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METABOLISM OF TRYPTOPHAN IN SOIL

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Summary—Production of auxins in soil is stimulated by the addition of L-tryptophan (L-TRP) as a precursor. Metabolism of L-TRP results in the formation of many products including niacin (kynurenine pathway) and serotonin (hydroxylation) in addition to auxins and is regulated by two distinct enzymes, L-TRP-2,3-dioxygenase (TDO) and indole-2,3-dioxygenase (IDO). The roles of IDO and TDO in L-TRP metabolism and indole-3-acetic acid (IAA) formation in soil were studied by the use of bactericides and specific enzyme inhibitors. The addition of a broad spectrum bactericide, chloramphenicol, to soil resulted in a significant decrease of L-TRP catabolism and stimulated hydroxylation of L-TRP. There was no evidence in this work to support the view that L-TRP is transformed through extracellular reactions in soil. Inhibition of TDO by the addition of benzaldehyde and norharman (a potent inhibitor of TDO and IDO) also reduced L-TRP catabolism in soil. Although IAA was detected in all soils treated with bactericides or enzyme inhibitors, production of this secondary metabolite (IAA) was not reduced by blocking the kynurenine pathway or hydroxylation. Auxins were not detected in soils tested with the addition of intermediates of the TDO and IDO pathways (kynurenine and 5-hydroxytryptophan, respectively). Incubation of D-TRP as a possible auxin precursor in California soils resulted in a significant decrease in metabolism compared with L-TRP additions as measured by CO_2 evolution and HPLC analysis. Rhizosphere bacteria isolated from lettuce (*Lactuca sativa*) were found to produce more IAA derived from L-TRP after they had been cultured on a medium containing L-alanine, L-asparagine and L-lysine compared with cultures that had been exposed to L-TRP or nutrient agar and then grown on a L-TRP supplemented minimal salts liquid medium. A soil which had extremely low yields of L-TRP-derived IAA increased production of auxin derivatives, including TRP-derived IAA, when incubated for 1 week with the amino acids, L-lysine, L-asparagine and L-alanine. The results suggest that synthesis of IAA in soil may not be the result of direct catabolism of L-TRP but may be linked to the cometabolism of L-TRP by the broad specificity of L-aminotransferases present in soil.

INTRODUCTION

Biological transformations of L-tryptophan (L-TRP) e.g. in soil, culture media, plants and animals) may undergo several possible pathways (Frankenberger and Brunner, 1983; Bender, 1989; Müller *et al.*, 1989). L-TRP can be converted into kynurenine (2-amino-4-[2-aminophenyl]-4-oxobutanoic acid) by tryptophan-2,3-dioxygenase (TDO; EC 1.13.11.11) and then utilized for synthesis of the vitamin, niacin, or for metabolic energy. L-TRP can be converted by an hydroxylase to 5-hydroxytryptophan (5-OH-TRP) with subsequent conversion to 5-hydroxytryptamine (5-OH-TAM, serotonin) and 5-hydroxy-indole-3-acetic acid (5-OH-IAA) or can be directly incorporated into peptides and proteins. The 5-hydroxy products, 5-OH-TRP and serotonin, can be reverted back into the kynurenine pathway by indole-2,3-dioxygenase (EC 1.13.11.17) (Bender, 1989). L-TRP can also be converted by soil microorganisms into indole-3-acetic acid (IAA) (Martens and Frankenberger, 1993) which may significantly influence plant growth and development (Frankenberger *et al.*, 1990; Frankenberger and Arshad, 1991a, b).

In order for organisms to utilize L-TRP as a sole nitrogen source, TDO must be induced to catalyze the formation of kynurenine (Bender, 1989). The kynurenine pathway is the major route of L-TRP metabolism for metabolic energy and while there are many aromatic L-TRP metabolites, none arising by this pathway has an intact indole nucleus (Bender, 1989). Martens and Frankenberger (1993) found that on an average, 80% of the L-TRP carbon added to soil was evolved as CO_2-C after exposure for 5 days, suggesting that the majority of L-TRP added to soil is utilized by the kynurenine pathway.

Production of biologically-active substances such as auxins by soil microorganisms may be important for growth and development of plants (Rovira, 1970). L-TRP is believed to be the primary precursor for formation of IAA in plants and microorganisms

Abbreviations used: IAA, indole-3-acetic acid; IS-HPLC; ion suppression reverse phase high performance liquid chromatography; TAM, tryptamine; IAAlD, indole-3-acetaldehyde; IAM, indole-3-acetamide; IAN, indole-3-acetonitrile; IAcry, 3-β-indoleacrylic acid; IAlD, indole-3-aldehyde; IBA, indole-3-butyric acid; TOL, indole-3-ethanol; ILA, indole-3-lactic acid; IM, indole-3-methanol; IPA, indole-3-propionic acid; IPyA, indole-3-pyruvic acid; IAA-Asp, 3-indoleacetyl-aspartic acid; IAA-Gln, 3-indoleacetyl-glycine; 5-OH-IAA, 5-hydroxyindole-3-acetic acid; 5-OH-TAM, 5-hydroxytryptamine; 5-OH-TRP, 5-hydroxytryptophan; TRP, tryptophan; TDO, tryptophan-2,3-dioxygenase; IDO, indole-2,3-dioxygenase; TAT, tryptophan aminotransferase.

