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Research Note

Auxin Production Is a Common Feature of Most Pathovars of *Pseudomonas syringae*

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We investigated indole-3-acetic acid (IAA) production by 57 pathovars of *Pseudomonas syringae* and related species. Most of those analyzed produced IAA, especially in the presence of tryptophan. Eight strains produced high IAA concentrations in the absence of Trp. The *iaa*M and *iaa*H genes of *P. savastanoi* pv. *savastanoi* were detected in a limited number of strains only, including the eight above-mentioned strains. Thus, IAA synthesis in most assayed strains of *P. syringae* and related species does not involve genes highly similar to *iaa*M and *iaa*H. In contrast, the *iaa*L gene encoding an IAA-lysine synthase was detected in most pathovars, and was often found on plasmids.

Additional keywords: Azospirillum, Erwinia, plant growth regulators, plant pathogens.

Most of the bacterial strains belonging to the *Pseudomonas syringae* group are plant pathogens. For years, the taxonomic position of this group and that of its constitutive members relied on a limited number of characters, including pathogenicity-related characters (Schaad 1982; Young et al. 1992). However, the emergence of numerical (Sneath 1984) and molecular techniques (Johnson 1984; Wayne et al. 1987; Woese 1992) led to a complete revision of the taxonomy of this group of bacteria. At present, Young et al. (1996) have listed over 50 pathovars of *P. syringae*, three pathovars of *P. savastanoi*, and three related species (*P. amygdali*, *P. ficuserectae*, and *P. meliae*) that fall within nine genomospecies (Gardan et al. 1997). Each genomospecies, which may eventually be regarded as a new species, contains strains exhibiting at least 70% DNA/DNA homology with the relevant type strain(s). As a consequence, several pathovars of *P. syringae* and related species have already been, or will be, renamed. For instance, *P. syringae* pv. *savastanoi* became *P. savastanoi* pv. *savastanoi*, *P. syringae* pv. *glycinea* became *P. savastanoi* pv. *glycinea*, and *P. syringae* pv. *phaseolicola* became *P. savastanoi* pv. *phaseolicola*. In this report, we will (i) retain only the current approved names for these plant pathogenic bacteria (Young et al. 1996), (ii) collectively term strains belonging to *P. savastanoi* and *P. syringae* pathovars and their related species as the *P. syringae* group, and (iii) include *P. viridiflava* with the three above-mentioned *P. syringae*–related species (Gardan et al. 1997). However, the *P. tremae* genomospecies, which includes only *P. syringae* pv. *tremae* strains, is excluded from this study since its taxonomic position still remains open to discussion (Gardan et al. 1997).

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For *P. savastanoi* pv. *savastanoi* strains, pathogenicity implies biosynthesis of plant growth regulators. The synthesis of hormones such as cytokinins and indole-3-acetic acid (IAA) leads to the formation of the characteristic knots on olive and oleander (Wilson 1935; Smidt and Kosuge 1978). In *P. savastanoi* pv. *savastanoi* oleander strains, IAA biosynthesis proceeds from L-tryptophan (Trp) to IAA via indole-3 acetamide (IAM). Two enzymes, a Trp-2 monoxygenase and an indoleacetamide hydrolase, are involved in this pathway (Kosuge et al. 1966). The synthesis of these enzymes is determined by two genes, called *iaa*M (for the monooxygenase) and *iaa*H (for the hydrolase) (Comai and Kosuge 1980, 1982; Comai et al. 1982). In *P. savastanoi* pv. *savastanoi* olive strains, *iaa*M and *iaa*H genes are located on the chromosome (Comai et al. 1982). These genes were cloned and sequenced (Comai et al. 1982; Palm et al. 1989) from a 52-kb plasmid of an oleander strain (Comai and Kosuge 1980, 1982; Comai et al. 1982). The *iaa*M and *iaa*H genes show 54 and 38% similarity, respectively, to *tms*1 and *tms*2, two genes responsible for IAA biosynthesis in the plant cells infected by *Agrobacterium tumefaciens* (Gielen et al. 1984; Klee et al. 1984; Yamada et al. 1985). Strains of *P. savastanoi* pv. *savastanoi* pathogenic on oleander also convert IAA to indole-3 acetyl-ε-L-lysine (IAA-Lys), a compound that is biologically less active than IAA (Hutzinger and Kosuge 1968) as estimated by the coleoptile elongation assay (Nitsch and Nitsch 1956; Surico and Iacobellis 1992). This conversion involves the enzyme IAA-lysine synthase, whose synthesis is encoded by the *iaa*L gene (Glass and Kosuge 1986, 1988; Roberto et al. 1990). In *P. savastanoi* pv. *savastanoi* oleander strains, this gene is located on the plasmid within 2,000 bp of *iaa*M and *iaa*H (Glass and Kosuge 1986).

