

Isolation and characterization of *Azospirillum* mutants excreting high amounts of indoleacetic acid

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Accepted February 15, 1983

HARTMANN, A., M. SINGH, and W. KLINGMÜLLER. 1983. Isolation and characterization of *Azospirillum* mutants, excreting high amounts of indoleacetic acid. *Can. J. Microbiol.* **29**: 916–923.

Mutants of *Azospirillum brasilense* Sp Cd, resistant to 5-fluorotryptophan (FT) excreted 3-indoleacetic acid (IAA), i.e., auxin, producing up to 16 µg/mL which was 30 times greater than the wild-type level. Under conditions of nitrogen fixation, the mutants excreted IAA up to 1 µg/mL, 10 times more than the wild type. However, none of the FT-resistant mutants of *Azospirillum lipoferum* Sp RG 20a excreted high levels of IAA. This was probably due to differences in the tryptophan and IAA biosynthetic steps between *A. brasilense* and *A. lipoferum* strains. Some of the FT-resistant mutants of *A. brasilense* Sp Cd showed a reduced feedback inhibition of anthranilate synthetase by tryptophan. The increased synthesis of tryptophan could explain the observed excretion of tryptophan and related metabolites. In addition, the IAA-overproducing mutants excreted other amino acids, probably owing to pleiotropic effects of deregulated tryptophan biosynthesis on amino acid metabolism. The growth patterns of some mutants excreting large amounts of IAA were almost identical to those of the wild type.

HARTMANN, A., M. SINGH et W. KLINGMÜLLER. 1983. Isolation and characterization of *Azospirillum* mutants excreting high amounts of indoleacetic acid. *Can. J. Microbiol.* **29**: 916–923.

Des mutants d'*Azospirillum brasilense* Sp Cd, résistants au 5-fluorotryptophane (FT) excrètent l'acide indole 3-acétique (IAA), i.e., auxine; ils en produisent jusqu'à 16 µg/mL ce qui est 30 fois plus que la quantité produite par le type sauvage. Lors de la fixation de l'azote, les mutants excrètent jusqu'à 1 µg/mL de IAA, soit 10 fois plus que le type sauvage. Cependant, les mutants d'*Azospirillum lipoferum* Sp RG 20a qui sont FT-résistants n'excrètent pas de quantités élevées de IAA. Ceci est probablement dû à des différences dans les étapes de la biosynthèse de la tryptophane et de l'IAA chez les souches de *A. brasilense* et de *A. lipoferum*. Quelques uns des mutants FT-résistants de *A. brasilense* Sp Cd montrent une rétroinhibition réduite de l'anthranilate synthétase par le tryptophane. L'augmentation de la synthèse du tryptophane pourrait expliquer l'excrétion du tryptophane et de métabolites apparentés que nous avons observés. En plus, les mutants surproducteurs de IAA excrètent d'autres acides aminés; cela est probablement dû aux effets pléiotropiques d'une biosynthèse dérégularisée du tryptophane sur le métabolisme des acides aminés. Les profils de croissance de quelques-uns de ces mutants qui excrètent de grandes quantités de IAA sont presque identiques à ceux du type sauvage.

[Traduit par le journal]

Introduction

In the past few years, *Azospirillum* species have been isolated from the rhizosphere of grasses and other plants in tropical and temperate regions (Dommergues et al. 1973; Döbereiner and Day 1976; Haahtela et al. 1981; van Berkum and Bohlool 1980). Attempts to demonstrate any beneficial effects of *Azospirillum* on plant growth in greenhouse and field experiments have yielded negative as well as positive results (for review see van Berkum and Bohlool 1980). An increase in nitrogen content of inoculated plants (Rennie and Larson 1979; Kapulnik, Kigel et al. 1981; Kapulnik, Okon et al. 1981; O'Hara et al. 1981; Schank et al. 1981) and nitrogen flux (¹⁵N) from *Azospirillum* to the plant (Rennie 1980) could be shown. However, the stimulating effects of *Azospirillum* on plant growth do not seem to be confined to their role in N₂ fixation, but may involve the influence of phytohormones on root growth (Tien et al. 1979; Umali-Garcia et al. 1980).

