The organism is a short rod (1·60 × 0·56 μm) with rounded ends, gram negative, non-spore former, non-acid fast, encapsulated and motile by a single polar flagellum. The colonies on P.D.A. are circular with entire margin, smooth, shining, moist with yellow pigment typical of the genus *Xanthomonas*.

The methods for biochemical and physiological characters were as described by Dye and Lelliott[1]. The results are as follows :

Gelatin liquefied, starch hydrolysed ; H_2S produced ; litmus milk cleared ; nitrates not reduced ; M.R. and V.P. tests negative ; indole negative ; catalase positive ; oxidase negative ; lecithinase and tyrosinase positive ; citrate utilised ; Tween 80 hydrolysed ; NaCl tolerance upto 3%. Acid but no gas from glucose, sucrose, fructose, galactose, lactose, trehalose, xylose, arabinose, cellobiose, maltose, ribose and mannose but not from mannitol, dulcitol, sorbitol, salicin, inocitol, melezitose, raffinose and rhamnose. The organism grows well on Kado's D5 medium[2] specific for xanthomonads but not on Kado's D4 medium specific for plant pathogenic pseudomonads. The optimum temperature for growth is 28°–30° C, optimum pH is 7·0. It is a strict aerobe.

In host range studies carried out under optimum conditions of infection with an average humidity of 85% and air temperature ranging from 22°–28° C, the organism infects *T. populnea* and *T. lampas*.

Since all the physiological and biochemical characters of the organism mentioned above conform to those of *Campestris* group of the genus *Xanthomonas* and as per International Standards for naming plant pathogenic bacteria advocated by Dye *et al.*[3], the organism is named as *X. campestris* pv. *thespesiae* pv. nov. The culture has been deposited in ITCC (Indian Type Culture Collection, Division of Mycology and Plant Pathology, New Delhi) under Accession No. ITCC P–33.

The authors are grateful to Dr. S. H. Godbole, Deputy Director and Head, Department of Microbiology, for his interest and encouragement. Thanks are also due to the Director, MACS, Pune, for providing necessary facilities.

June 10, 1981.

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ORGANOGENESIS IN CALLUS CULTURES OF *CROTALARIA MEDICAGENIA* LAMK.

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The root or shoot neoformation in undifferentiated cultures is dependent on a specific equilibrium between the auxins and the cytokinins ratio[1]. Cytokinins induced shoot buds formation in many cultures, as first shown by Skoog and Miller[2]. Several substituted purines bases have shown cytokinin activity; among them N_6 monosubstituted purines have proved most effective for bud induction even in root callus tissues[3]. Various species of *Crotalaria* in tissue culture have been studied for their organogenesis[4,5]. So far there is no report of organ induction from undifferentiated callus mass of *Crotalaria medicagenia*. This paper describes the root and shoot formation in callus cultures of *C. medicagenia* subjected to the influence of some synthetic cytokinins.

Callus tissues were raised from stem segments (5–10 mm) of *C. medicagenia* on modified Murashige and Skoog (MS)[5] medium supplemented with 2,4-D (2,4-dichlorophenoxy acetic acid) and kinetin. The calli were maintained for 18 months in dark growth chambers at 28° + 2° C.

Various cytokinins, *viz.*, kinetin (Kn), benzyl aminopurine (BAP), adenine (Ad) and adenine sulphate (Ads), and auxins, alpha-naphthalene acetic acid (α–NAA), 2,4-D were tested at different concentrations to study the organ formation. Differentiating cultures were maintained for a 16 hr light (3,000 lux) and 8 hr dark cycle. The temperature in the light cabinet was 30° C during light period and 28° C in dark period.

Initially 5–10 mm stem segments from different regions of the seedling were transferred in 2,4-D containing medium for callus induction. Callus initiation was observed on NAA and 2,4-D (each in 0·5 mg/l) containing MS medium within 5 days. Calli grew well after subsequent two or three subcultures. The callus was dark brown to yellowish brown, granular and friable. Frequent lateral root formation was observed on medium supplemented with NAA (5·0 mg/l) and Kn (0·1 mg/l) in explant and callus tissues. These roots were long, fibrous and brown in colour.

