Symbiosis, 32 (2002) 39–54
Balaban, Philadelphia/Rehovot

Production of Indole-3-Acetic Acid by Different Strains of *Azospirillum* and *Herbaspirillum* spp.

THARWAT EL-SAYED EL-DESOKY RADWAN[1], ZEINAT KAMEL MOHAMED[1], and VERONICA MASSENA REIS[2*]
[1]*Faculty of Science, Cairo University, Cairo, Egypt;*
[2]*Embrapa Agrobiology, km 47 Antiga Rio-SP, Seropédica, Rio de Janeiro, CP 74505, Brazil, E-mail. veronica@cnpab.embrapa.br*

Received July 23, 2001; Accepted September 30, 2001

Abstract

Forty-six different strains of the genera *Azospirillum* and *Herbaspirillum* were screened for their ability to produce indole compounds using colorimetric assay. All strains produced minor amounts of indole compounds in liquid, N-supplemented NFb-medium. Adding tryptophan to growth media, indole compounds production by the organisms was greatly increased. *Azospirillum* isolates produced higher amounts than *Herbaspirillum* isolates. Using a colorimetric assay the production of indole compounds in the presence of tryptophan strains ranged from 19.1 μM in *A. amazonense* strain Y6 to 378.7 μM by *A. brasilense* strain Cd, which produced the higher level among all strains tested. The indole compounds production by *Herbaspirillum seropedicae* ranged from 27.7 μM by strain Z 24 to 128.8 μM by strain Hawaii. Among *H. rubrisubalbicans* strains ranged from 43 μM by strain M1 to 163.3 μM by strain 198. Finally, comparing *Herbaspirillum*-like isolates, IAA production ranged from 31.2 μM by strain 5 isolated from rice to 210.5 μM by strain E 49 recovered from Oil Palm. These results were confirmed by both HPLC and bioassay techniques as compared to authentic IAA. Comparing four media for IAA production, bacterial strains had a different behaviour in semi-solid and liquid culture. NFb and JNFb semi-solid media were favourable for *Azospirillum* strains Cd and Br 17 but Potato and Dygs media strongly stimulated indole compounds production by *Herbaspirillum* in liquid cultures.

*The author to whom correspondence should be sent.

0334-5114/2002/$05.50

Keywords: Indole-3-acetic acid, diazotrophic bacteria, *Azospirillum*, *Herbaspirillum*, auxin

Abbreviations
IAA – Indole-3-acetic acid; O.D. – Optical density; Trp – Tryptophan

1. Introduction

Azospirillum has been isolated from the roots from numerous wild and cultivated grasses, cereals and legumes and from tropical, subtropical, and temperate soils world-wide. The importance of *Azospirillum* is due to its capacity to fix atmospheric nitrogen in addition to its ability to synthesise several phytohormones, enzymes, siderophores, polysaccharides and other substances, that can act to enhance various stages of plant growth. Okon and Labandera-Gonzalez (1994) concluded that *Azospirillum* spp. are capable of promoting the yield of agriculturally-important crops in different soils and climatic regions. Various strains of *A. brasilense* and *A. lipoferum* have been used to inoculate cultivars of different species of plants. The data indicate 60–70% occurrence of success with statistically significant increases in yield of the order of 5–30%.

Herbaspirillum seropedicae was originally isolated from rhizosphere soil, washed roots and surface sterilised roots of maize, sorghum, and rice (Baldani et al., 1986), but not from uncropped soil (Baldani et al., 1992). *H. seropedicae* was originally thought to be a new species of *Azospirillum* by its similar growth characteristics in semi-solid, N-free media. However, further analysis showed that it was a new genus (Baldani et al., 1986). Until now, this bacterium has been isolated from 13 members of the Gramineae family, normally colonising roots but it has also been found in the aerial parts of rice and maize as well as in stems of sugar cane, but not in leaves (Olivares et al., 1996). In 1991, Gillis et al. reclassified *Pseudomonas rubrisubalbicans*, which causes the mottled stripe disease in sensitive sugar cane varieties, as *H. rubrisubalbicans*. With this new reclassification, another group was identified as "species 3" which includes only non-diazotrophic bacteria and is mainly isolated from clinical material, such as wounds and faeces, although a few strains have been isolated from sugar cane, sorghum and maize. Recently a third group of diazotiophic strains which do not hybridise with the probes designed for identification of the two species described above, were isolated from several gramineous energy-plants and were characterised as a new species *Herbaspirillum frisingense* (Kirchhof et al., 2001). The strains tested in this paper, but not assigned to one of the known species, are named as *Herbaspirillum*-like strains.

