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Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria

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Abstract

Plant-growth-suppressive activity of deleterious rhizobacteria (DRB) may be due to production of metabolites absorbed through roots. Auxins produced in high concentrations in the rhizosphere by DRB contribute to reduced root growth. Selected DRB able to produce excessive amounts of auxin compounds for suppression of weed seedling growth may be effective for biological control of weeds. The objectives to this study were to assess the ability of DRB originating from weed seedlings to synthesize auxins from L-tryptophan (L-TRP), determine effects of DRB with or without L-TRP on seedling root growth, and characterize auxins produced from L-TRP using high performance liquid chromatography (HPLC). Auxins expressed as indole-3-acetic acid (IAA)-equivalents were produced by 22.8% of the DRB tested based on a colorimetric method. Under laboratory conditions, a DRB isolate classified as *Enterobacter taylorae* with high auxin-producing potential (72 mg L^{-1} IAA-equivalents) inhibited root growth of field bindweed (*Convolvulus arvensis* L.) by 90.5% when combined with 10^{-5} *M* L-TRP compared with non-treated control. Auxin derivatives produced by *E. taylorae* from L-TRP in broth culture after 24 h incubation identified by HPLC included IAA (102 $\mu g\ L^{-1}$), indole-3-aldehyde (IALD; 0.4 $\mu g\ L^{-1}$), and indole-3-lactic acid (ILA; 7.6 $\mu g\ L^{-1}$). Results suggest that providing L-TRP with selected auxin-producing DRB to increase phytotoxic activity against emerging weed seedlings may be a practical biological control strategy.

Introduction

Weed management involves integration of all available measures to reduce propagules in the soil (seedbank), prevent weed emergence and minimize competition from weeds growing with crops (Aldrich, 1984). Typically, dependence on a single weed control approach over time leads to a community of weeds adapted to survive that approach. Recognition of various side-effects of chemical herbicides led to the development of strategies to exploit microorganisms for biological weed control (Templeton and Smith, 1977). One such approach involves selecting microorganisms that can specifically inhibit the development of weed seedlings thereby hindering the establishment of weed populations competing with crops for growth requirements (Kennedy et al., 1991; Kremer et al., 1990). The most promising agents for biological control strategies focused on seeds and seedlings are rhizobacteria characterized by aggressive colonization of and subsequent establishment on roots. Currently, many of the traits that contribute to the competitiveness and colonization of bacteria in the rhizosphere are not well defined (Weller, 1988). Growth-inhibitory bacteria, or deleterious rhizobacteria (DRB), are commonly regarded as being non-parasitic, causing deleterious effects through production of harmful metabolites, which are absorbed by the root (Schippers et al., 1987; Suslow and Schroth, 1982). Metabolites that have been implicated in deleterious activity include hydrogen cyanide (Alstrom and Burns, 1989; Bakker and Schippers, 1987), phytohormones including indole-3-acetic acid (Loper and Schroth, 1986), and unidentified phytotoxins (Bolton et al., 1989; Fredrickson and Elliott,

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1985). Several DRB which reduce seed germination and seedling vigor, have been isolated from roots of various weed seedlings (Kremer et al., 1990).

Diverse soil microorganisms including bacteria (Selvadurai et al., 1991; Zimmer et al., 1988), fungi (Stein et al., 1990) and algae (Finnie and van Staden, 1985) are capable of producing physiologically-active quantities of auxins which may have pronounced effects on plant growth and establishment. For example, Loper and Schroth (1986) reported that two strains classified in the Enterobacteriaceae produced sufficient amounts of indole-3-acetic acid (IAA) to reduce root elongation when inoculated on sugarbeet (*Beta vulgaris* L.) seeds. Microorganisms inhabiting rhizospheres of various plants are likely to synthesize and release auxins as secondary metabolites because of rich supplies of substrates exuded from roots compared with non-rhizosphere soil (Kampert et al., 1975; Strzelczyk and Pokojska-Burdzeij, 1984). Also, Müller et al., (1989) revealed that the plant morphogenetic effects may be a result of different ratios of plant hormones produced by roots as well as rhizosphere bacteria.