Previous studies performed on a very limited number of strains of *P. syringae* pv. *syringae, P. savastanoi* pv. *phaseolicola, P. syringae* pv. *tabaci*, and *P. syringae* pv. *tomato* revealed that some of these produce IAA, especially when Trp is added to the culture media (Fett et al. 1987; Gardan et al. 1992). Genes responsible for IAA synthesis in *P. syringae* pv. *syringae* were cloned with a probe overlapping the *iaa*M/*iaa*H genes of *P. savastanoi* pv. *savastanoi* (White and Ziegler 1991). The deduced amino acid sequences of *iaaM* and *iaaH* of *P. syringae* pv. *syringae* share over 90% identity with the amino acid sequences deduced from the respective *iaa* homologues of *P. savastanoi* pv. *savastanoi* (Mazzola and White 1994). In most other pathovars, it is not known whether these genes are present (Ziegler et al. 1987; White and Ziegler 1991). However, the presence of *iaaM* and *iaaH* homologous sequences has been reported in *P. savastanoi* pv. *glycinea* (F. F. White and M. Mazzola, *unpublished;* quoted in Mazzola and White 1994). Furthermore, Gardan et al. (1992) showed that strains of *P. syringae* pv. *tomato* and *P. savastanoi* pv. *phaseolicola* produced compounds reacting with a Salkowski's formulation that is used for the colorimetric detection of indolic compounds such as auxin (Gordon and Weber 1951), though sequences similar to the *iaa*M/*iaa*H genes of *P. savastanoi* pv. *savastanoi* were not detected in these two strains. These results prompted us to examine the production of IAA by a wide range of strains of the *P. syringae* group. The results reported here deal with 57 pathovars of *P. syringae, P. savastanoi*, and related species belonging to eight out of nine genomospecies defined by Gardan et al. (1997)**.** Interestingly, these results show that most assayed strains do produce IAA. In these strains, however, genes involved in auxin production are not highly homologous to the *iaa*M and *iaa*H genes of *P. savastanoi*, since these sequence were not detected in approximately 80% of the *P. syringae* pathovars.