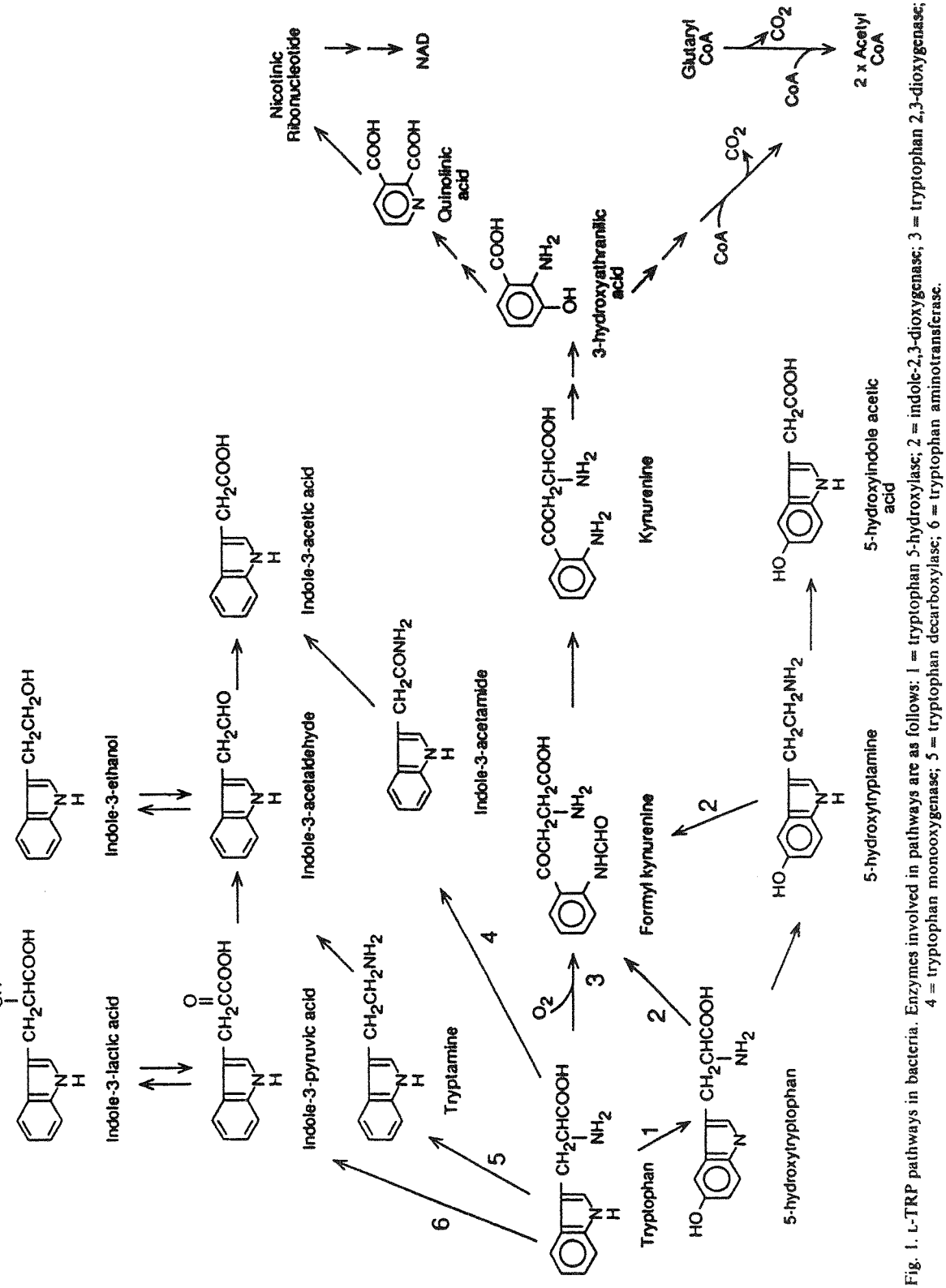

Fig. 1. L-TRP pathways in bacteria. Enzymes involved in pathways are as follows: 1 = tryptophan 5-hydroxylase; 2 = indole-2,3-dioxygenase; 3 = tryptophan 2,3-dioxygenase; 4 = tryptophan monooxygenase; 5 = tryptophan decarboxylase; 6 = tryptophan aminotransferase.

