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Tryptophan-dependent biosynthesis of auxins in soil

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Abstract

The presence of auxins in soil may have an ecological impact affecting plant growth and development. A rapid and simple colorimetric method was used to assess California soils for their potential to produce auxins upon the addition of L-tryptophan (L-TRP). The auxin content measured by colorimetry was expressed as indole-3-acetic acid (IAA)-equivalents. A substrate (L-TRP) concentration of 5.3 g kg^{-1}, glucose concentration of 6.7 g kg^{-1}, no nitrogen, pH 7.0, 40°C, shaking (aeration) and 48 h incubation time were selected as standardized conditions to assay for auxin biosynthesis in soil. IAA was confirmed as a major microbial metabolite derived from L-TRP in soil by use of high performance liquid chromatography (HPLC). Under standardized conditions, L-TRP-derived auxins in 19 soils varied greatly ranging from 18.2 to 303.2 mg IAA equivalents (auxins) kg^{-1} soil. This study suggests that the phenotypic character of the soil microbiota has more of an influence on auxin production than the soil physicochemical properties (e.g., pH, organic C content, CEC, etc.).

Introduction

Many soils contain compounds which exhibit strong auxin-like activity (Hamence, 1946; Parker-Rhodes, 1940; Sheldrake, 1971; Stewart and Anderson, 1942; Whitehead, 1963) and differ in their IAA synthesizing capacity depending on the fertility status and organic matter content (Chandramohan and Mahadevan, 1968; Hamence, 1946; Stewart and Anderson, 1942). Indole-3-acetic acid (IAA) is considered one of the major auxin-like products of soil.

The literature contains ample evidence that there are numerous microbiota actively involved in the synthesis of auxins in pure culture and in soil (Arshad and Frankenberger, 1992; Bric et al., 1991). Generally, microorganisms isolated from the rhizosphere and rhizoplane of various crops are more active in producing auxins than those from root-free soil (Brown, 1972; Dvornikov et al., 1970; Kampert et al., 1975; Purushothaman et al., 1974; Roberts and Roberts, 1939; Strzelczyk et al., 1977; Strzelczyk and Pokojska-Burdzeij, 1984). Auxin production in rhizosphere soil is most likely due to abundance of substrates and microorganisms. Narayanaswami and Veerraju (1969) found a 3-fold higher IAA content in the rhizosphere compared with non-rhizosphere environments. Likewise, Rossi et al. (1984) found that auxin-like components were greater in the rhizosphere soil of maize compared with non-rhizosphere soil, especially during seedling emergence. While a higher percentage of microorganisms isolated from rhizosphere soil than root-free soil are capable of synthesizing auxins, they can only influence plant growth if the released auxin is subjected to plant uptake and not metabolized

by other micoorganisms (Arshad and Frankenberger, 1991).

L-Tryptophan (L-TRP) is considered as the physiological precursor of auxins in higher plants and microbial biosynthesis. Purushothaman et al. (1973, 1974) reported active synthesis of auxins in soil amended with TRP. Frankenberger and Brunner (1983) confirmed this observation and provided unequivocal proof with HPLC-MS that IAA was produced in soil when incubated with TRP. In addition, Frankenberger and Poth (1988) separated and purified a soluble α-ketoglutarate-dependent L-TRP transaminase enzyme from cell-free extracts of a rhizobacterial isolate associated with *Festuca octoflora* Walt. This enzyme catalyzed the conversion of L-TRP to indole-3-pyruvic acid (IPyA), an intermediate of IAA synthesis.

The synthesis of IAA with application of TRP to soil can have dramatic effects on plant growth. Frankenberger and Poth (1987) reported that the growth of Douglas fir was dramatically increased by inoculation with the ectomycorrhizae, *Pisolithus tinctorius*, when L-TRP was applied at the rate of 0.34 to 34 μg kg^{-1} soil. In a culture study, this fungus was found capable of synthesizing auxin from L-TRP. Similarly, a physiological response of radish (*Raphanus sativus*) to TRP applied to soil under optimal nutritional conditions was observed by Frankenberger et al. (1990). We reported a significant positive effect of L-TRP comparable with pure auxins (IAA, indole-3-acetamide, indole-3-lactic acid, etc.) on growth parameters of radish when applied at low concentrations (ng to μg kg^{-1} soil) at the seedling stage.