Bacterial phytohormone production has been repeatedly postulated to be responsible for the plant growth stimulation caused by *Azospirillum* inoculation. The production of phytohormones seems to play an important role in *Azospirillum*-plant interactions. Several authors have published the amount of IAA produced by some strains of *Azospirillum*. Hartmann et al. (1983) found the IAA-production in *A. brasilense* to occur in late-exponential growth phase in pure cultures and *A. brasilense* wild type strains produced 227.4 to 284.4 µM in tryptophan supplemented minimal medium and the IAA-production of *A. lipoferum* was as low as 9.1 to 14.2 µM under the same conditions. Mascarua-Esparza et al. (1988) found that *A. brasilense* isolated from cactus produced IAA at rates of 207.6 to 426.6 µM; while *A. lipoferum* produced 39.7 to 99.5 µM. Fallik et al. (1989) identified IAA in *Azospirillum* tryptophan-free medium at rates of 0.18 to 0.23 µM and Iosipenko and Ignatov (1995) stated that the culture fluid of *A. brasilense* Sp 7 supplemented with 0.5 g/l NH_4Cl was found to contain 85.3 µM of IAA. They detected traces of cytokinin-like substances in root exudate medium in a period of only 4 to 6 days of culture growth.

In 1998, Bastián et al. detected IAA, GA_1 and GA_3 in cultures of *Herbaspirillum seropedicae*, grown in chemically-defined medium and since then no other publication in relation to phytohormone production by *Herbaspirillum* has been published.

The aim of this study was to investigate the production of IAA by several strains of diazotrophic bacteria using different methods: colorimetric, bioassay and HPLC and also to compare the effect of common media on the growth of these organisms to increase the production of IAA.

2. Material and Methods

Experimental organisms

As shown in Table 1, *Azospirillum* spp. and *Herbaspirillum* spp. were obtained from the bacterial collection of Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), National Centre of Agrobiology localised in Seropédica, Rio de Janeiro, Brazil. In addition, several strains isolated from rice and two strains isolated from Oil Palm (*Elaeis guineensis*) were tested which belong to the genus *Herbaspirillum*, but did not fit into the known species.

As reference bacteria, the following strains were used: *A. brasilense* Cd (ATCC 29729), *A. lipoferum* Br 17 (ATCC 29709), *A. amazonense* Y 6 (ATCC 35121), *Herbaspirillum seropedicae* Z 67 (ATCC 35892), *H. rubrisubalbicans* M 4 (LMG 2286).

Table 1. List of the strains used

No.	Bacterium species	Symbol	BR no.	Origin of the isolate
	A. brasilense			
1		Sp 109	11023	Rice – surface sterilised rots
2	ATCC 29145	Sp 7	11001	Digitaria – rhizosphere
3	ATCC 29729	Cd	11002	*Cynodon dactilon* – Israel
4		Sp 245	11005	Wheat – surface sterilised roots
5		Sp 245 (Nif⁻)	11009	Negative nitrogen fixer
	A. lipoferum			
6	ATCC 29707	Sp 59	11080	Wheat – roots
7	ATCC 29709	Br 17	11084	Maize – surface sterilised roots
8	ATCC 29708	RG 20 a	11115	
	A. amazonense			
9	ATCC 35119	Y 1	11142	*Digitaria decumbens* – surface sterilised roots
10	ATCC 35120	Y 2	11140	Weed grass – surface sterilised roots
11	ATCC 35121	Y 6	11141	*Pennisetum purpureum* – surface sterilised roots
12	*A. irakense* *H. seropedicae*			
13		R L 1		Sugar cane – washed roots
14		Hawaii		Sugar cane – roots – Hawaii
15		HCC 100	11387	Sugar cane – stem
16		HRC 55		Sugar cane – roots
17		HRC 61		Sugar cane – roots
18		HRC 80	11198	Sugar cane – roots
19		HPD 5		Weed plant – roots
20		ZAE 24		Rice – surface sterilised roots
21		ZAE 74		Rice – surface sterilised roots
22	ATCC 35892	Z 67	11175	Rice – surface sterilised roots
23		ZAE 94		Rice – surface sterilised roots
24		ZME 152	11178	Maize – surface sterilised roots
25		ZME 176	11179	Maize – surface sterilised roots
	H. rubrisubalbicans			
26	LMG 1278	M 1	11191	Sugar cane – leaves – Mauritius
27	ATCC 19308	M 4	11192	Sugar cane – leaves – USA
28	LMG 6415	M 5	11193	Sugar cane – leaves – Reunion
29		HCC 101	11510	Sugar cane – stems – Brazil
30		HCC 103	11504	Sugar cane – leaves – Brazil
31		HRC 51		Sugar cane – roots – Brazil
32		B 4362		Sugar cane – leaves – Brazil
33		IBSBF 198		Sugar cane – leaves – Mauritius