There are numerous soil microflora actively involved in the synthesis of auxins in pure culture and in soil (Arshad and Frankenberger, 1991). Some microorganisms produce auxins in the presence of a suitable precursor such as L-tryptophan (L-TRP). The effect of auxins on plant seedlings is concentration-dependent, i.e. low concentrations may stimulate growth while high concentrations may be inhibitory (Sarwar and Frankenberger, 1994). Different plant seedlings respond differently to variable auxin concentration (Holl et al., 1988) and type of microorganisms (Grayston et al., 1990).

The prospect of using rhizobacteria that produce excessive amounts of auxins in the rhizosphere to suppress growth of weed seedlings has not been investigated. Such rhizobacteria should selectively colonize weed seedling roots localizing excess auxin production thereby minimizing potential deleterious effects on crop seedling growth. Selection of deleterious rhizobacteria that specifically associate with germinating weed seeds and seedlings in soil has been demonstrated (Begonia and Kremer, 1994). Successful establishment of such DRB in weed rhizospheres would be more economical than chemical synthesis and/or field application of the growth- suppressive compounds (Arshad and Frankenberger, 1991). Investigations are needed to develop methods to maximize production of weed-suppressive compounds by DRB both in culture and in the rhizosphere. The objectives of this investigation were to: i) assess the ability of rhizobacteria isolated from weed seedling roots to synthesize auxins from L-TRP; ii) determine the effect of selected rhizobacteria on the root growth of various crop and weed seedlings with and without L-TRP; and, iii) tentatively identify and quantify the metabolites produced from L-TRP by rhizobacteria using HPLC.

Materials and methods

Seventy rhizobacterial isolates, originating from intact seedlings of economically important weeds collected from fields in mid-Missouri (Kremer et al., 1990), were compared for auxin (IAA-equivalent) synthesizing potential using L-tryptophan as the auxin precursor. Those isolates showing auxin- synthesizing potential were identified using the API 20E and API Rapid NFT diagnostic kits (Bio Merieux Vitek Inc., Hazelwood, Mo.). Each kit consists of a different set of 20 substrates that are inoculated with a suspension of a bacterial isolate. After a 48-h incubation, reactions are scored positive or negative and, along with the oxidase reaction of the isolate, used to determine a numerical code, which is matched to profiles in the identification codebook accompanying each kit. Classification of the bacteria (Table 1) was verified with gas chromatography-fatty acid methyl ester analysis (Sasser, 1990).

Reagents

L-TRP, IAA, IALD, ILA, tryptophol (TOL), indole-3-acetonitrile (IAN), ferric chloride, sodium phosphate (mono- and dibasic), glucose, yeast extract and agar were obtained from Sigma (St. Louis, MO); methanol, HPLC-grade water, ethyl acetate, perchloric acid and H3P04 from Fisher (Fair Lawn, NJ) and tryptone soy broth from Oxoid (Hampshire, England).

L-TRP broth

Fourteen grams agar, 0.204 g L-TRP and 4 g Difco nutrient broth were combined in 1 L deionized water, mixed and autoclaved at 121°C for 15 minutes.

Bacterial suspension

Individual isolates growing on nutrient agar plates were suspended in sterilized water to an optical density of 0.5 at 500 nm using a spectrophotometer (Ultrospec III, Pharmacia LKB, Cambridge, England).

Table 1. Comparative biosynthesis of auxins (IAA-equivalents) by different rhizobacterial isolates

Isolate[a]	Absorbance[b] (at 535 nm)	IAA-equivalent auxins (mg L^{-1})	Conversion[c] (%)
Klebsiella planticola 7.8.12.10	1.36	86.1	42.2
Pseudomonas sp.P-30	1.29	81.9	40.1
Enterobacter taylorae 3.8.12.7	1.14	72.2	35.4
Alcaligenes faecalis K-2	0.78	49.6	24.3
Pseudomonas paucimobilis Y-4	0.46	29.5	14.4
Xanthomonas maltophilia 7.8.12.7	0.36	22.9	11.2
Enterobacter sp.3.8.12.4	0.31	20.0	9.8
Enterobacter sp.3.8.12.10	0.30	19.4	9.5
Agrobacterium radiobacter N-24	0.23	14.8	7.3
Ts-55	0.20	13.1	6.4
Flavobacterium sp. N-19	0.18	12.0	5.9
Ts-54	0.18	11.7	5.7
7.8.12.8	0.17	10.9	5.3
Pseudomonas sp.P-40	0.14	9.2	4.5
261019	0.13	8.5	4.1
Pseudomonas sp. 23	0.06	4.0	2.0

[a] Numbers following species name are accession codes. Those isolates without species names could not be identified with the diagnostic tests used.
[b] Absorbance determined following addition of Salkowski reagent.
[c] % of L-TRP in culture medium metabolized to auxins.