Although soils have been tested for their auxin content in the presence or absence of TRP, a thorough investigation dealing with systematic studies of factors such as nitrogen and carbon levels stimulating the production of auxin in soil has not yet been conducted. Thus, the objectives of this study were to evaluate the environmental factors affecting auxin biosynthesis in soil and to assess California soils differing in their physicochemical properties for their potential to synthesize substrate-dependent auxins under standardized conditions.

Materials and methods

Surface samples (0–15 cm) of soils varying in physicochemical properties such as pH (5.91 to

Table 1. Properties of soils used and substrate (L-TRP)-dependent auxin biosynthesis

Soil	pH	Organic C (g kg^{-1})	Total N (g kg^{-1})	Cation exchange capacity (cmol[+] kg^{-1})	Clay %	Sand %	Waterholding capacity (mL kg^{-1})	IAA-equivalents (mg kg^{-1})
Santa Lucia	6.31	26.1	2.40	0.359	48	20	853	193.8
Sheephead	6.87	22.8	1.32	0.139	26	56	436	154.5
Tollhouse	6.82	21.0	0.83	0.111	21	67	424	106.2
Fallbrook	6.60	16.5	1.90	0.155	26	51	474	229.0
Cibo	7.42	16.2	1.94	0.455	54	27	620	103.2
Kitchen Creek	7.36	15.2	0.54	0.139	23	62	333	47.8
Altamont	7.45	11.6	1.68	0.268	46	21	540	303.2
Garey	5.91	11.1	1.31	0.083	21	47	363	113.7
Kimberly	7.61	10.9	1.21	0.112	26	67	400	263.2
Los Banos	7.96	9.7	0.96	0.124	37	20	800	99.2
Pico	8.55	9.1	1.18	0.177	26	60	500	137.9
Ramona	6.31	7.8	1.69	0.131	26	48	402	186.6
Oildale	7.70	7.1	0.87	0.091	23	66	353	168.6
Oceano	7.03	4.6	0.90	0.063	16	76	285	76.1
Domino	8.55	4.4	1.04	0.168	29	43	432	268.2
Redding	8.56	3.8	0.68	0.254	64	25	513	65.9
Kesterson	8.06	3.7	2.46	0.478	16	61	590	18.2
Milham	8.24	3.5	0.87	0.170	34	45	470	158.3
Hesperia	7.22	2.8	0.66	0.060	18	74	333	253.9

8.56), organic carbon (2.8 to 26.1 g kg^{-1}), total nitrogen (0.54 to 2.46 g kg^{-1}), cation exchange capacity (0.060 to 0.478 cmol $[NH_4^+]$ kg^{-1}), texture (16 to 64% clay and 20 to 76% sand) and water holding capacity (333 to 853 mL kg^{-1} soil) were collected (Table 1). The samples were air-dried and sieved (2-mm) prior to use.

Reagents

L-Tryptophan and IAA were obtained from Sigma (St. Louis, MO); ferric chloride and glucose from Mallinckrodt (Paris, KY); ammonium nitrate (NH_4NO_3), calcium chloride, perchloric acid, sodium phosphate (mono and dibasic) and trichloroacetic acid from Fisher (Fair Lawn, NJ); HPLC-grade methanol from Burdick and Jackson (Muskegon, MI); and HPLC-grade water from Baker (Phillipsburg, NJ).

Phosphate buffer (0.2 M)

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

Salkowski reagent

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

Method of assay

The following parameters were studied to determine optimum auxin production in soils under predetermined standardized conditions: substrate concentrations (L-TRP) from 0 to 13.3 g kg^{-1} soil; glucose from 0 to 66.7 g kg^{-1} soil; N as ammonium nitrate (NH_4NO_3) from 0 to 26.7 g kg^{-1} soil; pH from 6.5 to 8.5; temperature from 5 to 50°C (static incubation); incubation time from 0 to 72 h; and aeration (shaking vs. static).