Fifty-six strains of the *P. syringae* group (out of the 57 strains of our collection) were examined for IAA production with high-pressure liquid chromatography analysis of an ethyl acetate extract of the culture supernatants, according to a procedure that we developed (E. Glickmann, M. Elasri, A. Petit, and Y. Dessaux, *unpublished*). Unless otherwise stated, all strains were grown at 25°C in modified King B medium (KB; King et al. 1954), which consisted of the following: proteose peptone no. 3 (Difco-OSI, Elancourt, France), 10 g; K_2HPO_4, 1.15 g; $MgSO_4 \cdot 7H_2O$, 1.5 g; and glycerol 1.5% (vol/vol); pH was adjusted to 7.2 with HCl 2N. When appropriate, 0.5 g of Trp per ml was added to the media. All liquid cultures were aerated by shaking. Results presented in Table 1 are average values obtained from at least two independent experiments. In the absence of Trp in the growth medium, IAA concentrations were generally low (average value 1.3 µg/ml). No IAA or only trace amounts were detected in the supernatant of 26 out of the 56 assayed strains while five strains, 1392 (*P. syringae* pv. *syringae*), 2339 (*P. syringae* pv. *aceris*), 2897 (*P. syringae* pv. *myricae*), 1657 (*P. syringae* pv. *maculicola*), and 10971t (*P. syringae* pv. *ribicola*), produce IAA amounts greater than 5 µg/ml and three strains, 1670 (*P. savastanoi* pv. *savastanoi*), 2341 (*P. syringae* pv. *cannabina*), and 2899 (*P. syringae* pv. *photiniae*), synthesized IAA at concentrations over 2 µg/ml. In presence of Trp, all assayed *Pseudomonas* strains produced auxin in variable concentrations, the exceptions being *P. syringae* pv. *ciccaronei* strain 2342 and *P. syringae* pv. *persicae* strain 1573. The low level of IAA in the culture supernatant of strain 1573 could be related to its very poor growth in liquid KB medium. In agreement with this hypothesis, another *P. syringae* pv. *persicae* strain (CFBP1067), which grew as well as other assayed *Pseudomonas* strains in KB medium, produced IAA. Except for the two above-mentioned strains, in the presence of Trp, IAA concentrations in culture supernatants range from 0.7 to 69.7 µg/ml, the average value being 9.5 µg/ml. A few strains such as 1392 (*P. syringae* pv. *syringae*), 2339 (*P. syringae* pv. *aceris*), 1670 (*P. savastanoi* pv. *savastanoi*), 2215 (*P. syringae* pv. *delphini*), 1657 (*P. syringae* pv. *maculicola*), 10971t (*P. syringae* pv. *ribicola*), and 2341 (*P. syringae* pv. *cannabina*) generate high amounts of IAA. These include all five strains that produce IAA concentrations higher than 5 µg/ml without added Trp in their growth media. Among other assayed gram-negative bacteria (Table 1), only *Erwinia herbicola* pv. *gypsophilae* strain 11141, which harbors genes homologous to the *iaa*M/*iaa*H genes of *P. savastanoi* pv. *savastanoi* (Clark et al. 1993), and *Agrobacterium* strains C58 and K84 produce IAA, a result consistent with a previous report (Liu and Kado 1979).

We investigated the presence of sequences similar to *iaa*M and *iaa*H genes in the genome of 55 strains of the *P. syringae* group. This was assessed by Southern blot hybridization on genomic DNA preparations obtained by a modification of the protocol of Dhaese et al. (1979). The *iaa*M/*iaa*H probe consisted of a 2.2-kb *Bam*HI-*Eco*RI fragment from the plasmid pLUC2 that contains most of the *iaa*M and *iaa*H operon of *P. savastanoi* pv. *savastanoi* (Comai and Kosuge 1982). The *iaa*L probe generated from pLG87 was a 1,301-bp *Ple*I restriction fragment overlapping the only (but complete) sequence of the open reading frame ORF2 of the *iaa*L region (this open reading frame is sufficient to confer IAA-lysine synthase activity upon the recipient host; Glass and Kosuge 1986; Roberto et al. 1990). Probes were labeled by random incorporation of a digoxygenin-modified nucleotide (Boehringer-Mannheim, Mannheim, Germany). DNA was transferred onto a Hybond-N membrane (Amersham-Life Science, les Ulis, France) as previously described (Ausubel et al. 1989; Sambrook et al. 1989). Hybridization and detection of heteroduplex were made according to the manufacturer's instructions as modified by Dessaux et al. (1995) and Vaudequin-Dransart et al. (1995). Hybridization and washes were done at 61.5 ± 0.5°C. Washes were performed in a solution of 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% sodium dodecyl sulfate. This set of conditions should allow the detection of probe-DNA homology over 75%. Detection was performed with the NBT/X-pho colorimetric system or the more sensitive chemiluminescent substrates Lumigen-PPD and CSPD (Nucleic Acid Detection kit, Boehringer-Mannheim, Mannheim, Germany). Results are presented in Table 1.