(Gordon, 1954, 1958; Gibson *et al.*, 1972; Frankenberger and Brunner, 1983; Monteiro *et al.*, 1988). However, as evident from Fig. 1, L-TRP has many fates in biological systems (Frankenberger and Brunner, 1983; Bender, 1989). Purification of L-TRP-2,3-dioxygenase (TDO) from fungal, bacterial and mammalian sources indicates similarities in the enzyme, being specific only for L-TRP and resulting in the formation of identical products (Bender, 1989). L-TRP is a precursor in biological systems for many compounds, including niacin (nicotinic acid and nicotinamide) and actinomycins, in addition to IAA. Müller *et al.* (1989) suggested that the ability of soil microbiota to produce and release IAA in their environment is highly dependent on the catabolic pathway of L-TRP. The enzymes involved in catabolism of L-TRP may regulate the production of auxins in soil.

Martens and Frankenberger (1993) investigated the conversion of proposed intermediates of IAA production from L-TRP in soil. They found that addition of indole-3-ethanol, indole-3-pyruvic acid, indole-3-lactic acid or indole-3-acetaldehyde to Californian soils resulted in little to no IAA formation after exposure for up to 7 days. We also found that 3′-^{14}C-TRP added to soils resulted in the formation of labeled 5-OH-TRP, 5-OH-TAM and 5-OH-IAA by TRP-5-hydroxylase in addition to other indoles including IAA (Martens and Frankenberger, 1993). Since the conversion of L-TRP to IAA in soil is substrate dependent (Arshad and Frankenberger, 1991), the finding of competing metabolic pathways for L-TRP, including the kynurenine and 5-hydroxy pathways, is important for understanding the variability of IAA production noted for different soils (Sarwar *et al.*, 1992).

D-TRP may be transformed into IAA in plants (Kutácek and Kefeli, 1970; Law, 1987; McQueen-Mason and Hamilton, 1989; Tsurusaki *et al.*, 1990). Kuraishi and Sakurai (1988) observed that in some plant species, D-TRP was a more effective precursor of IAA than the L-isomer. An unusual aminotransferase having D-stereospecificity is required for this conversion. In bacteria, D-amino acids are used in the biosynthesis of bacterial cell walls and some antibiotics (McQueen-Mason and Hamilton, 1989). Clark (1974) reported that *Agrobacterium* and *Rhizobium* spp in pure culture formed IAA from both L- and D-TRP, but there are no published reports concerning D-TRP transformations in soil.

Our objective was to characterize D- and L-TRP-derived IAA biotransformations in soil and determine the major route of TRP metabolism.

MATERIALS AND METHODS

The auxins, enzyme inhibitors, and various pathway intermediates were obtained from Sigma Chemical Co. (St Louis, Mo) except for 3-indoleacetyl-aspartic acid and 3-indoleacetyl-glycine which were obtained from Research Organics (Cleveland, Ohio).

Surface samples of California soils (0–25 cm) were selected to obtain a range in chemical and physical properties (Table 1). Methods used to characterize these soils were described by Martens and Frankenberger (1991).

The production of IAA in soil after the addition of D- and L-TRP or various pathway intermediates was monitored as follows: substrate (800 μg) in 1 ml H_2O was added to 2.5 g soil in a 50-ml Erlenmeyer flask and kept at 30°C for various times with or without 250 μl toluene, 100 mg chloramphenicol, 65 μg *p*-chloromercuricbenzoic acid, 125 μg norharman or 250 μg benzaldehyde. The D- and L-TRP remaining and the resulting auxins produced were extracted with 4 ml 100 mM KH_2PO_4 (pH 7.0), shaken on a rotary shaker (200 rev min^{-1}; 4°C; 10 min) and an aliquot was filtered through a 0.22 μm Millipore GS filter (Bedford, Mass.).

In place of liquid–liquid partitioning with ethyl acetate, an on-line HPLC solid phase extraction system was employed in analyzing the soil extracts as described by Martens and Frankenberger (1991). Briefly, this involves addition of a calibrated aliquot (5–40 μl) of the filtered (0.22 μm) soil extract to 400 μl water and injection onto a 5 μm O.D.S. guard column (30 × 4.6 mm). Rinses with water and the mobile phase removed ionic interferences and the auxins were eluted with the mobile phase (45% methanol:65% H_2O, pH 2.53) onto a separator column (R-Sil C_{18}) for subsequent u.v. detection (280 nm). The auxins were identified by co-chromatography and u.v. spectral conformation with authentic standards.

Soil respiration (CO_2 evolution) upon addition of D- and L-TRP (0 or 2500 μg) was monitored by incubating 10 g soil samples with 1 ml H_2O for 0, 1, 2, or 5 days at 30°C in a 125-ml Erlenmeyer screw-top flask equipped with a Mininert™ gas sampling valve (Dynatech, Baton Rouge, La). A 1 ml headspace sample was separated on a Porapak Q column (Alltech Assoc. Inc., Deerfield, Ill.). The constituent peaks were detected by thermal conductivity on a gas chromatograph (Varian Associates Inc., Model 3700) with a He flow rate of 30 ml min^{-1}; a column temperature of 70°C, an injector temperature of 70°C, and a detector temperature of 110°C. Constituent peaks (CO_2) were confirmed through use of authentic external standards.

