Analysis of Biofilm Formation by *Gluconacetobacter diazotrophicus gum*D Mutant

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Gluconacetobacter diazotrophicus is an N_2-fixing endophyte isolated from sugarcane. The genome sequence of *G. diazotrophicus* has revealed an operon possibly involved in exopolysaccharide biosynthesis. This exopolymer may be involved in the bacterial biofilm maintenance that causes the xylem colonization. One of these genes is *gum*D (GenBank, GeneID: 5790498) is likely to encode the phosphate-prenyl glucose-1-phosphate transferase catalyzing the first step in gum biosynthesis in other organisms as *Xanthomonas campestris*, *Xylella fastidiosa* and *Gluconacetobacter* xylinus. The aim of this study was the construction of a *G. diazotrophicus* putative *gum*D knowkout mutant and the evaluation the capacity of this mutant in colonize glass wool. To study the *gum*D gene, vectors were constructed to inactivate this gene by allelic exchange mutagenesis strategy. The allelic exchange mutagenesis involves homologous recombination with two crossing overs that substitutes the wild-type copy of the target gene by a truncated copy interrupted by a selectable marker gene (kanamicin resistance). For biofilm assays, bacterial colonies were inoculated in 25 ml liquid modified LGI medium, pH 5.5, containing 1 mM glutamic acid and 0.1 g of glass wool and grown for 72 h at 30°C and 150 rpm. Briefly, the glass wool was washed with phosphate buffer, stained with 0.5% safranin solution and analyzed by optical microscopy. The disruption of the *gum*D gene influenced the adhesion capacity of *G. diazotrophicus* to the glass, used as a substrate, where was deficient in biofilm formation. This study appears to be the first to demonstrate that interruption of a gene required for exopolysaccharide synthesis can lead to reduced colonization of *G. diazotrophicus*.

Key words: Biofilm - Endophytes - Exopolysaccharide - Functional genomics - Phosphate-prenyl glucose-1-phosphate transferase.

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