Sequences homologous to the *iaa*M/*iaa*H genes of *P. savastanoi* pv. *savastanoi* were detected in only 11 strains, even though the experiment was performed under low stringency hybridization conditions. These strains belong to the genomospecies 1 (*P. syringae* pvs. *syringae* and *aceris*), 2 (*P. savastanoi* pv. *glycinea*, *P. syringae* pvs. *myricae* and *photiniae*, and *P. amygdali*), 3 (*P. syringae* pvs. *maculicola*, *ribicola*, and *viburni*), and 9 (*P. syringae* pv. *cannabina*). Interestingly, most of these strains (in pvs. *syringae, aceris, savastanoi, myricae,*

Table 1. Production of indole-3-acetic acid (IAA) by various *Pseudomonas syringae* pathovars and the relationship with the presence or the absence of the *iaa*M, *iaa*H, and *iaa*L genes

Genomospecies and control strains[a]	Pathovars and related species	CFBP no.	Production of IAA (HPLC detection)[b]		Hybridization[c]		Plasmids[d]
			KB without Trp	KB + Trp (2.5 mM)	*iaa*M/*iaa*H	*iaa*L	
1	*P. syringae* pv. *syringae*	1392	16.8	69.7	+	–	0/0
	P. syringae pv. *aptata*	1617	0.9	6.4	–	–	0/1
	P. syringae pv. *aceris*	2339	5.2	23.2	+	–	0/1
	P. syringae pv. *atrofaciens*	2213	0.7	7.1	–	–	0/0
	P. syringae pv. *dysoxyli*	2356	1.0	13.6	–	–	0/0
	P. syringae pv. *japonica*	2896	0.6	5.8	–	–	0/0
	P. syringae pv. *lapsa*	1731	0.5	4.6	–	–	0/0
	P. syringae pv. *panici*	2345	0.7	8.4	–	–	0/0
	P. syringae pv. *papulans*	1754	0.5	4.1	–	+/-	2/4
	P. syringae pv. *pisi*	2105	0.5	4.6	–	+	0/2
2	*P. savastanoi* pv. *savastanoi*	1670	2.6	16.4	+	+	3/4
	P. syringae pv. *aesculi*	2894	0.5	7.1	–	+	2/3
	P. syringae pv. *ciccaronei*	2342	ND	ND	–	+	4/7
	P. syringae pv. *dendropanacis*	3226	ND	1.2	–	+	2/3
	P. syringae pv. *eriobotryae*	2343	ND	1.6	–	+	1/1
	P. savastanoi pv. *glycinea*	2214	ND	2.9	+	+	NT/1
	P. syringae pv. *hibisci*	2895	TR	5.1	–	+/-	1/2
	P. syringae pv. *lachrymans*	1644t	0.7	8.3	–	+/-	1/3