Table 1. Continued

No.	Bacterium species	Symbol	BR no.	Origin of the isolate
	Herbaspirillum spp.			
34		5 rice		Rice – Embrapa Agrobiology
35		8 rice		Rice – Embrapa Agrobiology
36		10 rice		Rice – Embrapa Agrobiology
37		14 rice		Rice – Embrapa Agrobiology
38		22 rice		Rice – Embrapa Agrobiology
39		26 rice		Rice – Embrapa Agrobiology
40		27 rice		Rice – Embrapa Agrobiology
41		34 rice		Rice – Embrapa Agrobiology
42		36 rice		Rice – Embrapa Agrobiology
43		42 rice		Rice – Embrapa Agrobiology
44		29 sugar cane		Sugar cane – Embrapa Agrobiology
45		E 19		Oil palm – roots – Brazil
46		E 49		Oil palm – roots – Brazil

BR – Embrapa Agrobiology Type Culture; ATCC – American Type Culture Collection, Rockville; LMG – University Gent, Belgium.

Preparation of bacterial inoculum

A loopful from a pure colony of the bacteria was inoculated to 50 ml of Dygs medium (Rodrigues Neto et al., 1986) in a 125 ml Erlenmeyer flask and incubated for 48 h at 35°C with agitation. This medium was used for the multiplication of the bacteria and has the following composition (g/l): Glucose, 2; peptone, 1.5; yeast extract, 2; KH_2PO_4, 0.5; $MgSO_4.7H_2O$, 0.5; glutamic acid, 1.5; agar-agar for solid medium, 18; distilled water, 1000 ml and the pH was adjusted to 7. From this culture 2 ml were taken directly without washing as inoculum.

Culture conditions for testing IAA-production

The liquid NFB medium (Döbereiner, 1995) was used in 50 ml Erlenmeyer flasks. Washed cells were obtained by centrifugation at 10,000 g for 15 min at 4°C and washed 3 times (same conditions) in sterile 0.06 M phosphate buffer (pH 6.8) and resuspended in 30 ml of the buffer. The inoculated culture media were incubated on a shaker at 125 rpm at 35°C for *A. brasilense*, *A. lipoferum* and all *Herbaspirillum* strains and 30°C for *A. irakense*.

In addition, JNFb-, DYGS- (Rodrigues Neto et al., 1986) and potato medium were used alternatively. JNFb medium (Döbereiner, 1995) used for isolation and growth of *Herbaspirillum* spp. Yeast extract, biotin and bromothymol blue were omitted to avoid any colour interaction during the IAA-assay.

Assays for IAA were made at 48 hours for liquid media and after 7 days for semi-solid culture in three replicate cultures. Bacterial growth was measured by light absorption at 492 nm (O.D. 492) using a Perkin Elmer II spectrophotometer.

Determination of IAA in the culture medium

Colorimetric method

IAA was estimated in supernatants of the bacterial cultures originating from the liquid cultures which were prepared as follows: the bacterial culture was centrifuged at 15,000 g for 15 min at 4°C. Measurements were performed in accordance to the method described by Gordon and Weber (1951), and modified by Minamisawa et al. (1992). 1 ml of the bacterial filtrate was mixed with 2 ml of $FeCl_3$-$HClO_3$ reagent (0.01 M $FeCl_3$ in 35% $HClO_3$) where the presence of indole compounds produced a pink colour. The optical density was measured at 530 nm after 25 min of incubation using the Perkin Elmer Spectrophotometer II. The unknown concentration of IAA could be estimated from a previously prepared standard curve using 25, 50, 100, 150, 200, 300 µM of authentic IAA (Sigma).