Table 1. Properties of soils used

Soil	pH	Organic C	Total N	Clay	Sand	CEC*
			g kg^{-1}			
Sheephead	6.87	7.9	2.0	260	560	13.9
Altamont	6.45	12.3	2.6	460	210	26.8
Domino	8.55	7.4	1.1	290	430	16.8
Redding	6.56	2.0	1.2	640	250	25.4
Hesperia	7.22	8.6	2.9	180	740	6.6

*CEC, cation exchange capacity (cM$^+$kg^{-1} soil).

Three bacteria were isolated through an enrichment technique on a minimal salts medium (Frankenberger and Poth, 1988) from a soil–root interface of lettuce (*Lactuca sativa*) var. Grand Rapids with L-TRP serving as the sole N-source. These Gram-negative, motile bacteria were identified as *Pseudomonas aeruginosa* because of production of pyocyanin and fluorescein pigments on King's A and B medium, respectively (Bergan, 1981) and two *Agrobacterium* spp (Biolog Inc., Hayward, Calif.). The bacteria were then transferred to nutrient agar, or a minimal salts agar supplemented with L-asparagine, L-lysine and L-alanine or L-TRP. Isolated colonies from the different N sources were inoculated into a liquid minimal salts (1% glucose) media with L-TRP as the sole N-source and analyzed for IAA production at specified times.

RESULTS AND DISCUSSION

Studies were made to determine the fate of L-TRP in several California soils treated with bactericides and specific enzyme inhibitors. Figure 2 shows an IS–HPLC-u.v. chromatogram of 18 auxin derivatives and L-TRP separated by the method used for this work. IPA was utilized as an internal standard in the HPLC analyses of treated soils. Broad spectrum inhibitors including *p*-chloromercuricbenzoic acid, chloramphenicol and toluene were used to determine if L-TRP could undergo an extracellular transformation in soil. Chalvignac and Mayaudon (1971) extracted an extracellular enzyme from soil that converted L-TRP into IAA and IAM. *p*-Chloromercuricbenzoic acid is a well known inhibitor of sulfhydryl enzymes (Bender, 1989; Zollner, 1989). Chloramphenicol binds to 50S subunit of 70S ribosome and inhibits transpeptization (protein synthesis). Toluene is a plasmolytic agent which permits the assay of soil enzymes without interference from microbial proliferation. Norharman is a specific inhibitor of IDO and TDO while benzaldehyde inhibits TDO (Eguchi *et al.*, 1984).

Table 2 shows the results of the selected bactericides and enzyme inhibitors on the catabolism of L-TRP and IAA production. In most cases, the inhibitors substantially reduced L-TRP catabolism in the soils tested. The most effective inhibitors of L-TRP metabolism in soil were chloramphenicol (see Fig. 3) followed by *p*-chloromercuricbenzoic acid > norharman [see Fig. 4(b)] = toluene = benzaldehyde [see Fig. 4(a)]. There was no evidence in our work to support the view that L-TRP is transformed through extracellular reactions in soil with no significant differences in IAA formation in the presence or absence of the broad spectrum bactericides (Table 2). With the use of the specific enzyme inhibitors (norharman and benzaldehyde), the kynurenine pathway and hydroxylation of TRP should be blocked by inhibiting IDO and TDO. It was evident that IAA production was not substantially decreased in the presence of these inhibitors, and in some cases IAA production was stimulated, thus the conversion of L-TRP to IAA by the kynurenine pathway is not a major route for L-TRP-derived IAA formation in soils.