	P. syringae pv. *mellea*	2344	1.4	3.9	–	–	0/0
	P. syringae pv. *mori*	1642	1.0	5.0	–	+	0/1
	P. syringae pv. *morsprunorum*	2116t	TR	0.7	–	+	2/3
	P. syringae pv. *myricae*	2897	8.1	14.5	+	+	2/3
	P. savastanoi pv. *phaseolicola*	1390	ND	6.6	–	+/-	3/3
	P. syringae pv. *photiniae*	2899	2.4	6.5	+	+	3/3
	P. syringae pv. *sesami*	1671	TR	5.8	–	+	1/4
	P. syringae pv. *tabaci*	2106	TR	3.6	–	+	0/1
	P. syringae pv. *ulmi*	1407	0.9	4.8	–	+	3/5
	P. amygdali	3340	NT	NT	+	+	NT/2
	P. ficuserectae	3224	TR	2.6	NT	NT	NT/2
	P. meliae	3225	TR	1.7	NT	NT	2/4
3	*P. syringae* pv. *tomato*	2212	0.4	6.1	–	+	0/2
	P. syringae pv. *antirrhini*	1620	TR	8.9	–	+	0/0
	P. syringae pv. *apii*	2103	0.6	16.8	–	+	0/1
	P. syringae pv. *berberidis*	1727	0.4	11.4	–	+	0/0
	P. syringae pv. *delphinii*	2215	ND	25.7	–	+	0/0
	P. syringae pv. *maculicola*	1657	5.5	68.2	+	+	0/0
	P. syringae pv. *passiflorae*	2346	TR	5.8	–	+	0/0
	P. syringae pv. *persicae*	1573	ND	TR	–	+	2/4
	P. syringae pv. *philadelphi*	2898	TR	9.8	–	+	0/0
	P. syringae pv. *primulae*	11007t	TR	7.8	–	+	0/2 (or 3)
	P. syringae pv. *ribicola*	10971t	8.7	30.4	+	+	0/1
	P. syringae pv. *viburni*	1702	ND	9.9	+	+	1/2
4	*P. syringae* pv. *porri*	1908	TR	2.2	–	+/-	1/3
	P. syringae pv. *atropurpurea*	2340	0.5	3.2	–	+	0/1
	P. syringae pv. *coronafaciens*	2216	TR	2.9	–	+	NT/2
	P. syringae pv. *garcae*	1634	0.5	3.3	–	+	0/2
	P. syringae pv. *striafaciens*	1674	TR	3.5	–	+	0/0
	P. syringae pv. *oryzae*	3228	TR	1.6	–	+	0/0
	P. syringae pv. *zizaniae*	11040	1.1	4.8	–	+	0/0
6	*P. syringae* pv. *viridiflava*	2107	TR	5.3	–	–	0/0
	P. syringae pv. *primulae*	1660	ND	1.4	–	–	0/0
	P. syringae pv. *ribicola*	2348	TR	6.9	–	–	0/0
7	*P. syringae* pv. *helianthi*	2067	0.6	5.1	–	+	NT/NT
	P. syringae pv. *tagetis*	1694	1.1	9.4	–	+	0/2
8	*P. syringae* pv. *avellanae*	10963	ND	0.7	–	+	0/3
	P. syringae pv. *theae*	2353	1.5	9.7	–	+	0/3
9	*P. syringae* pv. *cannabina*	2341	3.7	27.1	+	+	4/8