Routinely, the L-TRP-derived auxin production assay was performed as follows: 3 g of soil were placed in a 50-mL Erlenmeyer flask and treated with 6 mL of phosphate buffer (0.2 *M*, pH 7.0) and 4 ml of a L-TRP solution (5.3 g L-TRP kg^{-1} soil). The flasks were covered with parafilm and incubated in darkness at 40°C for 12 h on a shaker (~150 rev min^{-1}). After incubation, the flask contents were treated with 2 mL of trichloroacetic acid (5 g 100 mL^{-1} H_2O) to terminate the reaction and 1 mL of calcium chloride (0.5 *M*) to facilitate filtration. The soil solution was then filtered through Whatman filter paper No. 2.

Investigations were also undertaken to determine abiotic production of auxin derived from L-TRP added to soil. The soil was sterilized by autoclaving three times at 121°C for 1 h on alternate days and the buffer solution was autoclaved at 121°C for 15 min, while L-TRP and glucose solutions were filter-sterilized through 0.22 μm pore membrane filters (Type GS, Millipore Corp., Bedford, MA) and added to soil under aseptic conditions.

Colorimetric determination of auxins

Three mL of the soil filtrate were added to a test tube and treated with 2 mL of the Salkowski reagent (color developing reagent). The mixture was allowed to stand for 30 min for color development. The intensity of the color developed was measured at 535 nm by using a spectrophotometer (Milton Roy, Rochester, NY). The amount of L-TRP-derived auxin content in soil was reported as IAA-equivalents (mg kg^{-1} soil) using standard IAA solutions.

High performance liquid chromatography

The presence of IAA in the soil filtrate was confirmed by an HPLC-UV analysis, according to the procedure described by Frankenberger and Brunner (1983). Briefly, the filtrate was partitioned with three volumes of acidic (pH 2.8–3.0) ethyl acetate (10 mL). The ethyl acetate fractions were separated from the aqueous phase in a 100-mL separatory funnel, reduced in volume and dissolved in 2 mL of methanol. Extracts were examined by reverse phase, ion-suppression HPLC with detection at 280 nm. An R-Sil C_{18} (ODS) 5 μm, 4.6 by 150 mm column (All-

tech Associates, Deerfield, IL) was employed for the analysis.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and treatment means were compared by determining the least significant difference (LSD) as described by Steel and Torrie (1980).

Results and discussion

Colorimetric methods have long been employed for the detection of IAA synthesized by plants and microorganisms, but have received little attention for soil analysis of IAA. A colorimetric method was used to determine auxin synthesis in 19 California soils and the effects of various environmental factors on TRP-derived auxin formation in three soils were thoroughly evaluated. The colorimetric method also forms chromophoric complexs with other auxin compounds synthesized by soil bacteria in addition to IAA, and thus the unit, IAA equivalents, were used to express auxin production.

Substrate concentration

Three soils were chosen to evaluate environmental conditions affecting auxin synthesis upon the addition of the precursor, L-TRP. Auxin biosynthesis in the Domino, Oildale, and Sheephead soils substantially increased upon the addition of up to 5.3 g L-TRP kg^{-1} soil (Fig. 1). However, L-TRP added beyond this amount (5.3 g kg^{-1} soil) did not increase auxin production significantly in soils. The presence of IAA in these soils was confirmed by HPLC-UV analysis (Fig. 2). Enhancement of soil auxin synthesis in response to 5.3 g kg^{-1} soil L-TRP was 61-, 30- and 12-fold compared with the untreated Domino, Oildale, and Sheephead controls, respectively. Sheephead soil which was relatively rich in native auxin content yielded 2.2- and 2.7-fold more IAA equivalents compared with Oildale and Domino soils, respectively, in the absence of L-TRP, but was the least

