Title: BIOFILM FORMATION BY *RHIZOBIU*M SPECIES IN DIFFERENT GROWTH MEDIA.

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Abstract:

Microbial biofilms are defined as complex communities formed by adhered microorganisms on solid or semisolid surfaces involved by exopolysaccharide (EPS). When bacteria start to fix on the surface of some substrate they develop a phase of structuration and activity of the biofilm, which is influenced in turn by several factors as cell growth, EPS production and excretion, pH, and temperature. For rhizobia strains, EPS plays an important role during the phase of recognition and first interactions between the host plant and the microsymbiont. Considering the role of EPS as adjuvant in the aggregation of rhizobia population, it can be considered as a significant factor in quorum-sensing behavior in soil and rhizosphere. The main aim of this work was the quantification of biofilm production as gum in different growth media by rhizobia strains. In order to study the biofilm formation four strains of *Rhizobium* were assessed: *R. tropici* (SEMIA 4077), *R. etli* (LMG17827), *R. giardini* biovar. *giardin*i (H152), and *R. leguminosarum* biovar. *plaseoli* (LMG8819). First, strains were inoculated in PGYL medium for 24 hs to obtain a great mass of cells used in the respective media for EPS production and evaluation. Bacteria were incubated in microplates in eight repetitions of three growth media: PSY (Peptone, salts, yeast extract) medium, PSY plus Diesel, and PSY plus calcium chloride. For biofilm quantification was performed the crystal violet test and optical density (O.D.) determination during six phases: 0, 6, 24, 36, 48, and 72 hs. Data have shown the great production of biofilm was achieved in PSY plus calcium chloride medium when compared to the others. Calcium chloride acts as a powerful crosslinking agent during biofilm formation. Probably, calcium chloride simulates a stressful environment that guide the bacterial cells to enhance their gum in order to overtake the bad effects on them. In spite of the biological role of EPS, this molecule can be considered an important biopolymer useful for industrial applications due its properties of viscosity.

Key-words: quorum-sensing, rhizobial gum, growth-curve, exopolysaccharide.

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