Salkowski reagent

Two mL 0.5 *M* $FeCl_3$ were mixed with 98 mL 35% perchloric acid to prepare the color developing reagent (Gordon and Weber, 1951).

Phosphate buffer

Solutions of Na_2HPO_4 and NaH_2PO_4 (0.2 *M* each) were prepared separately as stock solutions and appropriate volumes were mixed to prepare a buffer of pH 7.0.

Method of assay

Agar plates were prepared in triplicate for each isolate by transferring 24 mL-TRP broth combined with 1.2% molten agar and 1 mL, bacterial suspension in each sterile petri dish. Plates were swirled for 1 min to obtain uniform distribution of bacteria, allowed to solidify, and placed in the incubator at room temperature (24±1°C) for 24 hours.

At the end of incubation, one agar disk (approx. 0.12 cm^3) was removed from each petri dish using a cork borer and transferred to a test tube containing 3 mL Salkowski reagent. The agar disk rapidly dissolved in the reagent. The mixture was allowed to stand 30 min for color development and color intensity measured at 535 nm spectrophotometrically. The amount of L-TRP-derived auxins in the agar was reported as IAA-equivalents (mg L^{-1} agar) based on IAA standard solutions. Isolate 3.8.12.7, classified as *Enterobacter taylorae* and produced high amounts (72.2 mg L^{-1}) of auxins, was selected for further investigation.

L-TRP and rhizobacterium effects on bindweed seedling growth

Field bindweed (*Convolvulus arvensis* L.) was chosen as a representative weed for preliminary screening of effects of L-TRP and isolate 3.8.12.7 on seedling growth. Seeds were surface sterilized by immersing in 70% ethanol for 2 min, rinsing in sterile water, immersing in 1.25% sodium hypochlorite for 4 min, rinsing 4-6 times with sterile water and blotting on autoclaved filter paper. The surface-sterilized seeds were placed in petri dishes containing 1.5% agar for germination. Petri plates were wrapped with parafilm and incubated at 27°C overnight.

Treatments comprised a water control and different concentrations of L-TRP ranging from 10^{-8} to 10^{-2} *M* separately and in combination with 2 mL inoculum (0.5

Table 2. Effect of tryptophan concentration alone and in combination with inoculum on root growth of bindweed seedlings

Tryptophan (*M*)	Seedling root length	
	Without inoculum (mm)	With inoculum (mm)
Control	47.33 i[z]	10.67 d
10^{-2}	7.17 ab	5.67 a
10^{-3}	15.67 e	6.43 ab
10^{-4}	23.33 f	6.90 ab
10^{-5}	36.00 g	8.23 bc
10^{-6}	44.33 h	8.40 bc
10^{-7}	46.00 hi	11.67 d
10^{-8}	44.33 h	9.47 cd

[z] Treatment means sharing same letter(s) are statistically nonsignificant at $p \leq 0.05$ level according to Duncan's Multiple Range test. Data analyzed as a single set.

O.D. at 500 nm) added at plating. The bacterial isolate was grown on tryptic soy agar (Oxoid Ltd.) for 24 h. All treatments were supplemented with a basal dose of 1% (v/v) sodium phosphate buffer (0.2 *M*) of pH 7.0, 1% nutrient solution (Broughton and Dilworth, 1971) and 0.0025% (w/v) yeast extract before autoclaving. All treatments were replicated three times and applied on the same day.

After solidification of agar, pregerminated seeds of field bindweed with uniform radicle length (2-3 mm) were transferred aseptically to the treatment plates with sterilized forceps. Each plate received 15 seeds. The plates were wrapped with parafilm and incubated at 27°C in the dark for 48 hours. After incubation, seedling root length was measured and recorded (Table 2). Data were subjected to analysis of variance (ANOVA) and treatment means were compared by Duncan's Multiple Range Test (DMR).

