

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

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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 lignin¹. The enzyme is present in higher plants, basidiomycetes, ascomycetes, fungi imperfecti and streptomycetes but not in true bacteria and algae². 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 plants³. Indeed most infected plants display high PAL activity³.

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 medium⁴.

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 nm⁵. 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 Ou⁶ 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.

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