Biotransformation of Tryptophan to Indole-3-acetic Acid by *Xanthomonas campestris* pv. *campestris*

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Xanthomonas campestris pv. *campestris* produced indole-3-acetic acid when grown in liquid culture in the presence of certain minimum levels of tryptophan.

Introduction

Xanthomonas campestris pv. *campestris* is the etiological agent of black rot of crucifers, a disease of major economic importance which is a threat to the production of cruciferous crops in many areas of the world [1]. During a recent investigation of this pathogen for the production of phytotoxic metabolites in liquid culture we observed the biotransformation of certain amino acid constituents of the culture medium to their corresponding nor-carboxylic acids *via* a deamination process [2]. Several of these carboxylic acids, noteably 3-methylthiopropanoic acid and *trans*-3-methylthiopropenoic acid were previously reported as products of other xanthomonads and were described as phytotoxins [3, 4]. However, the relatively weak phytotoxicity expressed by high concentrations of these compounds is apparently due to the generation of excessively high hydrogen ion activities [2]. The carboxylic acid phytohormone indole-3-acetic acid (IAA) is a compound which expresses more selective and potent biological activity than those carboxylic acids previously described as products of *Xanthomonas campestris* [2–4]. IAA is known to be produced *in vitro* by a wide range of microorganisms, including plant pathogenic members of the Pseudomonadaceae [5]. Although in many cases this biochemical trait is unlikely to be involved in hyperauxiny during infection, in the case of *Pseudomonas savastanoi* (olive knot) IAA has been demonstrated to behave as a virulence factor in disease causation [6]. Here we report the results of a preliminary investigation of *X. c.* pv. *campestris* for the production of IAA.

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Results and Discussion

X. c. pv. *campestris* was cultured in modified Watanabe broth [2] in which l-tryptophan, at a concentration of 0.5, 0.05, or 0.005 g/l (media 1, 2, and 3 respectively), was substituted for l-methionine. HPLC analyses of incubated cultures demonstrated that tryptophan was rapidly utilised by the bacteria, such that its concentration was reduced to 2.9% of the original level of 0.5 g/l after 48 hr. After the same time period tryptophan was no longer detected in cultures initiated at a concentration of 0.05 g/l. The period of most rapid bacterial growth on all three media was between 24 and 48 hr. During this period the bacteria grown on media 1, 2, and 3 increased at very similar rates to give essentially the same cell density (Table). Coincidentaly, at time 48 hr TLC (Si gel, Et_2O) of EtOAc extracts of cultures grown on medium 1 afforded a UV quenching band, which possessed the same R_f value and UV absorption spectrum as an authentic IAA standard. Aliquots (10 µl) of 2–4 day culture filtrates (medium 1) were injected into a reversed phase HPLC system which enabled the resolution of a metabolite which co-chromatographed with authentic IAA. The identification of IAA as a component of cultures of *X. c.* pv. *campestris* was confirmed by MS and ^1H NMR analyses.

IAA was readily detected in cultures grown on media 1 and 2 by direct injection of the filtered culture fluid into the HPLC, and was quantified by comparison of the UV absorbance of a standard solution (monitored at 280 nm). After 96 hr the yield of IAA from cultures grown on medium 1 was ca 3 mg/l. This is equivalent to a conversion efficiency of 0.6% from 0.5 g/l of tryptophan precursor. IAA production on medium 2 after incubation for the same time period was approximately one order of magnitude less than on medium 1. Thus IAA yields from cultures grown on media 1 and 2 are proportional to the concentration of tryptophan precursor supplied to the bacteria. Cultures grown on medium 3 did not contain detectable levels of IAA despite attempts to isolate it by extraction of the acidified culture filtrate and subsequent concentration of the organic phase prior to HPLC analysis. In view of the amount of bacterial growth which occurred in this medium (Table), it seems probable that the relatively meagre amount of amino acid supplied to medium 3 would be utilised largely if not solely for primary metabolic events.

Table. Bacterial growth, utilization of tryptophan and production of IAA by *Xanthomonas campestris* pv. *campestris* in three different media.