Effect of isolate 3.8.12.7 on the growth of different plant seedlings

Seeds of red clover (*Trifolium pratense* L. cv. Kenstar), bindweed, velvetleaf (*Abutilon theophrasti* Medik.), redroot pigweed (*Amaranthus retroflexus* L.), green foxtail [*Setaria viridis* (L.) Beauv.], morning glory (*Ipomoea* sp.), corn (*Zea mays* L. cv. MoH24), soybean (*Glycine max* L. cv. Williams) and wheat (*Triticum aestivum* L. cv. Cardinal) were surface sterilized and allowed to germinate overnight on agar plates in the incubator as described previously. Treatments consisted of control, L-TRP (10^{-5} *M*), 2 mL bacterial suspension (0.5 O.D. at 500 nm) and L-TRP plus 2 mL bacterial suspension (Table 3). Controls received only sterile water and basal dose of buffer, nutrient solution and yeast extract as mentioned earlier. Treatments were combined with 0.9% agar and dispensed into petri plates in three replicates for each test seedling. Ten pregerminated seeds (2-3 mm radicle length) of each plant species were transferred aseptically to the plates containing the amended agars. Corn and soybean were tested using 5 seeds per plate because of their large seed size; therefore, two plates were considered as one replication to equalize the number of seeds tested among all plant species. All plates were sealed with parafilm and incubated at 26°C for 48 h.

After incubation, tap root length was measured and the data were subjected to analysis of variance. Treatment means were compared by using Duncan's multiple range (DMR) test at 95% confidence interval.

HPLC identification of L-TRP-derived metabolites in broth cultures of isolate 3.8.12.7

Metabolites produced in liquid cultures amended with L-TRP in the presence and absence of isolate 3.8.12.7 were analyzed by HPLC. The isolate, grown overnight on tryptic soy agar, was suspended in sterile water from which an 8-ml aliquot was added to 92 mL sterile L-TRP (10^{-5}*M*) in a 125-mL. Erlenmeyer flask to yield a cell concentration of 2.2×10^8 CFU mL. Control flasks received 92 mL sterile L-TRP plus 8 mL, sterile water. The experiment consisted of four treatments in three replicates including control, L-TRP, isolate and L-TRP plus isolate. Each treatment was supplemented with phosphate buffer, nutrient solution and yeast extract prior to autoclaving. All flasks were wrapped with parafilm and incubated at 27°C in the dark.

Extraction of metabolites was conducted at 24 and 48 h incubation. Half the volume (50 mL from each flask was transferred to beakers for filtration through 0.20 μm sterile filter membranes (Acrodisc, Gelman Sciences, Ann Arbor, MI). Flasks containing unused portions of cultures were returned to the incubator. Culture filtrates were extracted with ethyl acetate according to previous methods (Frankenberger and Brunner, 1983) with the following modifications. To 30 mL filtrate with pH adjusted to 8.0, 30 mL ethyl acetate were added in a 100-mL cylinder and mixed for 3 minutes. This procedure was repeated three times and, after reducing the pH to 2.8 to 3.0 with phosphoric acid, extraction was carried out an additional three times. Filtrates were combined in a beaker and evaporated to

Table 3. Effect of tryptophan (10^{-5} *M*) and isolate 3.8.12.7 applied alone or combined on seedling root growth of different plant species

	Root length (mm)								
Treatment	Red clover	Wheat	Bind-weed	Velvet-leaf	Pig-weed	Green foxtail	Morning glory	Corn	Soy-bean
Control	16.3 c[z]	57.8 d	47.5 d	59.7 d	31.2 c	30.9 c	48.2 b	59.1 c	25.7 b
TRP alone	16.7 c	48.6 c	38.8 c	53.2 c	31.5 c	28.4 c	49.6 b	41.5 b	29.3 b
Isolate 3.8.12.7 alone	8.2 b	33.5 b	10.0 b	36.6 c	14.6 b	8.5 b	13.9 a	44.5 b	25.4 b
TRP + isolate 3.8.12.7	3.7 a	19.1 a	4.5 a	17.1 a	7.3 a	3.8 a	9.1 a	30.5 a	19.4 a