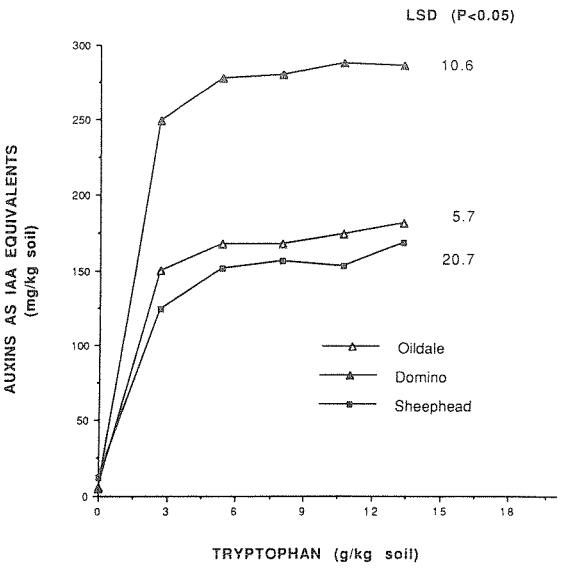

Fig. 1. Influence of substrate (L-tryptophan) concentration on production of auxin in soil when incubated in the dark (pH 7.0, 6.7 glucose kg^{-1} soil, no nitrogen for 12 h at 40°C).

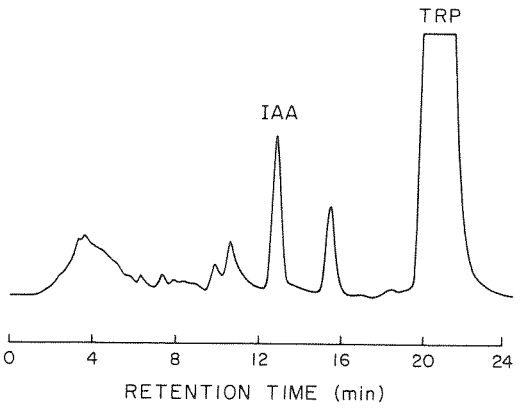

Fig. 2. HPLC chromatogram of an acidic extract of soil. Column, 5-μm R-Sil C_{18} (ODS); flow rate, 1 mL min^{-1}; mobile phase, isocratic 60% methanol in water acidified to pH 2.8 with H_3PO_4; sample injected, 20 μL; detection, 280 nm.

responsive to the L-TRP treatment. The most responsive was the Domino soil which contained 1.6- and 1.8-fold more IAA equivalents compared with the Oildale and Sheephead soil, respectively, in the presence L-TRP (5.3 g kg^{-1} soil). No significant increase in auxin content was observed in response to L-TRP when added at concentrations >5.3 g kg^{-1} soil which might be attributed to saturation of the reaction mix-

ture with the substrate. Similar results were found by Kaper and Veldstra (1958) who reported that production of IAA by *A. tumefacines* was increased by TRP addition up to 0.15%, but the reaction rate rapidly leveled off with higher concentrations of TRP.

Glucose concentration

The addition of glucose at 6.7 g kg^{-1} soil caused a rapid increase in L-TRP-dependent auxin synthesis in all three soils (Fig. 3). Further addition of glucose (>6.7 g kg^{-1}) either resulted in no change in the reaction rate or decreased auxin synthesis by approximately 18% in the case of the Domino soil. The increase in auxin synthesis upon application of 6.7 g of glucose kg^{-1} soil was 10.6-, 3.5- and 3.1-fold greater over the untreated controls of Domino, Oildale, and Sheephead soils, respectively. Sheephead soil yielded 2.0- and 1.3-fold more IAA equivalents than the Domino and Oildale soils, respectively, in the absence of glucose, whereas, its presence (6.7 g kg^{-1} soil) resulted in 2.0- and 1.7-fold greater IAA equivalents in the Domino soil compared with the Oildale and Sheephead soils, respectively. The enhancement of L-TRP-derived auxin biosynthesis by the addition of glucose to soils (Domino, Oildale and Sheephead) at a 6.7 g kg^{-1} soil level is most likely due to the enhanced heterotrophic bioactivity in soil by serving as a carbon and energy source. The Domino soil had a greater response to glucose which may be related to its lower organic carbon content compared with the other two soils (Oildale and Sheephead).

Nitrogen concentration

The addition of N as NH_4NO_3 had a strong inhibitory concentration-dependent effect on L-TRP-derived auxin formation in soil (Fig. 4). The highest bioactivity for auxin synthesis was observed in the absence of N in all three soils. The lowest level of auxin synthesis following an exogenous application of N was detected in soils with the highest N application (26.7 g kg^{-1} soil) which were 72.2, 66.7, and 61.8% less than the untreated Domino, Sheephead, and Oildale controls, respectively.