Bioassay method

According to the method described by Zimmer et al. (1988), wheat grains (*Triticum astivum* cv. BEM 16) were placed in a Petri dish and watered with 10 ml H_2O for 24 h at 4°C. The grains were then transferred to 2 layers of Whatman filter paper moistened with 6 ml of H_2O and incubated for 72 h at 26°C in the dark for germination. Twelve wheat root segments of 5 mm length were used, cut from the zone 2 mm to 7 mm from the tip and placed in a Petri dish containing one layer of filter paper moistened with 2.5 ml of the solution to be assayed for auxin content. The Petri dishes were incubated for 24 h at 26°C in the dark, and the wet weight of the root segments was determined afterwards from a standard curve prepared previously using IAA (Sigma). The test allowed IAA to be measured between 10^{-12} to 10^{-5} mg/ml of IAA.

High performance liquid chromatography method (HPLC)

IAA was assayed using the HPLC method described by Tien et al. (1979) which includes two steps as follows: (1) Extraction process: Bacterial cultures (50 ml), were centrifuged at 15,000 g for 15 min at 4°C, the supernatant fluid was acidified with 1 N HCl to pH 2.8–3.0. Aliquots of 10 ml were extracted with an

equal volume of ethyl acetate using a separating funnel, the lower aqueous layer was discarded, the upper organic fraction was filtered through 0.45 μm membrane filters, evaporated under vacuum. The residue was dissolved in 2 ml ethanol and chromatographed by high performance liquid chromatography (HPLC). (2) Injection process: The chromatograms of HPLC were produced by injecting 5–10 μl of the filtered extract onto a 10-μm reverse phase column in a LC 10 AD Schimazu Liquid Chromatography using a C_{18} column of 4 mm diameter and 30 cm length. The solvent system was 50% methanol in water, flow rate 1.5 ml/min, and the operating pressure was 1.600 lb/in^2 (108 atm). Detection was at 280 nm. Retention times for peaks were compared to those of authentic IAA standards. Quantification was made by area integration through the Schimazu Data Module microprocessor. Measurements for IAA were made at 48 hours for liquid media, when the cultures have reached the early stationary phase, and after 7 days for semi-solid cultures in three replicate vials.

3. Results and Discussion

Forty-six different strains were screened for their ability to fix atmospheric nitrogen and to produce indole compounds. As a result of the screening experiments, all strains were shown to produce indole compounds, but at different levels. Between genera, *Azospirillum* had the highest values (Table 2). The highest producer strain was *A. brasilense* Cd (378.7 μM in the presence of tryptophan). This is a well-studied isolate of *A. brasilense* and the strain which has been used most in physiology and inoculation studies. The three isolates of *A. amazonense* produced lower amounts similar to those produced by *Herbaspirillum* strains (Table 2). *A. irakense* showed an intermediate level of indole compounds production. Amongst *Herbaspirillum seropedicae* strains tested, the isolate Hawaii produced the highest value in the presence of tryptophan (128 μM) and the other ranged from 27.7 (strain Z 24) to 78.5 μM (strain HRC 80). The type strain Z 67 produced 51.3% less than Hawaii. Amongst the isolates of *Herbaspirillum rubrisubalbicans*, strains 198 and B 4362 produced 163.3 μM and 160.0 μM, respectively, in the presence of tryptophan (Table 2). The strains of *Herbaspirillum* genera that do not fit into the described species, strain E 49, isolated from Oil Palm, produced 210.5 μM in the presence of tryptophan (Table 2). All strains tested reduced drastically the production of indole compounds in the absence of tryptophan but still produced detectable concentrations of these substances.

It is apparent from the results, that all organisms could produce indole compounds without tryptophan addition to the culture medium when unwashed

Table 2. Indole compounds production by different *Azospirillum* strains grown in liquid NFb-medium in presence or absence of tryptophan (0.1 g l^{-1}) using unwashed inoculum. Colorimetric measurements of IAA were performed 48 hours after inoculation. Media of three replicates.

