

TITLE: OCCURENCE OF *SPHINGOMONAS* BACTERIA ASSOCIATED WITH APOPLAST FLUID OF SUGARCANE

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ABSTRACT:

The *Sphingomonas* genus comprehends Gram-negative, rod-shaped, strictly aerobic bacteria, which form mainly yellow-pigmented colonies, and contain on their outer membrane glycosphingolipids (GSLs), differently from other Gram-negative bacteria that contain lipopolysaccharide (LPS). Isolates from this genus have been described to associate with agronomically important plant species, presenting benefits such as bioremediation ability, protection against plant-pathogens and plant-growth promotion. The apoplast fluid composes ~3 % of sugarcane stem volume and contains ~12 % of sucrose. This intercellular space has been reported as the most suitable niche for establishment of bacterial endophytes, which include diazotrophs such as *Gluconacetobacter diazotrophicus* and *Herbaspirillum seropedicae*. Recent studies using culture-independent approaches have detected the presence of bacteria from the *Sphingomonas* genus within sugarcane tissues, but its diversity has been poorly evaluated. *Sphingomonas* isolation has been done by selection of yellow-pigmented colonies, and by using the antibiotics streptomycin and piperacillin. Here this approach was used with the apoplastic fluid from sugarcane stems of two commercial varieties (RB 867515 and IAC SP 5000) and spread in rich and minimal agar media containing the referred antibiotics. DNA was extracted from the yellow/orange-pigmented colonies of 57 isolates, and submitted to PCR using *Sphingomonas*-specific primers. The results showed that ~88 % of the isolates were positive to *Sphingomonas*. BOX-PCR analyses showed that there were seven main clusters (with internal similarity ≥ 70 %) and also other isolates, totalizing 30 phylotypes. Outcomes with results of this present study include knowledge of the occurrence of *Sphingomonas* within sugarcane plant tissues and possible new advances in sugarcane crop through development of new microbe-based inputs.

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Keywords: *Sphingomonas*, Sugarcane, Apoplast fluid, BOX-PCR, Isolation