Azospirillum brasilense strains were able to produce phytohormones such as cytokinins, gibberelins, and auxins. The effects of *Azospirillum* on root growth and plant weight of pearl millet resembled those due to the addition of a mixture of auxin, gibberelin, and kinetin (Tien et al. 1979; Reynders and Vlassak 1979). The excretion of auxin, i.e., 3-indoleacetic acid (IAA) in *A. brasilense* was dependent on the addition of tryptophan.

Considering tryptophan biosynthesis, the feedback inhibition of anthranilate synthetase by tryptophan is one important regulatory mechanism controlling the cellular tryptophan pool (Smith and Yanofsky 1962; Fantes et al. 1976). In general, three pathways are known for the conversion of tryptophan to IAA (Libbert et al. 1970; Clark 1974; Schneider and Wightman 1974; Comai and Kosuge 1980): (i) via indolepyruvic acid (transamination), (ii) via tryptamine (decarboxylation), and (iv) via indole acetamide (oxygenase).

In this paper we compare aspects of tryptophan and IAA biosynthesis in wild-type strains of *A. brasilense* and *A. lipoferum* and describe the isolation and

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properties of mutants which excrete IAA without an exogenous supply of tryptophan. Mutants excreting large amounts of IAA may help in understanding the role of auxin in the *Azospirillum*-plant association and may, perhaps, lead to an improved rhizocoenosis. A preliminary report of this work was presented at the *Azospirillum* workshop held at the University of Bayreuth (Hartmann 1982).

Materials and methods

Bacterial strains and growth conditions

Azospirillum brasilense Sp Cd, Sp 35, and Sp 7 (ATCC 29710, 29711, and 29145) and *Azospirillum lipoferum* Sp RG 20a, Sp Br 17, and Sp RG 6xx (ATCC 29708, 29709, and 29731) described by Tarrand et al. (1978) were obtained from the American Type Culture Collection. The typtophan auxotrophic *E. coli* K-12 mutants, T-3 and T-41, were kindly provided by Professor C. Yanofsky (Stanford University, CA, U.S.A.).

Azospirillum strains were grown aerobically at 30°C in tryptone-rich and minimal media as described by Albrecht and Okon (1980). To obtain N₂-fixing cultures, bacteria were grown in nitrogen-free liquid minimal medium with 0.05% oxygen in the gas phase. Growth was measured spectrophotometrically at 578 nm in an Eppendorf photometer.

Chemicals

Chorismic acid (barium salt), 5-fluorotryptophan, indoleacetic acid and other indole compounds, mercaptoethanol, pyridoxal-5-phosphate, Ehrlich and ninhydrin reagents were obtained from Sigma (St. Louis, U.S.A.). Xanthidrol was purchased from Fluka (Buchs, Switzerland), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) from EGA-Chemie (Steinheim, Federal Republic of Germany (FRG)), bovine serum albumin and ethylene diamine tetraacetic acid (Na₂-salt, EDTA) from Serva (Heidelberg, FRG). Silica gel thin-layer plates and all other chemicals of analytical grade were obtained from Merck (Darmstadt, FRG).

Mutagenesis and selection of 5-fluorotryptophan-resistant mutants

MNNG mutagenesis was performed according to Adelberg et al. (1965). Cells obtained from exponentially growing cultures treated with MNNG (100 µg/mL) for 30 min were washed twice and incubated overnight in tryptone broth. After washing, about 10⁷ cells were plated on minimal medium plates containing 5-fluorotryptophan (0.01 or 0.1 mg/mL). After 3 to 5 days about 10² resistant colonies appeared per plate.

Screening of tryptophan-excreting mutants

Those colonies were picked from fluorotryptophan-containing selective plates, which were surrounded by a growth area of FT-sensitive *Azospirillum*. This was taken as an indication for excretion of tryptophan metabolites by the central colony. These mutants were further screened in cross-feeding experiments using tryptophan auxotrophic *E. coli* K-12 mutants. The mutant T-41 (tryp-1) had an obligatory requirement for tryptophan, whereas the mutant T-3 (tryp-4) could grow on either anthranilate or indole (Smith and Yanofsky 1962). The growth of the mutant T-3 was much

better stimulated by tryptophan than the growth of T-41. To minimal medium plates seeded with about 10⁸ *E. coli* cells, 20 µL of overnight cultures of FT-resistant mutants were spotted. After incubation for 1 to 2 days at 30°C, growth stimulation zones were clearly visible.