Strains	With tryptophan		Without tryptophan	
	IAA (µM)	O. D. 492 nm	IAA (µM)	O. D. 492 nm
Azospirillum spp.				
Sp 109	246.8	1.46	31.0	1.42
Sp 7	266.7	1.37	5.8	1.12
Cd	378.7	1.48	15.6	1.29
Sp 245	221.4	1.47	9.8	1.29
245 nif⁻	26.5	1.59	7.0	1.56
Br 17	229.2	1.46	25.8	1.24
Y 1	50.3	1.69	29.3	1.50
Y 2	32.0	1.55	26.5	1.41
Y 6	19.1	1.54	8.5	1.43
A. irakense	178.1	1.58	14.2	1.22
LSD 1%	10.26	0.37	2.1	0.30
Herbaspirillum seropedicae				
RL 1	76.1	1.56	28.0	1.39
Hawaii	128.8	1.40	29.0	1.32
HCC 100	74.5	1.61	11.3	1.41
HRC 55	87.0	1.61	29.3	1.36
HRC 61	62.8	1.61	17.9	1.37
HRC 80	78.5	1.61	25.4	0.78
HPD 5	74.5	1.59	23.4	1.36
ZAE 24	27.7	1.08	14.8	0.21
ZAE 74	54.7	1.60	23.8	1.44
Z 67	62.8	1.62	23.0	1.46
ZAE 94	46.3	1.61	12.3	0.72
ZME 152	70.6	1.61	22.2	1.31
ZME 176	74.5	1.39	14.4	1.37
LSD 1%	7.5	0.37	3.6	0.31
Herbaspirillum rubrisubalbicans				
M 1	43.0	1.31	15.6	0.86
M 4	71.1	1.61	18.7	1.41
M 5	112.0	1.50	20.0	1.13
HCC 101	51.0	1.57	29.3	1.44
HCC 103	117.5	1.38	17.1	1.08
HRC 51	119.1	1.61	25.0	1.22
B 4362	160.0	1.56	19.1	1.21
IBSBF 198	163.3	1.35	8.2	0.94
LSD 1%	7.3	0.35	2.1	0.32

Table 2. Continued.

Strains	With tryptophan IAA (μM)	With tryptophan O. D. 492 nm	Without tryptophan IAA (μM)	Without tryptophan O. D. 492 nm
Herbaspirillum spp.				
5 rice	31.2	1.30	19.1	1.12
8 rice	78.5	1.61	23.1	0.97
10 rice	46.3	1.40	16.5	1.17
14 rice	39.5	1.47	11.7	0.90
22 rice	54.7	1.53	16.0	1.34
26 rice	102.0	1.45	35.0	0.94
27 rice	54.6	1.53	16.8	0.72
34 rice	129.2	1.54	31.2	1.21
36 rice	78.5	1.59	24.2	1.47
42 rice	50.7	1.60	24.2	1.51
29 sugar cane	137.0	1.44	19.5	1.19
E 19	54.6	1.61	22.6	1.40
E 49	210.5	0.67	26.0	0.30
LSD 1%	8.0	0.37	2.5	0.30

inoculum was used (Table 2). For example, strain Cd, grown without tryptophan but using unwashed cells produced 15.6 μM of IAA and 1.2 μM using washed cells. This could be caused by the large amount of the culture medium used as inoculum (2 ml) and the nutritional content of the inoculum was high as it was taken directly from the Dygs medium which is nutrient rich, and almost certainly contains compounds involved in IAA biosynthesis pathway(s). Even in the presence of tryptophan but using unwashed cells, all strains produced higher amounts of indole compounds indicating that other substances increment IAA production other than tryptophan.

Katzy et al. (1995) could not detect IAA production when *A. brasilense* was grown without tryptophan. They found high concentrations of IAA after addition of tryptophan to the bacterial medium. Supplementation of trepton instead of tryptophan reduced IAA production by a factor of three. They also found that *A. brasilense* mutants produced significantly less (50%) than the wild type strain.