A decrease in auxin production upon the addition of N (NH_4NO_3) could be attributed to less utilization of the added substrate (L-TRP) in the presence of available N (as NH_4NO_3) and C

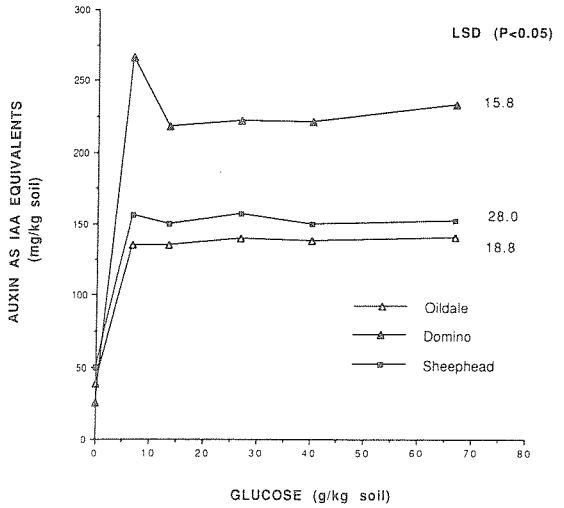

Fig. 3. Influence of glucose concentration on production of auxin in soil when incubated in the dark (pH 7.0, 5.3 L-TRP kg^{-1} soil, no nitrogen for 12 h at 40°C).

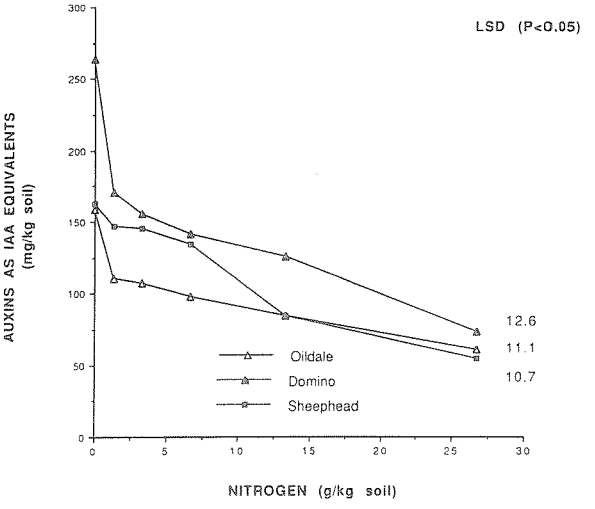

Fig. 4. Influence of NH_4NO_3 concentration on production of auxin in soil when incubated in the dark (pH 7.0, 5.3 L-TRP kg^{-1} soil, 6.7 g glucose kg^{-1} soil for 12 h at 40°C).

(as glucose) for microbial growth. A similar response to the addition of N was observed by Narayanaswami and Veerraju (1969).

pH

Tests with the three soils used in this study showed that, when soil samples were treated with phosphate buffer as in the assay method described, the pH of the soil-buffer mixture did not deviate more than ±0.1 pH unit. Variation in soil pH had a significant effect on L-TRP-derived auxin biosynthesis in soils (Fig. 5). Although auxin production from L-TRP was active over a wide range of the buffer pH, the optimal activity was observed at pH 7.0. At this pH, the Domino soil had 1.7-fold higher IAA equivalents than that of Oildale and Sheephead soils. Auxin biosynthesis in the Domino soil was more sensitive to an alkaline pH than the other two soils with a dramatic decrease in auxin production at pH above 7.0. The maximal L-TRP-derived auxin synthesis occurred at pH 7.0 which is also considered the optimal and most favorable pH for microbial activity. Other investigators have assayed auxin synthesis in soils maintained at pH ~7.0 (Chandramohan and Mahadevan, 1968; Kaper and Veldstra, 1958; Magie et al., 1963; Narayanaswami and Veerraju, 1969; Purushothaman et al., 1973).