Determinations of indole compounds and anthranilate

For rapid quantitative determination of indoleacetic acid, the colorimetric Salkowski reaction was performed (Tang and Bonner 1947). Extracellular indole compounds were measured using the xanthidrol assay of Dickman and Crockett (1956). Concentrations were calculated using L-tryptophan as the standard. While IAA gave one-third of absorption in the xanthidrol assay, as compared with L-tryptophan, the values for total indoles were corrected for the contribution of IAA using the data of the Salkowski reaction.

Anthranilic acid was first extracted from the acidified supernatants of cultures with ethylacetate and then reextracted in aqueous 0.1 M Tris buffer, pH 7.8 (Crawford and Gunsalus 1966). Concentrations were determined from measurements of the fluorescence at 320-nm excitation and a 390-nm emission wavelength in a Zeiss spectrofluorimeter PMQ 3.

Chromatographic analysis

Supernatant fluids (30 mL) of stationary phase cultures (48 h) were acidified with 1 M HCl to pH 2.8 and extracted twice with 10 mL ethylacetate. The combined extracts were concentrated to 1 mL by evaporation under vacuum at 37°C, and 0.1 mL was chromatographed on silica gel thin-layer plates with the solvent chloroform – ethylacetate – formic acid (5:4:1). The separated compounds were visualized by their fluorescence under ultraviolet (UV) and colour development with Ehrlich reagent.

Extracellular amino acids were identified using thin-layer chromatography and an amino acid analyser. Supernatant fluids (40 mL) of late exponential cultures (24 h) were evaporated to dryness at 37°C and dissolved in absolute ethanol (5 mL). The ethanol extracts were concentrated to 200 µL by air drying and 100 µL were chromatographed on silica gel thin-layer plates (solvent, *n*-butanol – acetic acid – water (8:2:2)). The separated components were identified after ninhydrin treatment. For amino acid analyses, the ethanol extract of evaporated culture medium (80 mL) was air dried, dissolved in 0.2 mL lithium citrate buffer (0.2 N, pH 2.2), and added to an amino acid analyser (Liquimate 3, kontron) employing a 4-mm φ column equipped with a high-efficiency cation exchange resin Durrum D1-6A. The analyser was run with a Dionex Pico-buffer System IV (Lithium, A-E) and standardized with authentic amino acids.

Enzyme assays

Anthranilate synthetase (chorismate pyruvate lyase; EC 4.1.3.27 (AS))

Cells were grown to late exponential phase (24 h) in minimal medium and washed with 0.1 M potassium phosphate buffer (pH 7.6), containing 5 mM magnesium chloride and 13 mM mercaptoethanol. They were concentrated 100-fold in the same buffer plus glutamine and were broken in a French press. The debris were removed by centrifugation at 4°C for 30 min at 8000 × *g*, and the supernatant was assayed immediately. AS was determined using the stop assay of Egan and Gibson (1970), as modified by Fantes et al. (1976). The