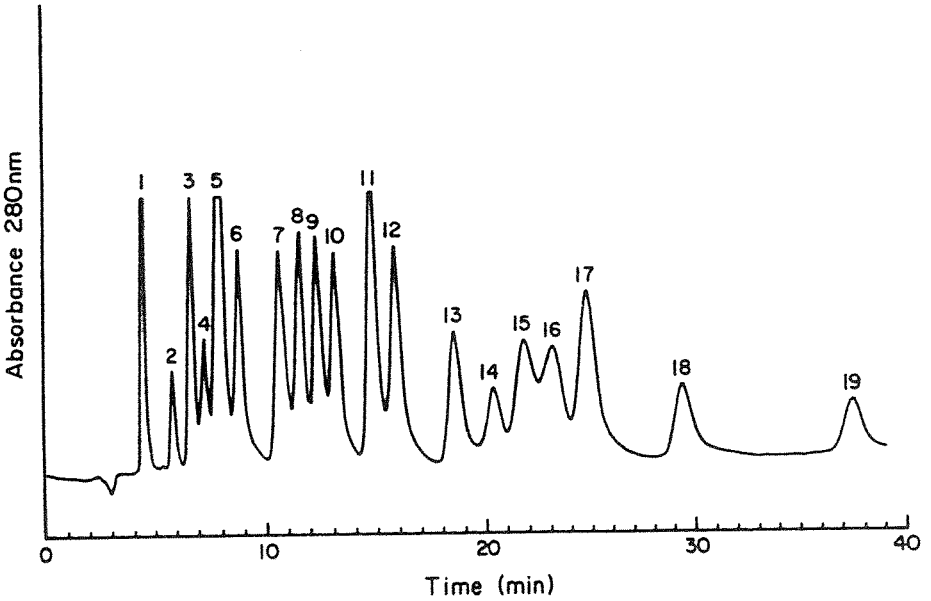

Fig. 2. Chromatogram of tryptophan and auxin derivatives detected by IS–HPLC (1 = 5-OH-IAA; 2 = IAA-Asp; 3 = IAA-Gln; 4 = 5-OH-TRP; 5 = IAM; 6 = ILA; 7 = 5-OH-TAM; 8 = IAA; 9 = IAALD; 10 = TOL; 11 = IALD; 12 = IAN; 13 = IPA; 14 = IPyA; 15 = TRP; 16 = IM; 17 = IAcry; 18 = IBA; –19 = TAM).

Table 2. Effects of selected bactericides and enzyme inhibitors on L-TRP metabolism and IAA production in soils*

Inhibitor	Time (days)	Soil: Sheephead L-TRP	Sheephead IAA	Domino L-TRP	Domino IAA	Hesperia L-TRP	Hesperia IAA
		mg kg^{-1} soil					
Control	0	306	ND†	303	ND	314	ND
	1	205	ND	135	16	228	2
	3	68	1.2	30	13	130	8
	7	ND	2.4	ND	1	ND	ND
p-Chloromercuricbenzoic acid	1	214	2	304	<1	307	8
	3	152	5	294	<1	225	<1
	7	4	3	264	1	115	<1
Toluene	1	201	<1	238	<1	315	1
	3	139	1	263	1	270	2
	7	110	1	48	3	200	7
Chloramphenicol	1	219	1	305	2	292	4
	3	167	3	299	1	262	5
	7	115	2	279	2	195	4
Norharman	1	222	2	321	2	312	9
	3	156	7	233	5	282	4
	7	30	4	216	9	118	3
Benzaldehyde	1	307	1	314	2	242	6
	3	130	3	275	5	163	5
	7	ND	<1	243	7	80	3

*2.5 g of moist soil with 1 ml water containing the specified amount of inhibitor and 800 μg L-TRP were exposed to the soil for the times specified.
†ND: not detected.

The addition of intermediates of the TDO and IDO pathway, kynurenine and 5-hydroxytryptophan, respectively, to soil resulted in their rapid disappearance within 3 days; the intermediates were not extractable from the soil matrix. At no time during soil exposure of these intermediates was IAA or any other auxin derivatives extracted from the five soils tested. IAA production in soils does not appear to be linked to the TDO and IDO pathway. Martens and Frankenberger (1993) reported that about 80% of the L-TRP carbon added to California soils was evolved as CO_2-C in <5 days suggesting that the IDO and TDO enzymes are active in the removal of L-TRP from the soil system.

While there are many aromatic L-TRP metabolites as a result of the kynurenine pathway, none arising by this pathway has an intact indole nucleus (Bender, 1989). This suggests that another mechanism must be present for synthesis of auxins. The enzyme, L-TRP aminotransferase (L-TAT) plays an important role in the initial transformations of L-TRP to auxins in the plant kingdom (Kutácek, 1985). L-TAT is an α-ketoglutarate-dependent conversion. Indole-3-pyruvic acid (IPyA) is the product of this catalysis (Fig. 1). Although L-TRP has been found to be a substrate of L-TAT, this enzyme has been reported to have a specificity 2–3-fold greater for other amino acids such as L-lysine, L-alanine and L-asparagine than for

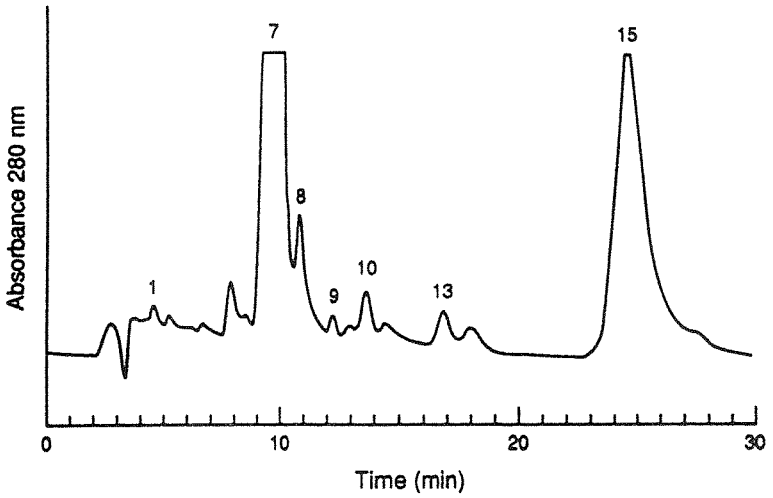

Fig. 3. IS–HPLC chromatogram of a Sheephead soil treated with 100 mg chloramphenicol and 800 mg L-TRP exposed for 5 days. Peaks are identified in Fig. 2.

