**INTRODUCTION:** Bacteria such as those of the family *Rhizobiaceae* contribute to the Biological Nitrogen Fixation in Legumes and thus can be used as inoculant. These bacteria promote the increase of the green mass and may decrease the supply of chemical fertilizers. The objective of this study was the biochemical characterization of rhizobia from nodules of *Crotalaria juncea*. MATERIAL AND METHODS: For the preparation of pots with bait plant we used soil collected from three municipalities in Goiás State: Porangatu, Rio Verde and Santo Antônio de Goiás. Plants were grown until the flowering period and the nodules were collected, surface sterilized and macerated in a mortar with sterile water (1:10). The extract was inoculated on plates with Semi-selective Medium 79 for the genus *Rhizobium*. Twenty four isolated colonies, with two patterns of rhizobium strains, were evaluated for the ability to grow on plates containing five sources of carbon (glucose, fructose, sorbitol, inositol, arabinose, nicotinic acid, malic acid and maleic acid) and assays for detecting seven enzymes activities (protease, lipase, esterase, urease, cellulase, amylase and catalase). Data were converted to a binary matrix and used for similarity analysis using the Jaccard coefficient (NTSYS), yielding a dendrogram. RESULTS AND DISCUSSION: The media that have led to better growth isolates were those containing inositol (44.4%), glucose (36.1%) and sucrose (34.7%). The enzymatic activity was observed for the enzymes protease, amylase and urease to 100%, 37.5% and 26.4% respectively. The dendrogram showed the separation of four groups with a similarity of 87%. It was also found that 9 isolates showed 100% similarity with the standards used *Rhizobium tropici* (BR322) and *Rhizobium galeagae* (10055). CONCLUSIONS: For the analysis, it was possible to observe the diversity of bacteria associated with roots of Crotalaria cultivated in Goiás.

Keywords: bacterial metabolism, *Rhizobium*, *Crotalaria*

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