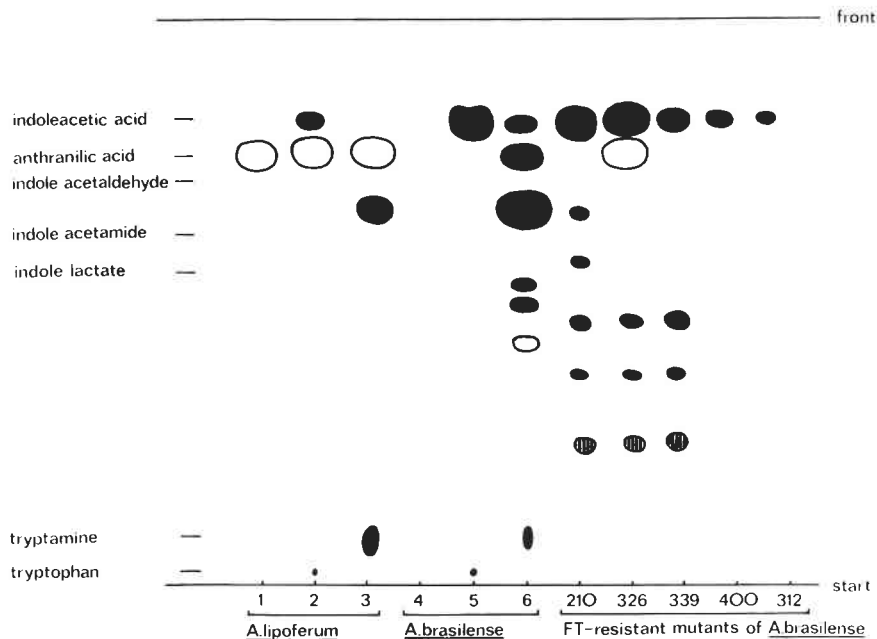


FIG. 1. Chromatograms of ethylacetate extracts of *A. lipoferum* Sp RG 20a, *A. brasilense* Sp Cd wild-type and FT-resistant mutants. Cultures were grown to stationary phase with 10 mM ammonium in minimal medium without further additions except tryptophan (2 and 5) and tryptamine (3 and 6), 0.1 mg/mL each. Equal amounts of extracts (0.1 mL) were added to silica gel plates, chromatographed in the solvent chloroform – ethylacetate – formic acid (5:4:1) and developed with Ehrlich reagent. ●, Blue or dark coloured; ○, yellow; ⊕, fluorescent.

reaction was started with 0.1 mL cell extract. After 30 min at 30°C the product, anthranilate, was extracted in 5 mL ethylacetate and measured fluorimetrically.

The activity of tryptophan synthetase (L-serine hydrolase; EC 4.2.1.20) was measured colorimetrically by following depletion of indole in the reaction with serine (Smith and Yanofsky 1962). Tryptophan monooxygenase (EC 1.13.12.3) was measured according to Comai and Kosuge (1980).

The *in vitro* determination of indoleacetic acid synthesis utilized stationary cultures, grown for 48 h in minimal medium. Cell extracts were prepared as described using the potassium phosphate buffer described above with 1 mM EDTA and 1 mM mercaptoethanol. Cell extract (0.1 mL) was added to 0.9 mL assay mixture to give in the final volume: 0.1 mmol potassium phosphate, pH 7.6; 1.0 μmol EDTA; 1.0 μmol mercaptoethanol; 10 μmol tryptophan; 10 μmol 2-oxoglutarate, and 0.05 μmol pyridoxal 5-phosphate. After 2 h at 37°C the assay was stopped by adding 0.1 mL 1 M HCl. The reaction products were extracted in 1 mL ethylacetate, and 0.1 mL of the extract was analysed for indole compounds using thin-layer chromatography. Protein concentrations were estimated by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

Results

IAA excretion in *A. brasilense* and *A. lipoferum* wild-type strains

As is shown in Fig. 1, indole lactate, indole acetaldehyde, and indole acetamide, which gave a

positive Salkowski reaction, did not occur in the culture supernatants, indicating that IAA was the main extracellular Salkowski-positive indole compound. Therefore, the Salkowski assay could be used for a quantitative determination of IAA in wild-type and mutant cultures.

IAA excretion of *A. lipoferum* strains was similar both under N_2 -fixing conditions and with 10 mM ammonium. IAA excretion by ammonium-grown cultures could be stimulated 10-fold by the addition of L-tryptophan, resulting in an extracellular level of about 2 μg/mL (Table 1). The excretion of IAA by strains of *A. brasilense* was increased with 10 mM ammonium and to very high levels by the addition of L-tryptophan (0.1 mg/mL).

Isolation of mutants excreting large amounts of IAA

After MNNG mutagenesis, colonies surrounded by a growth zone were isolated from FT-containing selective plates, assuming that these mutant colonies produced tryptophan, which competed with the toxic fluorotryptophan. Using *E. coli* K-12 T-3 as an indicator organism for tryptophan in a cross-feeding screening, 18 out of 54 mutants of *A. brasilense* Sp Cd showed growth stimulation zones of different diameters. In the case of *A. lipoferum* Sp RG 20a, no resistant colonies with growth zones on FT plates could be found. When some of these mutants were tested for cross-feeding on