Temperature of incubation

The effect of temperature on L-TRP-derived auxin biosynthesis was evaluated under static conditions. Maximal auxin producing bioactivity was observed at 40°C (Fig. 6). Although biosynthesis of auxin slightly increased up to 20°C, production rapidly increased from 25 to 40°C and then dropped off at 50°C. Incubation at 40°C promoted auxin biosynthesis in soil by 24.8-, 16.8-, and 13.9-fold compared with incubation at 5°C, and by 2.0, 1.4- and 1.9-fold compared with incubation at 50°C in the case of Oildale, Domino, and Sheephead soils, respectively. Maximal bioactivity for L-TRP-dependent auxin synthesis at 40°C indicates that the mesophilic soil microbiota are active in this transformation. Higher temperatures (>50°C) might have resulted in denaturation of the enzyme(s) involved in auxin synthesis since the auxin content was reduced sharply above 40°C. Chalvignac (1971) and Chalvignac and Mayaudon (1971) selected a

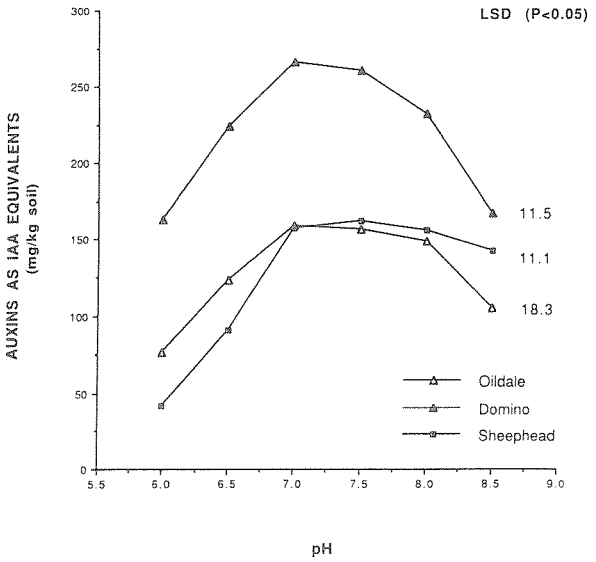

Fig. 5. Influence of pH of buffer on production of auxin in soil when incubated in the dark (5.3 g L-TRP kg^{-1} soil, 6.7 glucose kg^{-1} soil, no nitrogen for 12 h at 40°C).

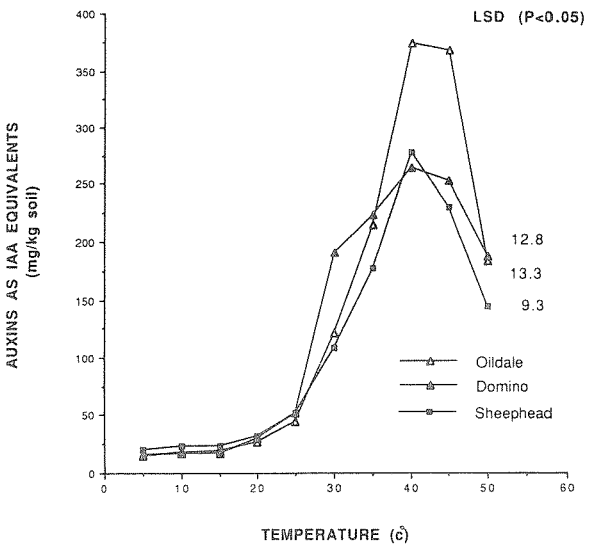

Fig. 6. Influence of temperature of incubation on production of auxin in soil (pH 7.0, 5.3 g L-TRP kg^{-1} soil, 6.7 g glucose kg^{-1} soil, no nitrogen for 12 h).

temperature of 37°C for assaying the enzyme(s) involved in auxin synthesis.

Aeration (static vs. shaking)

Shaking at 150 rev min^{-1} resulted in >2-fold greater L-TRP-derived auxin synthesis compared with static incubation under the optimal assay conditions (Fig. 7). The magnitude of increase in IAA equivalents in response to shaking was 2.5-, 2.2-, and 2.2-fold greater than the respective static controls of Domino, Oildale, and Sheephead soil, respectively. Under static incubation, the Domino soil yielded 1.4- and 1.6-fold more auxin content compared with Oildale and Sheephead respectively, whereas, under shaking conditions, the increases were 1.6 and 1.8-fold, respectively. Shaking of the reaction vessel promotes oxygen availability which is required for some enzymatic transformations of L-TRP to auxins. For instance, the intermediates, indole-3-acetataledhyde and indole-3-acetamide derived from L-TRP require oxidation for their conversion to IAA (Reinecke and Bandurski, 1987).