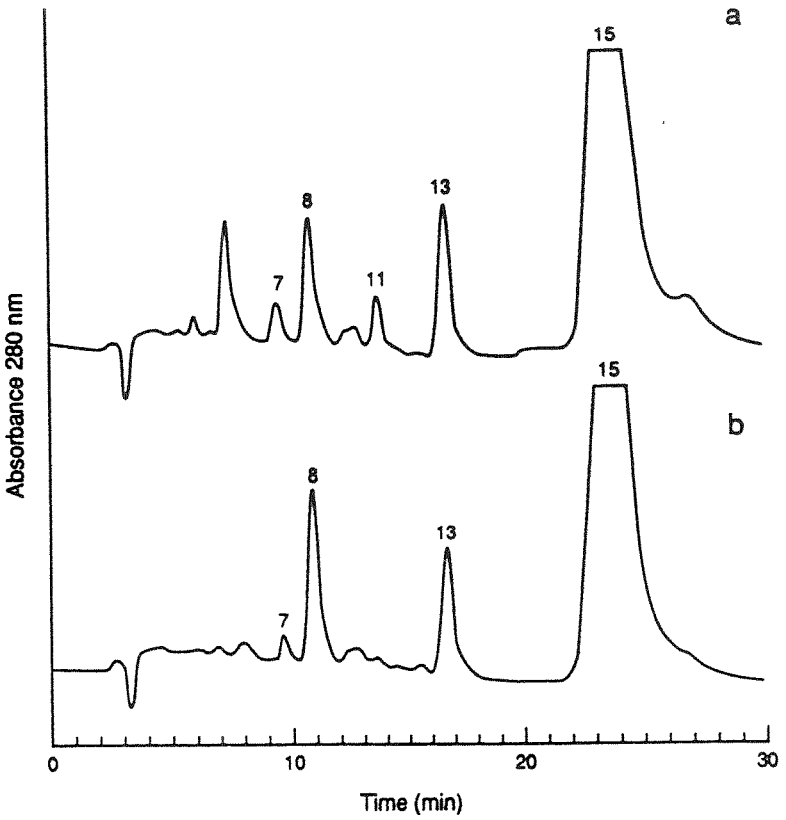

Fig. 4. IS–HPLC chromatograms of (a) a Domino soil treated with 250 mg of benzaldehyde and L-TRP (800 mg) exposed for 5 days; and (b) a Domino soil treated with 125 mg norharman and L-TRP (800 mg) exposed for 5 days. Peaks are identified in Fig. 2.

L-TRP (Kutácek, 1985). The broad specificity of this aminotransferase in plants was first reported by Truelsen (1972). Its lower affinity for L-TRP may be due to the substitution of the indole nucleus in L-TRP (indole-alanine) for the hydrogen in the alanine molecule. L-TAT has also been isolated from soil microorganisms. Frankenberger and Poth (1988) extracted a rhizobacterium from the grass *Festuca octoflora* Walt. which produced an L-aminotransferase which actively catalyzed the conversion of L-TRP to IPyA.

To investigate the broad specificity of L-TAT on IAA production in soil, three rhizobacteria were isolated from *L. sativa* L. var. Grand Rapids through an enrichment technique on a L-TRP-amended

Table 3. L-TRP-derived IAA production by rhizobacteria cultured previously on nitrogen-rich media*

		Bacteria								
		A. tumefaciens			*Agrobacterium* sp.			*P. aeruginosa*		
Treatment	Days	TRP	IAM	IAA	TRP	IAM	IAA	TRP	IAM	IAA
		mg l^{-1}								
Nutrient agar	0	304	ND†	ND	310	ND	ND	308	ND	ND
	3	207	1.0	ND	93	ND	ND	221	ND	2.0
	10	112	ND	ND	20	ND	ND	138	ND	ND
Tryptophan agar	0	310	ND	ND	310	ND	ND	312	ND	ND
	3	223	2.0	ND	170	ND	ND	223	5.0	4.0
	10	104	ND	ND	65	ND	ND	229	1.0	1.0
Amino acid agar	0	306	ND	ND	310	ND	ND	308	ND	ND
	3	213	81.2	11.0	235	ND	ND	200	60.7	21.1
	10	107	2.0	4.0	180	ND	4.0	236	4.0	8.0

*Cultures were grown on L-TRP-amended minimal salts agar, nutrient agar, or minimal salts agar amended with L-lysine, L-alanine and L-asparagine (amino acid agar) and then transferred into L-TRP minimal salts broth and monitored for auxin production at the times specified.

†ND: not detected.

