Phenylalanine ammonia-lyase in a corn pathogen *Ustilago maydis*

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Phenylalanine ammonia-lyase (PAL) is the entrypoint enzyme into phenylpropanoid metabolism in plants. Little is known about PAL in fungi. In order to explore the role PAL plays in the growth and survival of fungi, the structure and regulation of fungal PAL were investigated in the phytopathogen *Ustilago maydis*, the causal agent of corn smut. PAL was purified from liquid-cultured cells of *U. maydis* using ion-exchange and gel filtration chromatography, and preparative PAGE. Its native molecular mass was estimated as 320 kDa and its subunit molecular mass was 80 kDa. No isoforms of the enzyme were detected, and there was no evidence of glycosylation of the protein. The enzyme was most active at pH 8.8-9.2 and 30C and had a Km for L-phenylalanine of 1.05 mM. The enzyme did not deaminate L-tyrosine. The synthetic PAL inhibitor 2-aminoindan-2-phosphonic acid(AIP) strongly inhibited the enzyme, as did sulfhydryl reagents and carbonyl reagents, whereas t-cinnamic acid was only moderately inhibitory. Ustilago PAL activity had no requirement for metal ion cofactors, but was inhibited by heavy metal ions (Ag^{+}, Cu^{2+}, and Hg^{2+}). Polyclonal antibodies were raised against the purified PAL protein. Using degenerate oligonucleotide primers and polymerase chain reaction, a PAL clone was isolated from a *U. maydis* genomic library and 3047 bp of its nucleotide sequence was determined. It contained 495 bp of 5' untranslated sequence, a 2172 bp open reading frame encoding 724 amino acids, and 380 bp of 3' untranslated sequence. No introns in the PAL-encoding gene were detected. In *U. maydis*, PAL was shown to be encoded by a single gene.

This is the first work on the structure of a PAL gene from a pathogenic fungus. Substantial differences in PAL gene sequence and organization were found compared to PAL genes of other species. *U. maydis* PAL showed low amino acid sequence identity with other PALs (23-26% with plant PALs, 39-40% with yeast PALs). In *U. maydis*, PAL is constitutively produced at a low level but its regulation can be influenced by aromatic amino acids. L-tryptophan apparently induced the lyase enzyme. The inducibilty of PAL by L-tryptophan was also demonstrated in six other *U. maydis* strains and three Ustilago species tested. The enzyme is most readily induced during the early stationary phase of growth and the induced activity remains relatively constant during stationary stage. PAL induction was repressed by glucose but not by its reaction product

t-cinnamic acid. Induction did not require *de novo* protein synthesis, suggesting that some form of post-translational protein modification or a metabolic effect may be the basis of the induction of Ustilago PAL by L-tryptophan. PAL was detected only in cell extracts and not in the growth medium.