Symbiosis, 13 (1992) 147–150
Balaban, Philadelphia/Rehovot

Anthranilate and Indole-3-Acetate Production in *Azospirillum brasilense* Sp245 Mutant Derivatives

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Received October 13, 1991; Accepted January 26, 1992

Abstract

Several classes of mutants in anthranilate (Ant) and indole-3-acetate (IAA) production (Ant^+IAA^+, Ant^+IAA^- and Ant^-IAA^-) were obtained from *Azospirillum brasilense* strain Sp245 (Ant^-IAA^+) by chemical and transposon mutagenesis. Some of the Ant^+IAA^- mutants having the tryptophan-dependent anthranilate production also gained the ability to excrete a water-soluble brown pigment in the presence of phenylalanine. It is shown by using mobilization of T*n*5-Mob-mutagenized replicons that the genetic structures affected in the Sp245 Ant^+IAA^+ derivatives are different from that in the Ant^+IAA^- mutants.

Keywords: *Azospirillum brasilense* Sp245, anthranilic acid, indole-3-acetic acid, mutants

1. Introduction

The well-known ability of *Azospirillum* to synthesize IAA is thought to be one of the major factors influencing plant growth and yield (Cacciari et al., 1989; Fallik et al., 1989). Meanwhile, genes and pathways for IAA biosynthesis in *Azospirillum* are not well known (Hartmann et al., 1983; Abdel-Salam and Klingmüller, 1988, Ruckdäschel et al., 1988).

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0334-5114/92 /$03.50

In this study, several classes of *A. brasilense* strains Sp245 mutants in phytohormone production are described and the different location of these mutations in the Sp245 genome is demonstrated.

2. Materials and Methods

Bacterial strains and plasmids

A. brasilense strains used were the wild type strain Sp245 and its mutant derivatives obtained by using transposon or nitrosoguanidine mutagenesis. Rif^R plasmidless strains that were used as recipients for T*n*5-Mob-labelled *Azospirillum* replicons are *Agrobacterium tumefaciens* strain GMI9023 and *Pseudomonas putida* strain AC340 (trp). Plasmid RP4-4 ($Ap^RTc^RKm^S$) harboured by *Escherichia coli* strain C600 was used as a helper for mobilization of DNA molecules tagged with T*n*5-Mob.

Bacterial matings, growth media and testing of Ant and IAA production by thin layer chromatography were done as described previously (Katzy et al., 1990).

N-methyl-N'-nitro-N-nitrosoguanidine mutagenesis was done by using established methods (Miller, 1972).

3. Results and Discussion

We examined Ant and IAA production by approximately 20 Sp245 nitrosoguanidine induced mutants which gained yellowish pigmentation of colonies. These mutants were divided into three classes on the basis of tryptophan(trp)-dependent Ant and IAA phenotype (Fig. 1): Ant^+IAA^+ (three of the clones tested), Ant^+IAA^- (four of the clones) and Ant^-IAA^- (three of the clones). All the other clones tested turned out to be Ant^-IAA^+ (as the wild type strain Sp245). Two of the Ant^+IAA^- mutants also gained ability to excrete a water-soluble brown pigment in the presence of phenylalanine (Pig_{phe}^+), so a regulatory gene of the aromatic amino acid metabolism is likely to be affected in them.

A. brasilense wild type strain Sp245 did not produce such a pigment or Ant on the media with corresponding supplements. The identification of the mutant strains as derivatives of Sp245 was confirmed by testing their cell morphology, plasmid content and acetylene reduction activity.

Since we are interested in identifying genetic loci involved in the production of IAA, we subjected *A. brasilense* strain Sp245 to random T*n*5-Mob mutagenesis. Of approximately 200 Sp245 T*n*5-Mob-induced mutants, 17 were isolated

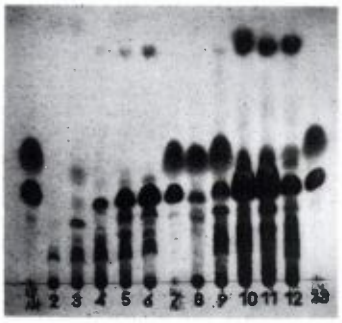

Figure 1. Chromatogram of ethyl acetate extracts of *A. brasilense* wild type strain Sp245 (Ant^-IAA^+) (lane 6) and its derivatives isolated after treatment with nitrosoguanidine: Ant^-IAA^- (lanes 2, 3 and 4), Ant^-IAA^+ (lane 5), Ant^+IAA^- (lanes 8 and 9) and Ant^+IAA^+ (lanes 10, 11 and 12). Equal amounts of extracts (2 μl) were added to Silufol UV-254 plates and chromatographed in the solvent chloroform:ethanol (16:1). IAA (lower spot, R_f 0, 33, 10 μg per lane) and Ant (upper spot, R_f 0, 48, 20 μg per lane) (Fluka) (lanes 1, 7 and 13) were used for control. [Spot on lane 9 having R_f similar to that of IAA differs from IAA by yellow colour after development of the chromatogram with Ehrlich reagent. The uppermost unidentified spot on lanes from 4 to 6 and from 10 to 12 have fluorescence under ultraviolet (254 nm) and a colour reaction with Ehrlich reagent resembling those of IAA].

which gained 5-fluoro-D, L-tryptophan resistance ($FTrp^R$) and excreted Ant and unaltered amount (30–32 μg/ml) of IAA (Ant^+IAA^+). FTrp resistance of $FTrp^RAnt^+IAA^+$ transposon-induced mutants seem to be due to an altered regulation of the enzyme anthranilate synthetase. Apparently, the enzyme was not sensitive to feedback inhibition by trp, since the mutants excreted anthranilate (up to 10 μg/ml) even in the presence of trp (200 μg/ml).

A T*n*5-Mob mutant with the $Ant^+IAA^-Pig_{phe}^+$ was chosen first on the basis of phenylalanine-dependent pigment production and then on its AntIAA phenotype using thin layer chromatography.

Transposon insertions in $FTrp^RAnt^+IAA^+$ mutants seem to be located in the chromosome since kanamycin resistance encoded by T*n*5-Mob was mobilized from them to *A. tumefaciens* or *P. putida* cells with a frequency below 10^{-8}. On the contrary, T*n*5-Mob insertion causing the $Ant^+IAA^-Pig_{phe}^+$ mutation is localized in an 85 MDa plasmid of strain Sp245 (Katzy et al., 1990).

All the mutants described in this study are not trp auxotrophs and have normal growth behaviour. Ant^+IAA^- mutants excreted Ant (up to 120 μg/ml) only in the presence of trp possibly due to the occurrence in strain Sp245

of an unusual pathway for trp breakdown. We speculate that coupling of the Ant^+ and IAA^- phenotype in a number of mutants may be due to dependence of IAA biosynthesis on the presence of unidentified products of trp breakdown throw anthranilate.

Further biochemical and genetic analyses of pathways for IAA biosynthesis in *Azospirillum* are necessary.

Acknowledgements

E.K. gratefully acknowledges the Organizing Committee of the *Azospirillum* V Workshop for the financial support.

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