TABLE 1. Comparison of the excretion of tryptophan metabolites in *Azospirillum lipoferum* and *Azospirillum brasilense* and in FT-resistant mutants of *A. brasilense* Sp Cd

Strains	Indoleacetic acid ($\mu\text{g}/\text{mL}$)			Total indoles ($\mu\text{m}/\text{mL}$)*		Anthranilate ($\mu\text{g}/\text{mL}$) with 10 mM NH_4^+
	Without NH_4^+	With 10 mM NH_4^+	With 10 mM NH_4^+ plus 0.1 mg/mL tryptophan	Without NH_4^+	With 10 mM NH_4^+	
<i>A. lipoferum</i>						
Sp RG 20a	0.2	0.2	2.5	0.6	1.4	7.2
Sp Br 17	0.3	0.2	2.3	0.7	1.6	0.2
Sp RG 6xx	0.3	0.1	1.6	0.8	2.0	7.3
<i>A. brasilense</i>						
Sp 35	0.1	0.7	40	0.8	2.4	0
Sp 7	0.2	1.0	50	0.8	4.5	0
Sp Cd	0.1	0.6	47	0.6	2.2	0
FT-326	0.8	16	44	2.3	19.0	5.0
FT-210	0.6	13	27	2.0	16.5	0
FT-400	0.4	10	48	2.3	14.2	0
FT-339	0.4	6.5	33	1.8	10.7	0
FT-335	0.3	3.9	46	1.6	6.7	0
FT-240	1.1	4.6	45	2.4	8.0	0
FT-312†	0.2	5.9	19	1.2	10.6	0

NOTE: Cultures with 10 mM ammonium were grown for 48 h to stationary phase (absorbance at 578 nm, 3.0), while cultures without NH_4^+ (nitrogen-fixing cultures) were grown for 2 weeks under microaerobic conditions (0.05% O_2) to stationary phase (absorbance of 1.5).

*Total indoles were determined with the xanthidol assay and corrected for the contribution of IAA as measured with the Salkowski reaction.

†Absorbance at 578 nm in stationary phase, 1.6.

indicator plates with the tryptophan auxotrophic *E. coli* mutant T-41, no growth stimulation was visible. The less sensitive indicator strain T-41 had to be used because of the anthranilate excretion by *A. lipoferum*, which cross-fed the indicator strain T-3.

Characterization of the IAA-overproducing mutants

Colorimetric determinations revealed that FT-resistant mutants of *A. lipoferum* showed no enhanced excretion of IAA and other indole compounds in minimal medium with 10 mM ammonium (data not shown). The results obtained for the FT-resistant mutants of *A. brasilense* with the highest level of IAA excretion are summarized in Table 1 (lower part). The excretion of total indoles in the mutants was increased up to fivefold relative to the wild-type level. IAA excretion produced up to 1 $\mu\text{g}/\text{mL}$ under N_2 -fixing conditions and up to 16 $\mu\text{g}/\text{mL}$ in cultures with 10 mM ammonium. In the presence of L-tryptophan (0.1 mg/mL), some of the mutants excreted IAA in similar quantities to the wild type, but in others (FT-312, FT-210, and FT-339) it was reduced (Table 1).

The overproducing mutants started to excrete indole compounds in the late exponential phase, while IAA was mainly excreted in the stationary phase (Fig. 2). The intermediary excretion of anthranilate up to 20 $\mu\text{g}/\text{mL}$ could only be found in the mutant FT-326. The growth curves of two mutants excreting large amounts of IAA

(FT-326 and FT-339) were similar to those of the wild type when grown in minimal medium with 10 mM ammonium. The growth rates of other mutants were reduced to different degrees. The mutant FT-312 showed a reduced growth yield in high ammonium medium. Under N_2 -fixing conditions, the growth of the mutants was similar to the wild type.

Chromatographic analyses of the excretion products

The excretion products were further analyzed by thin-layer chromatography (Fig. 1). While the wild type yielded no excretion products in ethylacetate extracts of the culture medium, the mutants excreted IAA, anthranilate (in FT-326), and other fluorescent or Ehrlich-positive indole compounds in minor amounts. None of these substances could be identified as indole lactate, indole acetaldehyde, indole acetamide, or indole pyruvate.