(*continued on next page*)

Table 1. (*continued from previous page*)

Genomospecies and control strains[a]	Pathovars and related species	CFBP no.	Production of IAA (HPLC detection)[b]		Hybridization[c]		Plasmids[d]
			KB without Trp	KB + Trp (2.5 mM)	*iaa*M/*iaa*H	*iaa*L	
	Agrobacterium tumefaciens	C58	ND	1.3	–	–	NT
	Agrobacterium sp. (biovar II)	K84	TR	2.3	–	–	NT
	Erwinia herbicola pv. *gypsophilae*	11141	ND	4.0	+	–	NT
	Alcaligenes faecalis	15	ND	ND	–	–	NT
	Escherichia coli	10161	ND	TR	–	–	NT

[a] All *Pseudomonas* strains were from the Collection Française des Bactéries Phytopathogènes (CFBP, INRA, Angers, France). *Agrobacterium* strains were taken from the collection of our laboratories. *Escherichia coli* strain 10161 and *Erwinia herbicola* pv. *gypsophilae* strain 11141 containing *iaa*M/*iaa*H genes were obtained from Louis Gardan. Genomospecies were as defined by Gardan et al. (1997).

[b] IAA concentrations were determined by high-pressure liquid chromagraphy (HPLC) from bacterial culture supernatants extracted with ethyl acetate (E. Glickmann, M. Elasri, A. Petit, and Y. Dessaux, *unpublished*). IAA concentrations are expressed in µg of bacterial supernatant per ml. Abbreviations: NT, not tested; ND, no IAA detected; TR, trace amounts ($\chi \leq 0.3$ µg/ml) of IAA detected.

[c] Presence of *iaa*M/*iaa*H- and *iaa*L-related sequences as assessed by Southern blot hybridization (for conditions see text): –, no signal; +/–, weak signal; +, strong signal.

[d] Plasmids hybridizing with *iaa*L / total number of plasmids. Plasmid separation from *P. syringae* strains was performed as indicated in the text with a modification of the procedure by Wheatcroft et al. (1990). Hybridization with the *iaa*L probe was performed on Southern blots of native plasmid DNA under conditions similar to those used to investigate the presence of *iaa*M, *iaa*H, and *iaa*L sequences in restricted genomic DNA preparations (see text).

photiniae, *maculicola*, *ribicola*, and *cannabina*) are among those that produced high amounts of IAA in the absence or in the presence of Trp. Our results also indicate that 81% of the assayed *Pseudomonas* strains that produce IAA do not harbor sequences highly related to the *iaa*M/*iaa*H genes. Therefore, IAA synthesis by these strains appears to be controlled by genes other than those homologous to the *iaa*M/ *iaa*H probe. Control hybridizations also were performed on genomic DNA extracted from several gram-negative bacteria (Table 1). As expected, no sequence homologous to the *iaa*M/*iaa*H genes was detected in *A. faecalis* and *Escherichia coli* whereas the *iaa*M/*iaa*H genes of the *E. herbicola* control strain generated clear hybridization signal with the probe. The *tms*1/*tms*2 genes of the C58 T-DNA were not detected under our experimental conditions. This observation is in agreement with the expected detection threshold (approximately 75%) provided by our experimental conditions.

We also investigated the presence of *iaa*L-sequences in the genomes of 55 strains of the *P. syringae* group by Southern blot hybridization. Results are presented in Table 1 for the *Pseudomonas* strains and for the gram-negative control strains. Remarkably, 43 out of 55 assayed *Pseudomonas* strains possess sequences related to the *iaa*L probe, but the results vary according to the genomospecies. *iaa*L-related sequences were detected in all pathovars of the genomospecies 3 (12 strains) and 4 (7 strains), and in most assayed strains (17 out of 18) of genomospecies 2. In contrast, only two out of 10 strains of the genomospecies 1 appear to harbor *iaa*L-related sequences and none of the three assayed strains of the genomospecies 6 contain *iaa*L-related sequences. No *iaa*L-related sequences were detected in the genomes of *E. herbicola* strain 11411, *A. tumefaciens* C58, *A. faecalis* 15, and *E. coli* 10161 (Table 1). Related sequences also were not detected in *Rhizobium meliloti* strain Rm41 and several other *Pseudomonas* strains belonging to *P. fluorescens*, *P. putida*, *P. corrugata*, and *P. stutzeri* (data not shown). Interestingly, the presence of *iaa*L-related sequences is not correlated with the presence of the *iaa*M/*iaa*H homologues in the assayed *Pseudomonas* strains as deduced from the results obtained with strains of the *P. syringae* pvs. *syringae* and *aceris*.