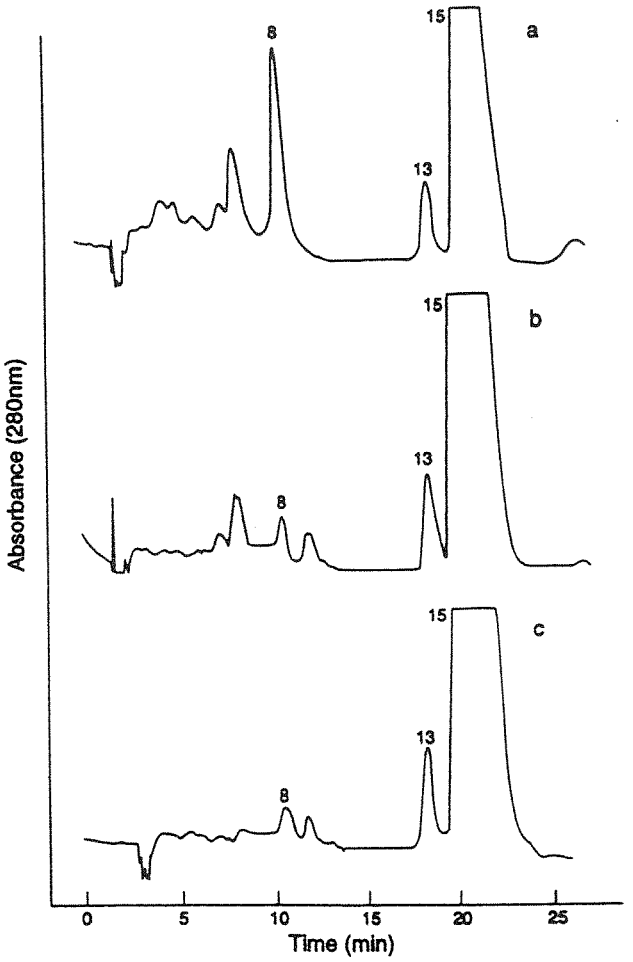

Fig. 5. IS–HPLC chromatograms of (a) a *P. aeruginosa*, (b) *A. tumefaciens*; and (c) *Agrobacterium* sp. grown on L-alanine, L-lysine and L-asparagine-amended minimal salts agar before inoculation in L-TRP-amended broth and exposed for 10 days. Peaks are identified in Fig. 2.

minimal salts media. The colonies were streaked onto nutrient agar to check purity and streaked onto nutrient agar, L-lysine, L-asparagine, and L-alanine-amended minimal salts agar (AMA) or L-TRP-amended minimal salts agar. The bacteria were then transferred into L-TRP-amended minimal salts broth and the broth was analyzed at 0, 3 and 10 days for IAA production. *P. aeruginosa* produced IAA from all three nitrogen sources (Table 3), but previous growth on the AMA resulted in more than a 9-fold increase in IAA and IAM formation (Table 3; Fig. 5). The *Agrobacterium* spp produced IAA only after

Table 4. Effects of previous additions of specific amino acids on L-TRP-derived IAA production in soil*

		Amino acid							
	Exposure to L-TRP	L-Lysine		L-Alanine		L-Asparagine		Control	
Soil	(days)	L-TRP	IAA	L-TRP	IAA	L-TRP	IAA	L-TRP	IAA
		mg kg^{-1} soil							
Domino	0	314	ND†	308	ND	304	ND	312	ND
	1	218	12	235	10	250	8	190	6
	3	130	14	112	16	100	16	34	13
	5	30	18	ND	12	10	13	ND	12
Redding	0	300	ND	302	ND	308	ND	303	ND
	1	180	3	192	2	175	3	120	ND
	3	100	4	90	3	75	4	6	1
	5	20	2	ND	1	ND	3	ND	1

*The soils were exposed to 100 μg amino acid g^{-1} soil for 5 days at ambient temperatures before addition of 800 μg L-TRP in 1 ml water to 2.5 g moist soil and kept for times specified.
†ND: not detected.

previous growth on the AMA but *A. tumefaciens* substantially increased production of both IAM and IAA when grown previously on AMA compared with nutrient agar or L-TRP-amended agar (Table 3; Fig. 5). The considerable increase in auxin formation when the bacteria had previously been grown on AMA compared with other N sources suggests that these amino acids may stimulate (or induce) the activity of L-TAT.

Stimulation of soil L-TRP dependent auxin production by previous addition of the three amino acids as found for the rhizosphere organisms was also noted in soil. Addition of the amino acids, L-alanine, L-asparagine or L-lysine (100 μg amino acid g^{-1} soil) to soil for 5 days resulted in an increase in the formation of IAA in two soils tested (Table 4). No IAA was produced when the L-lysine, L-asparagine or L-alanine were added without L-TRP to the soils. The Domino soil was less responsive to L-amino acid additions than the Redding soil and the higher rate of IAA production in the Domino soil suggests it may possess high L-TAT activity. Auxin formation in the nutrient-rich rhizosphere soil has been reported to be higher than in root-free soil (Narayanaswami and Veerraju, 1969). This increased auxin formation in the rhizosphere may be due to the influence of root exudates. Plant root exudates have been found to contain amino acids as well as other growth promoting substances. Twenty-three amino acids have been detected in root exudates from 15 different plant species (Rovira, 1970). Alanine, lysine and asparagine were among the amino acids reported to be present in high amounts in root exudates.