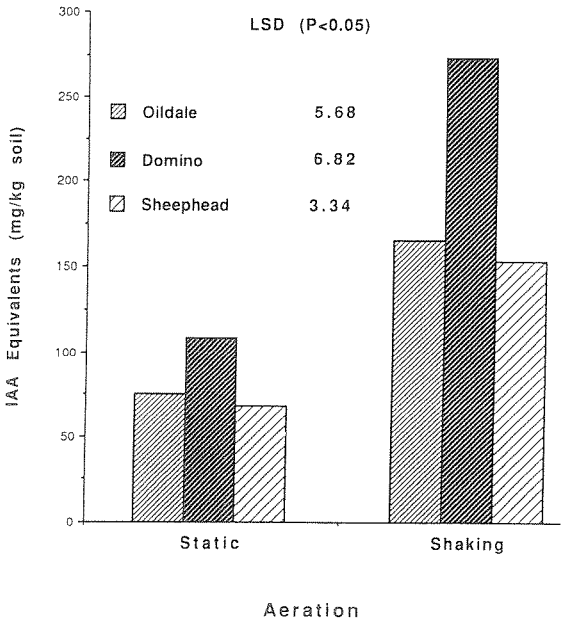

Fig. 7. Influence of aeration on production of auxin in soil (pH 7.0, 5.3 g L-TRP kg^{-1} soil, 6.7 g glucose kg^{-1} soil, no nitrogen for 12 h at 40°C).

Enhanced microbial production of auxin derived from TRP by shaking a synthetic medium was also reported by Bailey and Gentile (1962).

Time of incubation

A time course study revealed that L-TRP-dependent auxin production increased up to 48 h in all three soils (Fig. 8). The lack of linearity of the time course is most likely a result of several reaction rates of the enzymes involved in the conversion of TRP to IAA. Oildale and Sheephead soils yielded similar levels of IAA equivalents after 24 h of incubation, while auxin synthesis was greatest in the Domino soil. At >48 h of incubation, a decrease in soil auxin content (Domino and Oildale soils) was noted which may be the result of rapid decomposition of auxin exceeding formation. A standardized 12-hour incubation period was chosen to assay other California soils to allow a rapid assay for analysis and to avoid subsequent dilutions to read the color intensity with a spectrophotometer within the working range. The time course for the maximal level of auxin production in soils tested here is less than that reported by Brown and Walker (1970) who found a 7-d maximal rate with shaken *Azotobacter chroococcum* cultures.

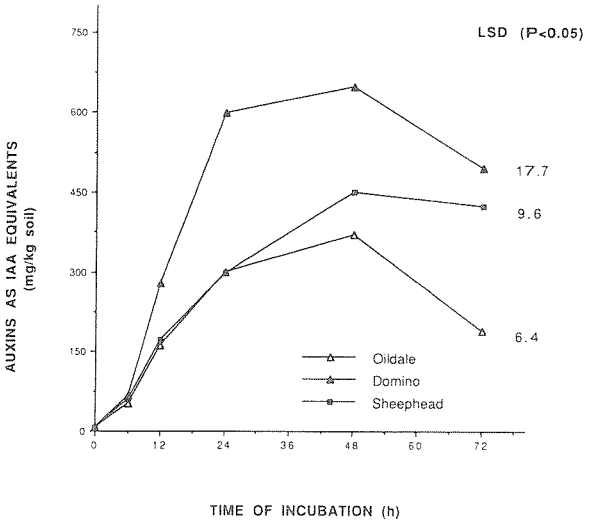

Fig. 8. Influence of time of incubation on production of auxin in soil (pH 7.0, 5.3 g L-TRP kg^{-1} soil, 6.7 g glucose kg^{-1} soil, no nitrogen at 40°C).

Auxin biosynthesis in California soils

This study indicated that auxin synthesis in soils can be increased under the following conditions:

microorganisms and rich with organic C released as root exudates. Because of the intimate contact between the rhizosphere microbiota and plant roots, it is highly likely that substrate-dependent

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