Time (hr)	0			24			48			96		
Medium[a]	1	2	3	1	2	3	1	2	3	1	2	3
Growth ($A_{640\ nm}$)	0.25	0.25	0.28	0.42	0.41	0.40	1.55	1.56	1.52	1.68	1.30	1.24
Tryptophan concn[b]	100	100	100	69	43	Nd	2.9	Nd	Nd	Nd	Nd	Nd
IAA concn ($A_{280\ nm}$)[c]	Nd	Nd	Nd	Nd	Nd	Nd	50.5	1.23	Nd	234[d]	22.5	Nd

[a] Media, 1, 2 and 3 were composed of Watanabe broth [2] containing 0.5, 0.05 and 0.005 g/L of l-tryptophan instead of methionine
[b] Expressed as percentage of original level
[c] Expressed as area units of absorption peaks
[d] Equivalent to 3 mg/l of culture filtrate
Nd = not detected

From a consideration of the very low levels of IAA found in healthy plant tissues (10^{-6}–10^{-7} M), hormonal imbalance might be expected to occur if only trace amounts of this compound were produced by a pathogen during infection. A peak co-chromatographing (HPLC) with authentic IAA was detected in extracts of healthy leaf tissue of cabbage (*Brassica oleracea* L.) cv. Golden Acre. An examination of diseased leaf tissue failed to indicate the presence of IAA, although in this case the HPLC profile contained additional peaks, with retention times similar to that of IAA, which could have masked that corresponding to IAA if it was present at concentrations equivalent to those in healthy tissue. Notwithstanding, failure to detect increased levels of IAA in infected tissue is not unexpected since any production by the pathogen *in vivo* would be dependent on the availability of free tryptophan.

The biotransformation of tryptophan to IAA by *X. c.* pv. *campestris* is analogous to that previously described for the conversion of phenylalanine to phenylacetic acid [2], itself a naturally occurring auxin [7]. Discussion on the biogenesis of IAA by *Xanthomonas campestris* is complicated by the fact that two different biosynthetic routes to IAA are known to operate in species of the closely related genus *Pseudomonas* [5, 6]. The production of IAA has not previously been reported within the genus *Xanthomonas*. All four pathovars of *Xanthomonas campestris* previously examined possess the ability to carry out the biotransformation of methionine to trans-3-methylthiopropenoic acid [2]. Pathovars of *Xanthomonas campestris* are very similar metabolically, as well as immunologically, and can only be distinguished with certainty by their host reaction [8]. It seems likely that the ability to produce IAA is widespread within *X. campestris* and, indeed, may be a characteristic of all 111 pv.'s of this species.

Experimental

Bacterial cultures

Xanthomonas campestris pv. *campestris* was a gift from Louie DiNitto, ARCO Seed Co., Brooks, Oregon U.S.A. Watanabe broth had the composition as in [2] except that l-tryptophan at concentrations of 0.5, 0.05, and 0.005 g/l was substituted for methionine to give media 1, 2, and 3 respectively. 500 ml Erlenmeyer flasks containing 150 ml of medium received 2% (volume) inoculum from a 72 hr liquid shake culture in late logarithmic phase which contained *ca* 10^8 colony forming units/ml. Incubation was at 28 °C on a gyratory shaker operating at 170 rpm.

Isolation and identification of IAA from bacterial cultures

After incubation for 3–4 days cultures were harvested (pH *ca* 6), acidified to pH 3.5 (6 N HCl) and partitioned twice against equal volumes of EtOAc. The organic phases were bulked and reduced to dryness *in vacuo*. The residue was taken up in a small volume of solvent for TLC (Si gel, Et_2O) to afford IAA, R_f 0.68. IAA was further purified by HPLC [reversed phase C-18 uBondapak, MeCN–H_2O (acidified to pH 3.5 with HCl), 4:7; flow rate 1.1 ml/min]. EIMS and ^1H NMR (CD_3OD) were identical to those of authentic IAA (ex Sigma Chem. Co.). IAA was quantified by direct HPLC analysis of aliquots (10 µl) of culture filtrate (0.2 µ, Gelman filter disc). IAA Rt = 6.06.

Examination of cabbage tissue for IAA

Cabbage leaves (cv. Golden Acre) were inoculated at a number of sites with 1 µl of a suspension of *X. c.* pv. *campestris* (*ca* 6.4×10^5 colony forming units/ml). Three leaf discs (1 cm diameter) of infected or non-infected control leaves (*ca* 160 mg fr. wt) were homogenised in 50% aqueous Me_2CO and the extracts reduced to a very small volume *in vacuo* at 40 °C. The residue was partitioned between Et_2O and H_2O adjusted to pH 3.5 (HCl). The Et_2O phases were reduced to dryness and taken up in a small volume of MeOH for HPLC (column as above, MeCN–H_2O, 1:9 to MeCN–H_2O, 5:5 over 20 min., 1.0 ml/min., Rt = 24.44).

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