmid has regions that are homologous to the *lps/cal* and *fla/swa* loci of p120, whereas the 115-MDa plasmid (p115) of strain Sp7 has regions that are homologous to the *fla/laf* and *mot/swa* loci of p85 [1]. According to their electrophoretic profiles, p85 and p115 have a smaller copy number than p120 and pRhico [1].

The functional role of p115 is as yet unknown. Analysis of the S variant of strain Sp7 (Sp7-S), which lost its 115-MDa plasmid as a result of long-term cultivation at 40–42°C [3], showed that the R–S dissociation in strain Sp7 is associated with redistribution of the contributions from the two *O*-specific polysaccharides (*O*-PSs) of the R and S cell variants to the topography of the cell wall [4] rather than with changes in the length of *O*-PS. The plating of Sp7-S cells after they had been stored at –70°C for several years gave rise to colonies with R, RS, and S morphology.

The aim of this work was to analyze the DNA and phenotype of eight derivatives of Sp7-S and derivative strain Sp7.K2 of Sp7, which spontaneously lost one of its *O*-PSs [4]. Investigations along these lines may provide insight into genetic events associated with the phenotypic heterogeneity of cell populations of *A. brasilense.*

Our experiments showed that the bacterial strains that produce colonies with R (strains Sp7.1–Sp7.4), RS (strain Sp7.5), and S (strains Sp7.6–Sp7.8 and Sp7.K2) morphologies have an altered plasmid composition (see table). The use of cloned fragments of Sp245 plasmids [5] as probes made it possible to perform a hybridization analysis of the DNA of Sp7-S and Sp7.K2 derivatives. It should be noted that horizontal gel electrophoresis in a Tris–acetate buffer with the use of a nonradioactive ECL DNA hybridization kit from Amersham (United Kingdom) was ineffective for the analysis of plasmids with molecular masses greater than 300 MDa, whereas vertical gel electrophoresis in a Tris–borate buffer showed the occurrence of such plasmids in all the strains under study (data not shown). Full plasmid profiles of strains Sp7 and Sp7-S can be found in our earlier publications [3, 5]. Like pRhico [1], the new replicon found in strains Sp7.1–Sp7.8 and Sp7.K2 was found to hybridize with 8.3- and 15-kb *Bam*H1 fragments containing *fla/swa* and *lps/cal* loci from p120. Hybridization analysis of the *Bam*H1 fragments of the total DNA of strain Sp7.K2 and the derivative strains of Sp7-S with the use of the aforementioned probes did not reveal any differences between these strains. In common with pRhico, the new plasmid of strains Sp7.1, Sp7.3, Sp7.5–Sp7.8, and Sp7.K2 did not hybridize with the 2.4-kb *Eco*RI fragment of p85. However, like p115 of strain Sp7, the 131-MDa plasmid of strains Sp7.2 and Sp7.4 was able to hybridize with the 2.4-MDa *Eco*RI fragment of p85 (see figure). Presumably, the new plasmid identified in almost all the strains under study is a derivative of pRhico, whereas that of strains Sp7.2 and Sp7.4 is a hybrid of pRhico and a fragment of p115. The DNA region that is homologous to this fragment occurs in strains Sp7 and Sp245 in two copies, one of which is located in p115 and p85 [1]. It is likely that this DNA region is able to migrate. Hybridization analysis of the total DNA of almost all the strains with the 2.4-kb *Eco*RI fragment of p85 showed the presence of two *Eco*RI fragments of the same size (~2.3 and 2.8 kb), as in the case of strain Sp7. The total DNA of strains Sp7.7 and Sp 7.3 exhibited the presence of one hybridization signal (either strong or weak) at the level of the 2.8-kb *Eco*RI fragment. It is probable that, in strain Sp7-S, one of the fragments of p115 has been integrated into the chromosome, making the genome unstable and prone to plasmid rearrangements. The genetic instability of strain Sp7-S may also be due to the specific method of its derivation (incubation at an elevated temperature). It should be noted that soil bacteria are often exposed to elevated temperatures in their natural habitats.

