Title: GIBBERELLIN OXIDASE ACTIVITIES IN BACTEROIDS OF DIFFERENT *RHIZOBIUM SPECIES.*

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Abstract:

Gibberellins (GAs), diterpene phytohormones widely distributed in higher plants, are also present as secondary metabolites in some fungi and bacteria. Their biosynthesis consists in two cyclization reactions from the C20 precursor geranylgeranyl diphosphate (GGDP) followed by several oxidative steps. GGDP cyclization gives *ent*-kaurene, the first committed intermediate in the pathway which is further oxidized to the bioactive gibberellins. In contrast to plants and fungi, there is few information about GA biosynthesis in bacteria. Enzyme activities of this pathway have been demonstrated at a significant level only in bacteroids of *Bradyrhizobium japonicum*, a soybean (*Glycine max*.) symbiont. This microorganism contains a gene operon that encodes the enzymes of GA biosynthesis which is expressed under low oxygen tension as found in root nodules of soybean plants. In this work GA 20-oxidase activity was investigated in bacteroids of different *Rhizobium* species in order to find out if the GA pathway is present in other species within the rizobiaceae besides *B. japonicum*. This oxidase catalyzes the oxidative loss of the methyl group at C20 that produces the 19,10-γ-lactone function essential for phytohormone activity. *ent*-Kaurenoic acid oxidase activity was tested for some strains as well. Bacteroids were isolated from root nodules of legume plants grown from seeds inoculated with specific *Rhizobium* strains under controlled laboratory conditions. ^{14}C-labelled GA precursors (*ent*-^{14}C-kaurenoic acid or ^{14}C-GA_{12}) were added to a bacteroid suspension and the labelled products were isolated after incubation, by partition in ethyl acetate, solid phase chromatography and HPLC in a C18 column. Labelled C20 oxidation products were formed efficiently from ^{14}C-GA_{12} (that has a methyl group at C20) by some of the tested *Rhizobium* strains, mainly *Rhizobium phaseoli*, while a lower activity or absence of activity was found for other *Rhizobium* species. *ent*- Kaurenoid or GA products formed from *ent*-^{14}C-kaurenoic acid were not hydroxylated at C3 or C13 in contrast to the products formed by plant or fungal GA oxidases.

Key words: Gibberellins, *Rhizobium*, oxidases

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