The excretion of ninhydrin-positive substances by the mutants is shown in Fig. 3. The ethanol extract of the wild-type culture was blank, but the mutants revealed fluorescent (FT-210 and FT-326), red-coloured (FT-339), and a variety of ninhydrin-positive spots. In most of the mutants the dominant amino acid was tryptophan, followed by very low levels of phenylalanine, methionine, glutamine, leucine, tryptamine, etc. This result was corroborated by amino acid analysis of the ethanol extracts. While tryptophan was excreted up to

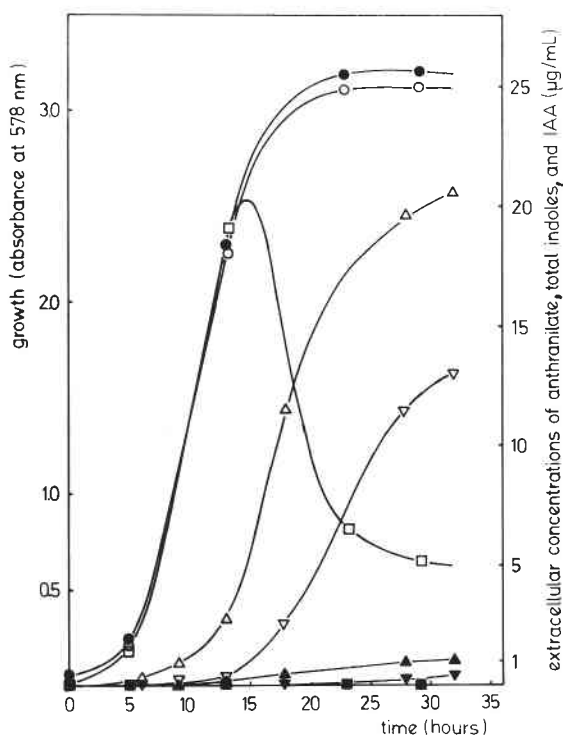


FIG. 2. Growth (●, ○) and excretion of anthranilate (■, □), total indole compounds (▲, △), and indoleacetic acid (▼ ▽) in *A. brasilense* Sp Cd wild type (closed symbols) and the FT-resistant mutant FT-326 (open symbols). Colorimetric and fluorometric measurements were performed in the supernatants of cultures growing in minimal medium with 10 mM ammonium. The concentration of total indoles was calculated from the values of the xanthidol assay and corrected for the contribution of IAA.

0.5 µg/mL, the other amino acids were found at concentrations of 6 ng/mL and lower.

Tryptophan and IAA biosynthesis in wild-type and mutant strains

In *A. lipoferum* Sp RG 20a and *A. brasilense* Sp Cd, the anthranilate synthetase (AS) was highly sensitive to feedback inhibition by tryptophan. With 0.1 mM tryptophan, AS activity was inhibited more than 20 times (Fig. 4). In some IAA-overproducing mutants, the feedback inhibition of AS was reduced 5–10 times; in one case (FT-312), the feedback control of AS was almost normal. The specific activity of AS (0.15 nmol·min⁻¹·mg protein⁻¹) was found slightly reduced in the mutants possibly owing to the presence of tryptophan in the extracts. The specific activity of tryptophan synthetase was similar in *A. lipoferum* Sp RG 20a (7 nmol·min⁻¹·mg protein⁻¹) and in *A. brasilense* Sp Cd wild type and mutants (4 nmol·min⁻¹·mg protein⁻¹). Interestingly two *A. lipoferum* strains excreted anthranilate up to a concentration of 7 µg/mL when