Mean values within columns followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's Multiple Range test

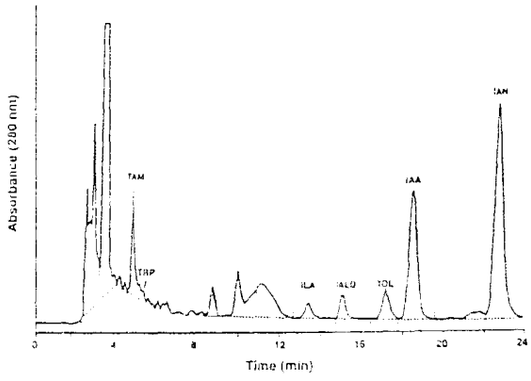

Fig. 1. HPLC chromatogram of an ethyl acetate extract of culture filtrate from *Enterobacter taylorae* 3.8.12.7. L-TRP-L-tryptophan, TAM-tryptamine, ILA-indole-3-lactic acid, IALD-indole-3-aldehyde, TOL-tryptophol, IAA-indole-3-acetic acid, IAN-indole-3-acetonitrile.

near dryness at 45°C on a hot-plate. The residues were dissolved in 3 mL methanol and filtered through a 0.2 μm membrane prior to injection onto the HPLC.

L-TRP-derived metabolites were separated using an isocratic HPLC method on a Beckman Model 338 HPLC system (Beckman Instruments, Inc., San Ramon, CA). The system consisted of two Model 110B pumps operated at a 1 mL min^{-1} flow rate, a Model 507 autosampler with a 100-μL sample loop, and a Model 166 variable-wavelength UV detector set to 280 nm. An ultrasphere octadecyl (C-18) reversed-phase column (ODS, 5 um, Beckman) with dimensions of 250- by 4.6-mm (i.d.) was used. The mobile phase was 35% methanol:water (pH 2.53) at an ambient temperature of 26±1°C. Sample peaks were tentatively identified by comparing their relative retention times with that of a standard chromatogram (Fig.1) using IAN as a reference peak. Identified peaks were quantified by preparing standard curves using peak area for each metabolite separately.

Results

Comparative biosynthesis of auxins (IAA-equivalents) by different rhizobacteria.

Rhizobacterial isolates were tested for L-TRP-dependent biosynthesis of auxins in agar medium. Of 70 rhizobacterial isolates tested, only 16 (22.8%) exhibited presumptive auxin production based on pink color development using Salkowski reagent (Table 1). The remaining isolates showed no detectable activity in utilizing L-TRP for auxin synthesis. The isolates showing positive reactions for auxin production varied greatly in their potential to synthesize L-TRP-dependent auxins (IAA-equivalents), ranging from 4.0 to 86.1 mg L^{-1} of agar medium. Isolate 7.8.12.10 possessed the greatest potential for synthesizing auxins from L-TRP with 21.6-fold higher auxin content compared with isolate #23, the poorest in generating auxins among the 16 auxin-producing isolates (Table 1). This study confirms that L-TRP addition promoted auxin synthesis among a wide range of bacterial isolates. Based on colorimetric determination of auxin synthesis, isolate 3.8.12.7 (*E. taylorae*) with high auxin-generating potential (72.2 mg $^{-1}$ corresponding to 35.4% of added L-TRP) was selected for further investigation.

Effect of L-TRP concentrations on root growth of field bindweed seedlings

The effect of L-TRP concentrations ranging from 10^{-8} to 10^{-2} *M*, alone and combined with bacterial inoculum on root growth of bindweed seedlings was evaluated. Concentrations > 10^{-6} *M* L-TRP significantly inhibited root growth (Table 2). Root length inhibition by L-TRP ranged from 84.8 to 34.5% over the 10^{-2} to 10^{-5} *M* concentrations compared to the control. Addition of only inoculum to the control caused 77.4% reduction in root length of field bindweed. Supplementing L-TRP with inoculum further inhibited root growth. Root growth was significantly reduced by $\geq$ 10^{-6} *M* L-TRP supplemented with inoculum. Based on these results, we selected the 10^{-5} *M* L-TRP level for further study to determine selectivity for growth suppression among weed and crop seedlings.