The production of IAA was dependent on the tryptophan addition. These results are in accordance with the literature for *Azospirillum* and are also shown to be true for *Herbaspirillum* strains in this report. Many investigators stated that tryptophan is essential for the production of IAA in *Azospirillum* species (Baca et al., 1994; Bar and Okon, 1992; De-Francesco et al., 1985;

Hartmann et al., 1983; Lebuhn and Hartmann, 1994; Prinsen et al., 1993; Ruckdaschel et al., 1988 and 1991; Ruckdaschel and Klingmüller, 1992; Tien et al., 1979; Zimmer et al., 1988).

HPLC quantification of IAA-production confirmed the results obtained using the colorimetric method (Table 3). Bastián et al. (1998) showed that *H. seropedicae* strain Z 67 produced 0.047 µM of IAA in NFb medium assayed by GC-SIM system for HPLC analysis and that only a fraction corresponded to the free IAA. Horemans et al. (1986) measured IAA production of *A. brasilense* R07 by HPLC-fluorimetry and observed that in the presence of tryptophan, this strain produced 290.1 µM and 2.45 µM in absence of this precursor. Also, *A. amazonense* (strain Y 1) produced lower amounts of IAA. Fuentes-Ramírez et al. (1993) reported that cultures of *Gluconacetobacter diazotrophicus* (formerly *Acetobacter*) produced amounts at rates of 108.1 to 369.7 µM of IAA using colorimetric Salkowsky assay. On the other hand, estimates obtained from HPLC analyses ranged from 0.79 to 13.76 µM of IAA in culture medium and these amounts were in accordance of those obtained by Crozier et al. (1988) for the genus *Azospirillum*.

Table 3. Bioassay of IAA-concentration in tryptophan-supplemented NFb liquid medium inoculated with diazotrophic bacteria compared to that of authentic IAA and HPLC analysis. For the bioassay the fresh weight of 12 wheat root segments were used. Media of three replicates.

Bioassay with IAA		Bioassay with diazotrophic bacteria			HPLC
IAA (µM)	Fresh weight (mg)	Bacterial strains	Fresh weight (mg)	Bacterial IAA (µM)	IAA (µM)
0	30	Control	30	0.00	0.00
25	55	Sp 109	122	207.36	205.23
50	74	Cd	154	331.10	325.36
100	93	Br 17	129	229.40	224.28
150	107	Y1	61	37.49	35.19
200	123	Z 74	73	43.74	41.59
250	139	Z 67	79	58.18	56.18
300	154	M 4	81	67.48	65.94
400	171	B 4362	101	128.09	125.31
500	186	34 rice	98	110.12	108.46
600	89	29 sugar cane	97	109.73	106.89
LSD 1%	6		8	10.608	

Table 4. Effect of different culture media on indole compounds production (μM) using liquid culture shacken at 125 g during 48 hours and semi-solid media. Nitrogenase activity was measured by Acetylene Reduction Assay (ARA, nmoles of ethylene/hour/vial) after 7 days in semi-solid condition. Media of three replicates.

Bacterial strain	Culture media	Liquid media IAA (μM)	Semi-solid IAA (μM)	ARA
Cd	NFb	340.15	550.25	167.14
	JNFb	308.17	521.24	120.00
	Potato	92.33	210.91	0.28
	Dygs	88.69	190.73	0.00
Br 17	NFb	233.12	496.12	199.00
	JNFb	221.14	483.51	104.00
	Potato	138.07	129.45	0.15
	Dygs	82.36	110.91	0.00
Z 67	NFb	77.3	35.84	85.25
	JNFb	118.71	40.31	105.12
	Potato	151.83	76.83	0.00
	Dygs	180.21	120.68	0.00
M 4	NFB	101.12	36.80	91.95
	JNFb	109.12	44.75	106.63
	Potato	205.94	100.75	0.00
	Dygs	235.83	157.30	0.00
34 rice	NFb	125.12	37.81	95.02
	JNFb	157.00	43.82	126.23
	Potato	192.51	113.62	0.00
	Dygs	255.52	212.73	0.00
LSD 1%		9.74	10.07	4.12

Liquid NFb and JNFb media received 0.1% of NH_4Cl. NFb and JNFb media contained 0.01% of tryptophan in both conditions.