Preliminary tests of cytokinins for differentiation showed that callus could be induced bud formation very readily on medium contained high Kn (2·5 mg/l), with or without addition of NAA (0·1 mg/l). Different concentrations of BAP, Ad and Ads were tested

TABLE I

Effect of phytohormones on organogenesis of Crotalaria medicagenia *callus*

Phytohormone mg/l	Callus	Organogenesis	
		Bud	Root
NAA 5·0	+	−	+++
NAA 1·0	+	−	++
NAA 0·5 + 2,4-D 0·5	+++	−	+
NAA 0·2 + Kn 1·0	−	++	−
NAA 0·2 + BAP 0·5	−	+++	−
NAA 0·2 + Ad 10·0	−	++	−
NAA 0·2 + Ads 10·0	−	+	−

"+" sign indicates the degree of response.
"−" = No response.

TABLE II

Intensity of bud formation on C. medicagenia *callus in response to different concentrations of cytokinins*

Cytokinin mg/l		Frequency of bud formation
Ad	0·0	−
Ad	5·0	−
Ad	10·0	++
Ad	20·0	++
Ads	5·0	−
Ads	10·0	+
Ads	20·0	+
Kn	1·0	++
Kn	2·5	++
BAP	0·5	+++
BAP	1·0	+++
BAP	2·0	++

"+" sign indicates the degree of response,
"−" = No response.

for the organ formation of callus tissues (Tables I and II). The comparative study showed that BAP markedly stimulated bud formation at the tested concentrations (0·5, 1·0 and 2·0 mg/l). Among them, 0·5 and 1·0 mg/l BAP were apparently significant (Fig. 1, Table II). But further growth of shoots was greatly inhibited at higher concentration (2·0 mg/l) BAP in the medium. Large number of small leafy shoots and embryoid-like structures were observed within 30 days along the periphery of the callus and a few penetrated into the medium. At the initial stage shoot apex regions looked like small green humps which were composed of distinctly differentiated shoot apical meristem and leaf primordia. The embryoids were formed generally from the surface of the callus. These were small, globular, brown and sometimes

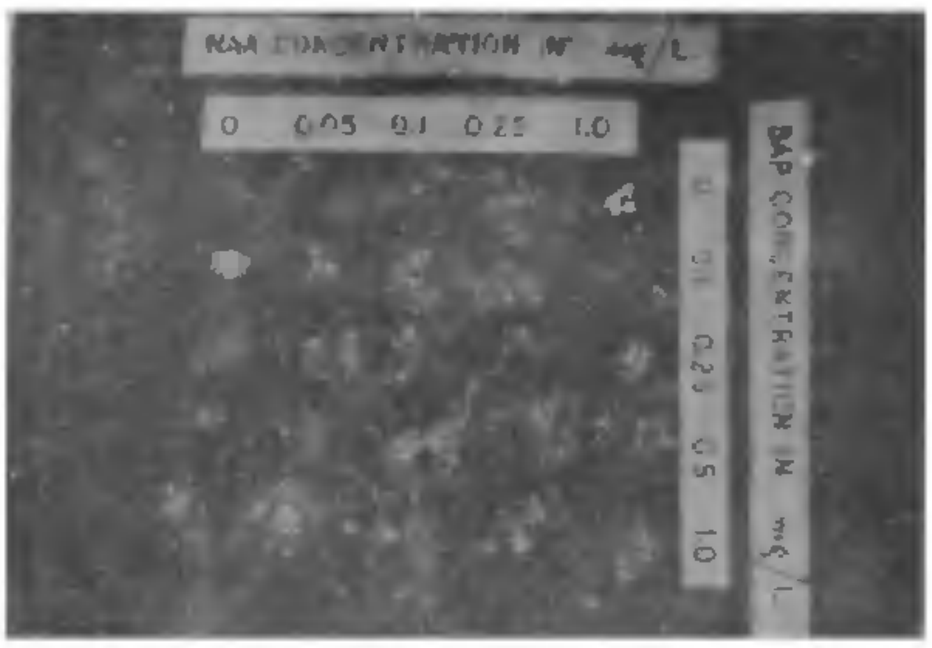

FIG. 1. Effect of different BAP and NAA levels on growth and organ formation of *Crotalaria* callus cultured on MS medium

FIG. 2. Shoot and shoot-bud formation in callus after 60 days growth on MS medium supplemented with 0·5 mg/l BAP and 0·2 mg/l NAA.

green in colour. The maximum number of full developed shoots were observed on the 60th day. Once the shoot buds were formed, they were followed by several shoot apices with rosette leaves (Fig. 2). The callus tissues turned hard and compact and granular in texture.