Table 5. Metabolism of D- and L-tryptophan in soil monitored by CO_2-C evolution*

Soil	Isomer	Day (s) 1	2	5
		% CO_2-C recovery		
Hesperia	L-TRP	3.0	33	72.5 (12.1)
	D-TRP	2.5	17	46.4 (6.2)
Altamont	L-TRP	20.0	50.0	85.3 (5.6)
	D-TRP	<1	<1	5.6 (2.5)
Sheephead	L-TRP	<1	31.2	80.4 (8.3)
	D-TRP	<1	<1	5.6 (0.5)
Redding	L-TRP	1.0	51.2	78.2 (0.8)
	D-TRP	ND†	<1	1.0 (0.0)
Domino	L-TRP	6.0	54.1	85.7 (12.3)
	D-TRP	1.3	1.5	2.1 (1.0)

*Soil samples (10 g) were kept at 30°C for 1, 2 and 5 days after addition of 2250 μg D- and L-TRP. Values are corrected for CO_2-C evolved from the control soil (no compound added). Value in parentheses indicates amount of IAA produced after exposure for 5 days (mg IAA kg^{-1} soil).
†ND: not detected.

While L-TRP has been demonstrated as a precursor of IAA formation, D-TRP may also serve as a substrate in IAA formation in plants (Law, 1987; McQueen-Mason and Hamilton, 1989; Tsurusaki

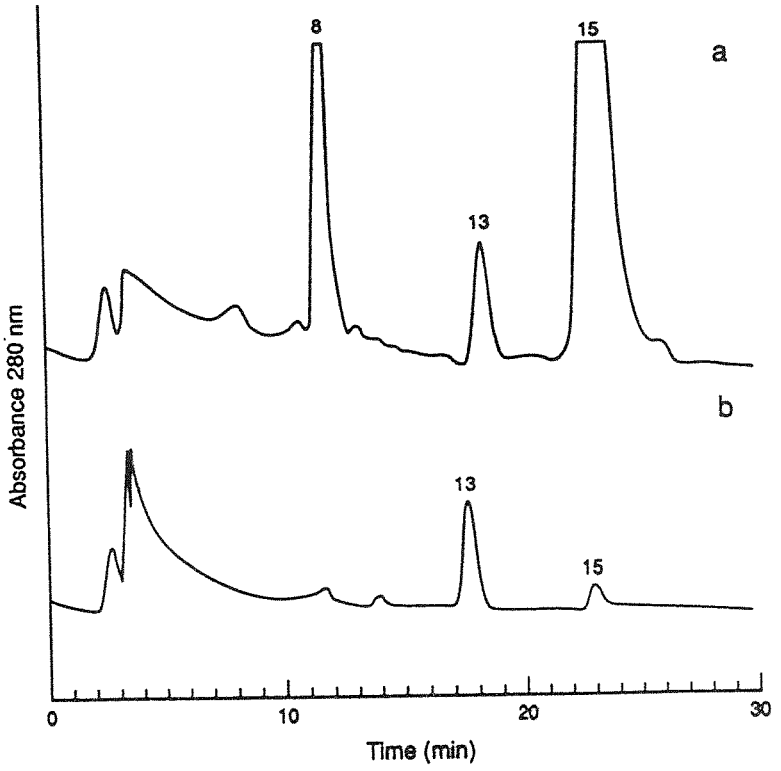

Fig. 6. IS–HPLC chromatograms of (a) an Hesperia soil treated with 800 mg D-TRP exposed for 5 days, and (b) an Hesperia soil treated with 800 mg L-TRP exposed for 3 days. Peaks are identified in Fig. 2.

et al., 1990). Clark (1974) reported that with *Agrobacterium* and *Rhizobium* spp, IAA was formed from both D- and L-TRP additions. In our study, IAA was not detected without the addition of either isomer of TRP. When D-TRP was added to soils in our study, IAA was produced in all soils except the Redding soil (Table 5). A substantial reduction in metabolism of D-TRP when compared with L-TRP was noted in the soils tested when monitored by CO_2-C evolution (Table 5). HPLC analysis indicated that D-TRP-C not recovered in the headspace as CO_2-C was recovered as D-TRP in soil solution (Fig. 6). D-TRP has been reported to be a substrate of IDO, resulting in the synthesis of D-kynurenine which is then utilized for metabolic energy in the cell (Bender, 1989). D-TRP may hold promise as a soil additive for promotion of plant growth and yield by functioning as a slow-release precursor for IAA formation in the rhizosphere.

In summary, our work shows that the L-TRP added to soil is primarily catabolized by the kynurenine pathway and hydroxylation. L-TRP-dependent IAA formation in soil may be regulated by an L-aminotransferase whose broad specificity for L-amino acids also includes L-TRP as a substrate. The activity of the enzyme, L-TAT is stimulated by the amino acids, L-asparagine, L-lysine and L-alanine. This stimulation of L-TAT by amino acids may explain why rhizosphere soil has a higher content of auxin when compared with root-free soil.

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