The variation in the amounts of IAA synthesized by *Herbaspirillum* may be attributed to the fact, that the experimental conditions may not be appropriate for all the isolates to synthesize IAA. Strain variation and the composition of the culture media are also important in this respect.

The bioassay technique proved that the tested strains produced IAA and this product was biologically active as IAA stimulates root cell elongation and fresh weight (Table 3). This positive effect is optimal at lower concentrations of IAA and higher amounts inhibit root growth leading to shortening of both the main and lateral roots. This finding is in accordance with the results by

Sarwar and Frankenberger (1994), Torry (1976), Zimmer et al. (1988), and Zimmer and Bothe (1988).

In experiments to detect the most suitable medium for bacterial growth, nitrogen fixation and indole compounds production by the type strains mentioned above, each strain was cultivated in four different media of different compositions (Table 4). Growth, indole compounds production and nitrogenase activity (ARA) varied upon using media of different composition. Nitrogen fixation was completely inhibited in all organisms in semi-solid Dygs- and Potato-media. This is due to the fact that these media are rich in nitrogenous substances which inhibit nitrogenase activity. Also, indole compounds production by *Azospirillum* was lower in these two media in both liquid and semi-solid conditions (Table 4). These substances excreted by *A. brasilense* and *A. lipoferum* in semi-solid NFb medium was 2.6 and 3.8 fold, respectively, as compared to the IAA produced in semi-solid Potato medium. This situation was different for the *Herbaspirillum* strains tested, where indole compounds production decreased in the order Dygs, Potato, JNFb and NFb media under both liquid and semi-solid conditions (Table 4). In liquid Dygs medium, the increment of its production for *H. seropedicae, H. rubrisubalbicans* and *Herbaspirillum*-like 34 rice was 2.33, 2.33 and 2.04 fold, respectively. This behaviour suggests that an important precursor for auxin synthesis by this generus was absent when minimal media is used (NFb and JNFb) and it is present in the enriched media. Also, in contrast to *Azospirillum*, all liquid media tested produced more indole compounds than semi-solid media.

NFb medium – either as N-free semi-solid or N-amended liquid medium – proved to be the most suitable for indole compounds biosynthesis by *Azospirillum*. This may be attributed to its suitable formula, specially in semi-solid media which stimulated optimal nitrogenase activity and in turns, favour enzyme system controlling auxin biosynthesis, giving rise highest levels of it.

The superiority of JNFb over NFb medium for IAA-production of *Herbaspirillum* spp. reflects, that these media were formulated for the isolation and characterisation of this genus. These two media differ in pH which is lower in JNFb (final pH 5.8) as in NFb (final pH 6.8). *Azospirillum brasilense* is known to be sensitive to initiate growth in acid condition, decreasing the growth rate.

Lower indole compounds production in the N-free semi-solid medium in relation to liquid culture by *Herbaspirillum* spp. might be due to: no correlation between nitrogenase enzyme and enzyme system controlling auxin production; lower growth rate in semi-solid compared to liquid condition; O_2 tension favours nitrogenase activity but inhibits enzyme(s) controlling IAA biosynthesis and favour other promoting substances or other minor IAA biosynthetic pathways. In N-amended medium or Dygs and potato media, the

growth rate was higher giving rise to higher indole compound amounts by the huge number of growing cells.

The results obtained showed that production of indole compounds differ between strains and that *A. brasilense* is the most effective one among four species of *Azospirillum* and three species of *Herbaspirillum*. The amounts of auxin produced could be detected and quantified using a simple colorimetric assay. Also, with the objective to produce inoculant using bacteria plus medium mixed with a carrier, the amount of indole compounds produced can be reflected in a better root development, specially in a dry climate or tropical soils, where stress conditions are quite common. Combining optimal bacterial selection and inoculant formulation, the increment of root promoting effects may be enhanced.

Acknowledgements

The authors wish to thank Dr. Jose Ivo Baldani (EMPRAPA, Seropédica) for the helpful discussion of these results and Dr. Anton Hartmann (Neuherberg, Germany) for suggestions on the manuscript. This work was performed in a co-operative association with Embrapa Agrobiology and Faculty of Science, Cairo University to complete the Ph.D. Thesis of the first author. This work was partially supported by the Pronex II/FINEP.

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