The results described above showed that *Crotalaria* callus have vigorous regeneration potentiality. All the four tested cytokinins (BAP, Kn, Ad and Ads) stimulated bud initiation, while NAA at higher concentrations promoted root formation in isolated callus tissues. Adenine (6-aminopurine) may act as a precursor to endogenous cytokinins in organogenetic phenomenon[6,7]. The BPA, in conjunction with small amount of NAA, evoked better response in bud induction has its parallel in response to the callus of *Citrus* tissues[8,9]. The formation of lateral roots in explant and callus tissues with addition of NAA and callusing in place of organ induction with addition of 2,4-D, agree with earlier results obtained with *C. juncea* and *C. burhia*[4,5]. Thus it appeared that *C. medicagenia*

cultures required BAP for the bud initiation, NAA for the root formation and 2,4-D for the induction of undifferentiated callus mass.

June 22, 1981.

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DO PHYTOPATHOGENIC AND SYMBIOTIC BACTERIA CONTAIN PHENYLALANINE AMMONIA LYASE?

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L-PHENYLALANINE ammonia lyase (EC 4.3.1.5; PAL) which catalyses the non-oxidative deamination of L-phenylalanine to *trans*-cinnamic acid is regarded as the first major enzyme involved in the synthesis of cinnamic acids, flavonoids, and lignin[1]. The enzyme is present in higher plants, basidiomycetes, ascomycetes, fungi imperfecti and streptomycetes but not in true bacteria and algae[2]. We have not found any publication to substantiate the claim on bacteria. This prompted us to search for PAL in phytopathogenic and symbiotic bacteria, as we believe that the enzyme from the bacterium during infection may contribute to the phenol pool of the infected plants[3]. Indeed most infected plants display high PAL activity[3].

Erwinia amylovora, *Pseudomonas glycinea*, *P. solanacearum*, *P. tabaci*, *Rhizobium leguminosorum*, *R. trifolii*, *Xanthomonas citri*, *X. oryzae*, *X. phaseolicola* and *X. translucens* obtained from the culture collection of C.A.S. in Botany were screened for PAL activity. Of these, *P. tabaci* and *X. citri* did not grow on L-phenylalanine (2 mM) amended Dye's medium[4].

Preinduced 2-day old cultures (O.D. 1 at 640 nm) were suspended in replacement medium containing 4 mM L-phenylalanine in 25 mM Tris-HCl buffer pH 8·8 and incubated with shaking for 4 hr. Cells were removed by centrifugation at 6,000 g for 40 minutes; washed with the buffer, sonicated at high speed at 1 amplitude for 3 minutes, recentrifuged at 7,700 g for 45 minutes at 4° C and the clear supernatant was used as enzyme extract. Reaction mixture in a total volume of 3 ml contained 1 ml of the buffer, 1·5 ml of enzyme extract and 0·5 ml of L-phenylalanine, final concentration 25 μM. The reaction was terminated at 20 hr by adding 1 ml of 1N HCl and extracted twice with 5 ml of peroxide free ether. Ether was evaporated till dryness and the residue was dissolved in 3 ml of 0·05 N NaOH and the product of reaction, cinnamate was measured at 278 nm[5]. Suitable controls were kept. The samples did not absorb at 278 nm indicating the absence of deamination of L-phenylalanine. In no case did we find any reaction, despite repeated attempts with different bacterial species, enzyme and substrate concentrations. Nor did we succeed in separating the cinnamate by tlc. Therefore we confirm that phytopathogenic and symbiotic bacteria do not contain PAL. Nor did they produce the enzyme inductively.

Reddy and Ou[6] claimed that *X. translucens* f. sp. *oryzicola*, which incites leaf streak disease in rice, produced the enzyme. The method used by them is strictly qualitative and as they have not presented any details of the results, we doubt about the validity of the claim.

We believe that accumulation of L-phenylalanine ammonia lyase at the infection site, which is reported in plants infected by bacterial parasites[7,8] is essentially due to the host activity.

We thank Dr. R. Sridhar, Central Rice Research Institute, Cuttack, for drawing our attention to the paper of Reddy and Ou. P.A. thanks University Grants Commission for the award of a post-doctoral fellowship.

August 12, 1981.

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