The location of the *iaa*M, *iaa*H, and *iaa*L genes in the bacterial genome was investigated by Southern blotting of native plasmid DNA preparations. Plasmid separation was performed by a modification of the protocol designed by Wheatcroft et al. (1990) and Geniaux et al. (1993) for the separation of *Rhizobium meliloti* plasmids in horizontal gel. One milliliter of an overnight culture in KB medium at OD_{600} = 0.3 was centrifuged (8,000 × *g*, 5 min, 4°C), and the pellet resuspended in 1 ml of 5 M NaCl. Fifty microliters of 5% Na-Sarcosyl was added, and gently mixed by inverting the tube. The mixture was immediately centrifuged under the above conditions, and the supernatant was discarded. The pellet was then resuspended and the extraction was carried out as indicated by Wheatcroft et al. (1990). All operations were performed on ice. Plasmid separation was achieved in a 0.7% agarose gel, during 5 h at 5 V/cm. The buffer was cooled at 0°C with ice and re-circulated from one side of the tank to the other. Plasmids were detected in 37 out of 56 assayed *Pseudomonas* strains (Table 1). The number of plasmids ranged from 0 (for instance in the *P. syringae* pv. *syringae* strain 1392) to eight (in the *P. syringae* pv. *cannabina* strain 2341). Nineteen out of 20 assayed strains of the genomospecies 2 harbor plasmid(s), but only half of those from the genomospecies 1, 3, and 4 possess plasmids, while no plasmids were detected in the strains of genomospecies 6.

Southern blots performed with the *iaa*M/*iaa*H probe involved the only strains that genomic DNA preparations generated a hybridization signal with this probe (see Table 1). Results (not shown) indicate that the *iaa*M/*iaa*H genes are most often located on the chromosome of these strains. Thus, *P. savastanoi* strain 1670 isolated from olive likely harbored chromosomal copies of the *iaa*M/*iaa*H genes as already observed for other *P. savastanoi* strains isolated from this host plant (Comai et al. 1982; Glass and Kosuge 1986; Palm et al. 1989). The chromosomal location found in our *P. syringae* pv. *syringae* strain 1392 is in agreement with a previous report (White and Ziegler 1991). However, the *iaa*M/*iaa*H genes can also be located on plasmids, as observed in the oleander strains of *P. savastanoi* pv. *savastanoi* (Comai and Kosuge 1980). A similar location was observed for strains 2897 (*P. syringae* pv.

myricae) and 2899 (2897 (*P. syringae* pv. *photinae*), where the probe obviously hybridized to plasmids. This result is consistent with the fact that plasmids often play an important role in plant-bacteria interactions, as exemplified by the detection of genes involved in the coronatine toxin production by strains of the *P. syringae* pvs. *tomato* and *glycinea* (Cuppels and Ainsworth 1995).

Results obtained in hybridization experiments performed with the *iaa*L probe vary according to the genomospecies. Hybridization of the probe with plasmids was observed in strains belonging to the genomospecies 2. This was the case for at least 14 out of 17 strains that harbored plasmid(s) and in which the genome contained *iaa*L-related sequences. In contrast, plasmid-borne *iaa*L-related sequences were detected only in two strains of genomospecies 3 and in one strain of genomospecies 4. Interestingly, four out of the eight plasmids of the *P. syringae* pv. *cannabina* strain 2341 hybridized to the *iaa*L probe. Hybridization of the *iaa*L probe to multiple plasmids also occurred in several other strains. This latter phenomenon could be related to plasmid recombination such as co-integration or deletion, a hypothesis supported by the detection of numerous insertion sequences in plant-pathogenic *Pseudomonas* strains (Soby et al. 1993). The detection of *iaa*L-related sequences in strains of the *P. syringae* group is an interesting peculiarity of our study since it is the first time that such sequences have been widely detected in pathovars other than *P. savastanoi* pv. *savastanoi*. The synthesis of IAA-Lys, detected in culture supernatants of *P. savastanoi* pv. *savastanoi* strains (Hutzinger and Kosuge 1968; Glass and Kosuge 1986), supposedly regulates the amount of IAA produced by the bacteria, a feature that seems to be necessary for "optimal" growth of the strains in planta and the full expression of their virulence (Glass and Kosuge 1988; Surico and Iacobellis 1992). Overall, the results of the analysis of the plasmid content and of the location of the *iaa*M, *iaa*H, and *iaa*L genes are in good agreement with those previously reported in the literature for a limited number of strains (Coplin 1982).