Effect of isolate 3.8.12.7 on the growth of different plant seedlings

Different plant seedlings reflected a wide range in response (measured as root length) to the application of isolate 3.8.12.7 alone and in combination with L-TRP (Table 3). Root length inhibition by the combination of inoculum and L-TRP ranged from 90.5% for bindweed to 24.8% for soybean compared with their respective controls. Root inhibition of other seedlings varied from 87.4 to 48.4% for green foxtail and corn, respectively. Inoculation with the isolate alone also affected plant seedlings. Root length of weeds was strongly inhibited by 78.9% in bindweed, 72.5% in green foxtail and 71.2% in morning glory, while lesser inhibition was observed in crop plants, including wheat, corn and soybean with 42, 24.7 and 1.2%, compared with their respective controls.

The 10^{-5} *M* concentration of L-TRP variably affected seedling root lengths. Maximum inhibition (29.8%) was observed with corn, bindweed (18.3%) and wheat (15.9%) but root lengths of the weed species velvetleaf and green foxtail was reduced only 10.9 and 8.1%, respectively, when compared with their respective controls. Other seedlings including red clover, pigweed, morning glory, and soybean did not respond significantly to applied L-TRP. In brief, all treatments had a significant negative effect on all plant seedlings except soybean where individual applications of L-TRP or inoculum did not differ from controls.

HPLC identification of auxin metabolites in broth cultures of Enterobacter taylorae *8.12.7*

Analysis of liquid cultures of *E. taylorae* (Table 4), indicated that L-TRP metabolites were not identified in controls after 24 and 48 h of incubation.

Treatments involving the addition of L-TRP alone produced TOL (11.2 μg L^{-1}) over 24 h whose concentration increased to approximately five times after 48 h of incubation. After 48 h, two additional metabolites (IALD and ILA) were identified in the culture supernatant. Only a very low quantity of IAA (11.5μg L^{-1}) was found in the culture flasks containing inoculum alone after 24 h but IALD and ILA (each 2.4 μg L^{-1}) were identified at 48 h of incubation. The synthesis of IAA after 48 h increased 1.4-fold over the 24 h incubation.

The production of auxin derivatives by *E. taylorae* in broth culture was confirmed by HPLC analysis (Fig.1). When *E. taylorae* was incubated with 10^{-5} *M* L-TRP, IAA (102.9 μg L^{-1}), TOL (20.5 μg L^{-1}), IALD (0.38 μg L^{-1}) and ILA (7.6 μg L^{-1}) were released after 24 h with production increasing 2.2-, 2.7-, 46.8- and 4.8- fold, respectively, by 48 h. These results indicated that the major metabolite released in the combination treatment was IAA, which corresponded to 10.8% of added L-TRP.

Discussion

There is ample evidence that numerous soil microorganisms are actively involved in the synthesis of auxins in pure culture and soil (Arshad and Frankenberger, 1991). Generally, microorganisms isolated from the rhizosphere and rhizoplane of various plants are more active in producing auxins than those from root-free soil (Kampert et al., 1975) probably because of higher fertility status (Chandramohan and Mahadevan, 1968). Rhizobacteria generally produce auxins only in the presence of a suitable precursor such as L-TRP (Loper and Schroth, 1986). We found that comparative production of auxins in a minimal salts medium amended with L-TRP was variable among diverse rhizobacterial strains (Table 1). This large variation may be because of inherent properties of the individual bacteria and environmental factors prevailing in the bioassay, which has been reported for analyses of auxins in different soils (Sarwar et al., 1992).

The selected strain originating from the rhizosphere of giant foxtail was typical of bacteria from rhizo-

Table 4. Root growth inhibitory metabolites produced in culture by *Enterobacter taylorae* 3.812.7 detected by HPLC

		Incubation time			
		24h	conversion	48h	conversion[a]
Treatment	Metabolite	($\mu g L^{-1}$)	(%)	($\mu g\ L^{-1}$)	(%)
Control	IAA	nd[b]	-	nd	-
	TOL	nd	-	nd	-
	IALD	nd	-	nd	-
	ILA	nd	-	nd	-
L-TRP	IAA	nd	-	nd	-
	TOL	11.18	0.55	54.93	2.69
	IALD	nd	-	1.71	0.08
	ILA	nd	-	18.57	0.91
INOC	IAA	11.46	0.56	15.57	0.76
	TOL	nd	-	nd	-
	IALD	nd	-	2.37	0.12
	ILA	nd	-	nd	-
L-TRP +	IAA	102.93	5.04	221.32	10.84
INOC.	TOL	20.49	1.00	54.56	2.67
	IALD	0.38	0.02	17.80	0.87
	ILA	7.61	0.37	33.21	1.63

[a] % of L-TRP in culture medium metabolized to auxins.