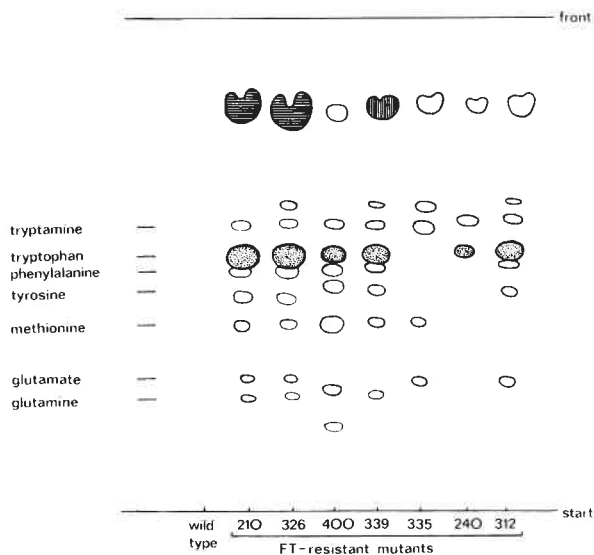


FIG. 3. Chromatograms of ethanol extracts of evaporated culture supernatants of *A. brasilense* Sp Cd wild-type and FT-resistant mutants. Cultures were grown to late exponential phase in minimal medium. Equal amounts of extracts (0.1 mL) were added to silica gel plates, chromatographed in the solvent butanol – acetic acid – water (8:2:2), and developed with ninhydrin reagent. ⊕, Brown; ○, violet; ⊗, fluorescent; ⊙, red.

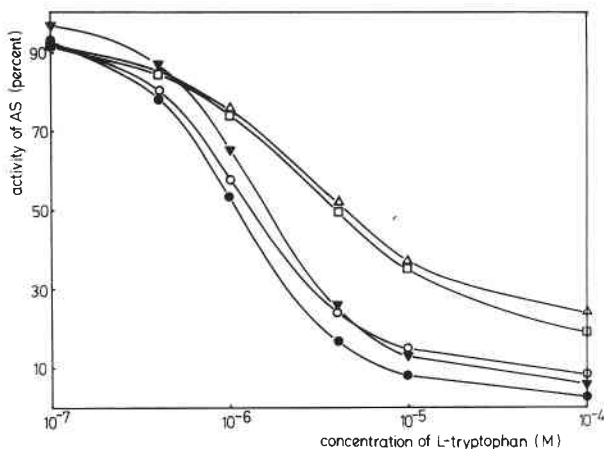


FIG. 4. Feedback inhibition of anthranilate synthetase (AS) by tryptophan in *A. lipoferum* Sp RG 20a (▼), *A. brasilense* Sp Cd wild type (●), and the FT-resistant mutants 326 (□), 400 (△), and 312 (○). Activities are given in percent activity of AS without tryptophan in the assay.

grown with 10 mM ammonium, while no *A. brasilense* strain showed this phenomenon (Table 1). This probably reflected differences in the tryptophan biosynthetic enzyme activities in the two *Azospirillum* species.

Azospirillum brasilense Sp Cd, but not *A. lipoferum* Sp RG 20a, could convert tryptamine to IAA, when it

was added to minimal medium cultures. Besides other compounds IAA was identified chromatographically in small amounts (Fig. 1). Both species could not use indole acetamide to produce IAA. A tryptophanoxxygenase was not detectable in crude extracts. Crude extracts of *A. brasilense* Sp Cd and *A. lipoferum* Sp RG 20a could convert tryptophan to IAA when 2-oxoglutarate was present (transamination pathway). IAA was identified by thin-layer chromatography of ethylacetate extracts (data not shown).

Discussion

The results obtained with wild-type strains of *A. brasilense* and *A. lipoferum* indicated that the two *Azospirillum* species differed with respect to tryptophan and IAA biosynthesis in the following respects (i) While *A. brasilense* produced high quantities of IAA in the presence of tryptophan, in *A. lipoferum* IAA was barely detectable; (ii) mutants excreting IAA in high quantities could be readily isolated in *A. brasilense* Sp Cd, whereas no similar mutants could be obtained in *A. lipoferum* Sp RG 20a; (iii) *A. lipoferum* excreted high amounts of anthranilate under high nitrogen supply, but no anthranilate was detected in the case of *A. brasilense*; and (iv) the tryptamine-dependent pathway of IAA biosynthesis was present only in *A. brasilense*. In both species a 2-oxoglutarate-dependent transamination of tryptophan may be involved in the first step of IAA biosynthesis. The pathway via indole acetamide was not detectable in *Azospirillum*.

