

The Major Protein of *Gluconacetobacter diazotrophicus* is a Porin-like protein.

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*Gluconacetobacter diazotrophicus* is a diazotrophic Gram-negative bacterium that colonizes roots, stems and leaves of sugarcane and promotes plant growth by fixing atmospheric N<sub>2</sub> and by phytohormone synthesis. We report here on the most abundant *G. diazotrophicus* protein that is observed on 1D and 2D electrophoresis gels and which was identified as an Outer Membrane Lipoprotein (ORF GDI2185) by MALDITOF-TOF analyses followed by genome databank search. The secondary protein structure including the presence of transmembrane and periplasmic domains was predicted using bioinformatics tools. Also, an automated homology modeling program which provides protein structures was used. These analyses suggest that the protein contains two domains: an eightstranded  $\beta$ -barrel domain, as is typically found in porins and a periplasmic anchor domain, that may interact with the peptidoglycan layer. A PCR fragment, containing the ORF GDI2185, was amplified from de PAL5 genome, cloned into the pGEM-T easy vector (Promega) and mutagenized *in vitro* using the commercial EZ::Tn5 <kan-2> transposon insertion kit, (Epicentre). Mutant plasmids containing Tn5 insertions in the centre, at the 3' end and after the predicted stop codon of the ORF GDI2185 were selected and used to transform PAL5. No insertional mutants in the ORF GDI2185 could be generated, although various transformants with Tn5 insertion after the stop codon were obtained, suggesting that ORF GDI2185 plays an essential role and that mutations are lethal. Supporting this idea, our analyses suggest that this porin-like protein is probably involved in the nutritional functions of the bacteria. Additional studies are necessary to understand the precise role of this important protein in *G. diazotrophicus*.

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