[b] Not detected.

spheres of many plants, which provide favorable environments for gram negative-bacteria (Curl and Truelove, 1986). The auxin-producing *E. taylorae* rapidly colonized green foxtail seedling roots (data not shown) and is representative of other enterobacterial isolates found in the rhizosphere, many of which actively produce auxins (Haahtela et al., 1990). Recently, an isolate of *E taylorae* detrimental to grassy weed species was reported and is under consideration as a potential weed biocontrol agent (P A Harris, pers. commun.).

L-TRP applied to bindweed seedlings under sterile conditions decreased root length at high concentrations (10^{-5} to 10^{-2} *M*) but had little effect at lower levels (10^{-8} to 10^{-6} *M*). Inoculum added to the medium greatly inhibited root growth, a characteristic of DRB (Schroth and Hancock, 1982) which may be due to metabolite production or aggressive colonization of root surfaces, both affecting normal root physiology. The inhibitory effect of *E. taylorae* on root length was enhanced by addition of L-TRP, likely due to production of auxins (Loper and Schroth, 1986), as this isolate demonstrated a strong auxin producing potential with added L-TRP (Table 1).

Production of different L-TRP metabolites during incubation such as IAA, TOL, IALD, and ILA by *E. taylorae*, was determined by HPLC. HPLC analysis confirmed that IAA was the major metabolite produced along with small amounts of IALD and ILA. Formation of TOL in the bioassay appeared to be the result of chemical transformation of L-TRP since addition of inoculum with L-TRP did not increase the level of TOL, indicating the isolate was unable to produce TOL (Goran et al., 1985). In contrast, several enzymes in plants have been reported to be involved in TOL metabolism (Brown and Purves, 1976). Reduction in bindweed root length was most likely due to production and release of high amounts of IAA disrupting the hormonal balance critical to the growth and development of root tissues (Loper and Schroth, 1986). Production of IAA at 24 h and IALD at 48 h incubation by *E taylorae* occurred in the absence of the precursor, L-TRP (Table 4). This suggested possible involvement

of constitutive enzymes in production of the metabolites, which agrees with Smidt and Kosuge (1978) who reported constitutive production of IAA by the phytopathogen *Pseudomonas syringae pv. savastanoi*. The production of ILA in culture flasks might be partially due to enhancement of L-TRP metabolism by the isolate under reduced aeration in static culture (Kaper and Veldstra, 1958).

Differential root responses to inoculation with *E. taylorae* in the bioassays were most likely due to synthesis of auxins (IAA) above a threshold level specific for each plant type (Scott, 1972). The response of roots to exogenous auxins not only varied across plant type but also among cultivars of the same plant. Our study illustrated a strong deleterious effect of the isolate, *E. taylorae*, which was enhanced with supplemental L-TRP (Table 3). Differential responses of plant seedlings to applied L-TRP under sterile conditions may be attributed to the transformation of L-TRP to TOL which is non-toxic to plants (Goran et al., 1985). This may explain the subtle beneficial effects of L-TRP alone on red clover, pigweed and morning glory whereas root length inhibition of other seedlings might be due to uptake and metabolism of TOL to toxic derivatives by plant enzymes (Brown and Purves, 1976).

Our investigation emphasizes the potential for manipulating the weed seedling rhizosphere using specific DRB with detrimental activity. The strategy of providing L-TRP for use by the introduced DRB as a precursor of auxins appears to be a practical method of increasing phytotoxic activity against emerging weed seedlings in soil especially in the presence of crops relatively insensitive to applied L-TRP such as soybeans (Table 3). The amount and persistence of the precursor required to sustain growth suppressive effects on weed seedlings in the soil is the focus of current studies.

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