The resistance of an organism to an amino acid analogue may be due to several mechanisms. The uptake of the toxic antimetabolite could be blocked, the protein synthesis could select out the analogue, or it could be degraded. Alternatively, an altered regulation could lead to an increased biosynthesis of the corresponding amino acid. The overproduction of the amino acid in the cell, and often its excretion into the medium could compete with the toxic analogue. Our screening procedure selected for these overproducing mutants out of the bulk of resistant mutants. The isolated FT-resistant mutants excreted tryptophan and indole compounds including IAA. The IAA overproduction of the mutants was not limited to the addition of tryptophan or conditions of high nitrogen supply as in the wild type, but occurred to a smaller extent also in nitrogen-limiting conditions. In the mutants FT-326, FT-339, and FT-400, the enzyme anthranilate synthetase was less sensitive to feedback inhibition by tryptophan than in the wild type, resulting in the excretion of tryptophan into the culture medium. This appeared to be the mechanism of FT resistance in these mutants. Similar results have been reported for FT-resistant mutants of *Hansenula polymorpha* (Denenu and Demain 1981) and α -methyltryptophan-resistant mutants of *Pseudomonas savas-*

tanoi (Smidt and Kosuge 1978). In the mutant FT-312 another mechanism of IAA overproduction operated since the feedback control of AS was normal.

The excretion of anthranilate in some *A. lipoferum* wild-type strains indicated a defect in the conversion of anthranilate to tryptophan, except tryptophan synthetase. This and the low activity of IAA biosynthesis from tryptophan may be the reasons for the failure to isolate IAA-overproducing mutants of *A. lipoferum* Sp RG 20a. In contrast, the occurrence of mutants with a weak feedback control of AS as overproducing mutants in *A. brasilense* Sp Cd indicated that the regulation of AS was the most important regulatory step. The fact that tryptophan synthetase was unaltered in these mutants indicated no regulatory function for this enzyme in *Azospirillum* as in *Rhizobium leguminosarum* (Holmgren and Crawford 1982).

Interestingly, some IAA-overproducing mutants also excreted a variety of unidentified indole compounds. Probably the increased tryptophan production led to a general increase in the synthesis of indole compounds. Secondary effects of the deregulation of AS may explain the observed differences in the composition of the excreted substances. Small quantities of a variety of amino acids were also detected in the culture supernatants of the mutants. This was possibly due to the pleiotropic effect of deregulation of glutamine-dependent AS and an increased tryptophan pool. It is further supported by the observation that some revertants of glutamate synthase defective mutants in *A. brasilense* Sp 6 become FT resistant (Barberio et al. 1982). Another explanation could be that the overproduction of tryptophan might influence the general amino acid transport systems leading to the efflux of various amino acids.

Auxin production has been described in different microorganisms (Clark 1974; Barea and Brown 1974; Scott 1972). IAA production is responsible for the induction of stem galls in oleander by *Pseudomonas savastanoi* (Smidt and Kosuge 1978). Interestingly, in *Agrobacterium tumefaciens* (Liu et al. 1982) and in *P. savastanoi* (Comai and Kosuge 1980), the genes for IAA biosynthesis are located on large plasmids. Libbert et al. (1969) found IAA at markedly reduced levels in plant roots growing without microorganisms. A tremendous stimulation in the growth of lateral roots and root hairs after inoculation with *A. brasilense* was attributed to an effect of IAA and other hormones produced by the bacteria (Umali-Garcia et al. 1980). The reported differences in the tryptophan and IAA metabolic pathways may be involved in the *Azospirillum* - plant root association. We speculate that the different IAA production may be one of the factors determining the specificities of the two *Azospirillum* species for different plants (Baldani and Döbereiner

1980). The FT-resistant mutants may prove useful in assessing the role of auxin and nitrogen fixation in the stimulation of plant growth. It may well be that the property of FT-resistant mutants to excrete various amino acids under particular conditions may also have an additional influence on plant growth. Our initial experiments with axenic cultures of *Setaria italica* growing on vermiculite under nitrogen-free conditions showed increased plant height and dry weight with the IAA-excreting mutant FT-400 as compared with the wild-type strain. However, further experiments are needed utilizing other mutants and plant species to test their potential for practical application.

Acknowledgements

The skilful technical assistance of Mrs. M. Ohlraun, Miss G. Siegl, and Mr. M. Stanzel is gratefully acknowledged. We thank Dr. I. Rosnitschek, Department of Plant Physiology, University of Bayreuth, for performing the amino acid analysis and Dr. D. M. Whale for reading the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft.

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