From the data presented above, auxin production is a common feature of most pathovars of *P. syringae*. Interestingly, our assayed strains clearly fall in two groups. The first group contains eight strains that produce high concentrations of IAA in the presence or absence of Trp and that harbor genes homologous to the *iaa*M/*iaa*H genes of *P. savastanoi*. In agreement with the original results of Comai and Kosuge (1982) and White and Ziegler (1991) on *P. savastanoi* pv. *savastanoi* and *P. syringae* pv. *syringae*, we speculate that IAA synthesis in the strains of this first group involves the *iaa*M/*iaa*H genes and therefore proceeds via indole-3 acetamide (IAM). A second group, which contains most of the assayed strains, produces IAA but does not harbor genes highly related to the *iaa*M/*iaa*H genes of *P. savastanoi*. IAA synthesis in this group of bacteria might be unspecific and result from the nonenzymic conversion of Trp to IAA in the culture medium: Epstein et al. (1980) showed that up to 30% of input Trp can be converted to IAA under certain experimental conditions. In our conditions, however, we never observed nonspecific conversion of Trp to IAA. Spontaneous transformation of Trp to IAA is also unlikely because several strains of the second group produce low but detectable amounts of IAA in the absence of added Trp in the medium. Furthermore, the conservation of the *iaa*L sequence in such strains would be more difficult to understand if IAA production was only artefactual. Because we recently detected indole pyruvate (IPyrA) in the culture supernatants of strains of the second group and characterized an enzymic activity converting Trp to IPyrA (and vice-versa) in cell-free extracts of one of these strains (E. Glickmann, M. Elasri, A. Petit, and Y. Dessaux, *unpublished*), we suggest that IAA biosynthesis proceeds, at least in some isolates of the second group, via a pathway involving IPyrA as an intermediate, and not via IAM. Such a pathway has already been described in *Azospirillum brasilense* and *Erwinia herbicola*, which both produce IAA via IPyrA and indole acetaldehyde (Manulis et al. 1991; Costacurta et al. 1994; Brandl and Lindow 1996).

The involvement of IAA in pathogenicity has been unambiguously demonstrated for *P. savastanoi* pv. savastanoi. Interestingly, other pathovars such as *P. syringae* pv. *amygdali* and *P. syringae* pv. *myricae*, which induce proliferation of plant tissues on other host plants, also harbor *iaa*M/*iaa*H genes. The reverse proposal, however, is not true. Various pathovars harboring the *iaa*M/*iaa*H genes (e.g., *P. syringae* pvs. *syringae, aceris, photinae, viburni, ribicola,* and *maculicola,* and *P. savastanoi* pv. *glycinea*) do not induce proliferation of plant tissues, but rather necrotic diseases. Thus, pathogenicity in strains of *P. savastanoi* pv. *glycinea* and *P. syringae* pv. *syringae* relies on the presence and expression of the *hrp* genes (Huang et al. 1988; Lindgren et al. 1988; reviewed in Willis et al. 1991; Van Gijsegem et al. 1993; Bonas 1994). However, the latter strain is able to produce IAA at concentrations up to 70 µg/ml (4×10^{-4} M) in KB medium supplemented with 2.5 mM Trp. Very likely, these IAA concentrations are not produced in planta by most of the assayed *Pseudomonas* strains. However, even lower concentration of IAA could potentially induce biological effects. Previous studies performed with *Pseudomonas* strains suggested that IAA production by these strains may inhibit some plant defense mechanisms (Robinette and Matthysse 1990). IAA production also has been associated with the epiphytic survival of the bacteria or with toxin production as demonstrated for *P. syringae* pv. *syringae* strains on *Phaseolus vulgaris* (Mazzola and White 1994). These data, taken together with our results, demonstrate that the generalized production of IAA by plant-pathogenic *Pseudomonas* spp. might play various and substantial biological and ecological roles that remain to be elucidated.

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