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0026-2617/05/7404-0495

Plasmid composition and some phenotypic properties of parent *A. brasilense* strains Sp7 and Sp7.K2 and derivative strains Sp7.1–Sp7.8 of Sp7-S

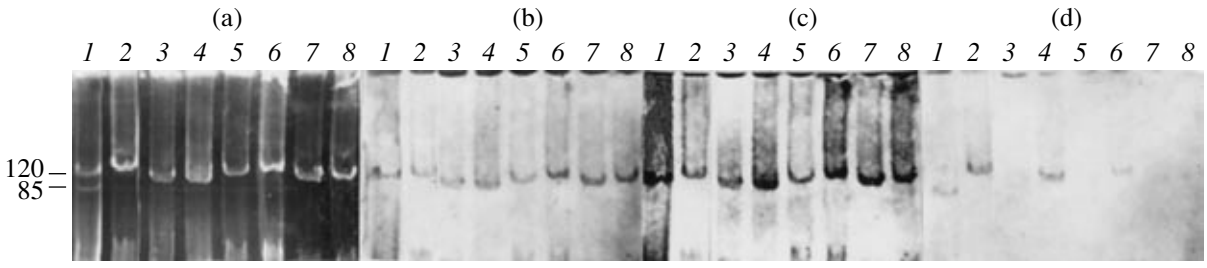
Strain	Molecular mass of plasmids in MDa	Ampicillin MIC (μg/ml) for growth on MMA	SDS MIC (μg/ml) for growth on			CTAB MIC (μg/ml) for growth on PA	Bacterial growth in the presence of 20 μg/ml CTAB		
			MMA	GMA	PA		MA without malate	MSM	GSM
Sp7	90, 115, >300	500	200	100	300	300	+	±	±
Sp7.1	124, >300	500	200	100	300	500	+++	±	+
Sp7.2	131, >300	>2000	100	100	250	500	++	±	+
Sp7.3	121, >300	>2000	100	100	250	300	–	±	±
Sp7.4	131, >300	500	200	100	300	300	++	±	+
Sp7.5	94, >300	500	100	200	300	500	+++	+	±
Sp7.6	90, >300	>2000	100	200	250	300	+	+	±
Sp7.7	107, >300	1000	100	200	250	300	++	±	±
Sp7.8	124, >300	1000	200	100	300	500	++	+	+
Sp7.K2	94, >300	2000	100	100	250	300	±	±	±

Note: Abbreviations [5]: MA stands for mineral agar; MMA, mineral agar with malate; GMA, mineral agar with glycerol; and PA, potato agar. Bacterial growth on agar media in streaks was evaluated according to the following criteria: "–" (no growth), "±" (poor growth), "+" (moderate growth), "++" (good growth), and "+++" (very good growth).

The question arises as to how plasmid dynamics can affect the phenotype of *A. brasilense* (see table). One of the reasons for the enhanced ampicillin resistance of strain Sp7.K2 and some derivative strains of Sp7-S may be the amplification of the respective genes of pRhico [2]. The dynamic organization of pRhico may be determined by the loci coding for transposase and DNA invertase [2]. The derivative strains of Sp7-S did not lose their ability to swim and swarm. Moreover, the collective migration rate of the derivative strains of Sp7 and Sp7-S from the point of inoculation into an MM medium containing 0.3 or 0.4% Bacto agar was 1.7–2.7 times higher than that of the Sp7 parent strain. Many loci of pRhico contain code for the formation of carbohydrate-containing polymers at the cell surface, which may play the role of a lubricant during bacterial swarming [2]. Therefore, it is not surprising that alterations in the mass and structure of pRhico are associated with changes in the morphology of azospirillum colonies produced on solid and semisolid media. Nearly all the strains under study, except for Sp7.K2, can adsorb calcofluor on the cell surface.

Plasmid rearrangements influence the resistance of azospirilla to cationic (such as cetyltrimethylammonium bromide (CTAB)) and anionic (such as sodium dodecyl sulfate (SDS)) surfactants. The ability of strains Sp7.1, Sp7.2, Sp7.4, Sp7.5, Sp7.7, and Sp7.8 to grow on mineral agar with CTAB as the carbon source instead of malate (see table) needs further investigation.

To the best of our knowledge, we are the first to describe the profound dynamics of plasmids and the phenotypic changes related to plasmid rearrangements in bacteria of the genus *Azospirillum*.



Homology of plasmids from the parent strain Sp7, its derivative strain Sp7.K2, and the derivative strains of Sp7-S to fragments p120 and p85 from *A. brasilense* Sp245. Plasmids from Sp245 (lanes 1), Sp7.2 (lanes 2), Sp7.K2 (lanes 3), Sp7 (lanes 4), Sp7.1 (lanes 5), Sp7.4 (lanes 6), Sp7.5 (lanes 7), and Sp7.7 (lanes 8) before hybridization (a) and after hybridization with the peroxidase-labeled 15-kb *Bam*H1 fragment of pOmegon-Km-*lps*348X (b) and 8.3-kb *Bam*H1 fragment of pOmegon-Km-*fla*048X (c) containing p120, as well as with the 2.4-kb *Eco*RI fragment of p85 from pEK051X (d) [5]. The numbers on the left-hand side indicate the molecular mass of the plasmids in MDa.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research and by the Russian Presidential Foundation "Leading Scientific Schools of Russia," grant no. NSh-1529.2003.4.

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