Relative expression of hypothetical protein-related genes for *Bradyrhizobium diazoefficiens* CPAC 7.

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ABSTRACT
From the proteomic map of *Bradyrhizobium diazoefficiens* strain CPAC 7 we selected nine hypothetical protein-related genes and evaluated their relative expression by Real-time quantitative PCR (RT-qPCR). The experiment was performed with two treatments (induced or not with 5 µM genistein). Six out of nine protein-related genes were up-regulated in the presence genistein.

INTRODUCTION
Bradyrhizobium diazoefficiens strain CPAC 7 (Delamuta *et al.*, 2013) is a microsymbiont that fixes nitrogen in association with soybean (*Glycine max* L.) and is used in commercial inoculants in Brazil; therefore it has high ecological and economical importance. It is estimated that more than 30% of the genome of *B. diazoefficiens* type strain USDA 110 encodes hypothetical proteins. The hypothetical protein nomenclature is used when the existence of a gene is supported only by the prediction of gene-finding softwares, and when they do not show significant homology with any characterized gene (Batista *et al.*, 2010). However, these proteins can bring interesting information to gene bioprospection. In these sense, our goal was to study the relative expression of some hypothetical protein-related genes from strain CPAC 7 in response to genistein.

MATERIAL AND METHODS
Strain CPAC 7 was grown in AG medium until exponential phase in two treatments (Induced or not with genistein 5 µM). Total RNA was isolated using Trizol and employed for cDNA synthesis using Superscript III^{TM} reverse transcriptase (Invitrogen^{TM}). Platinum® SYBR Green®qPCRSuperMix-UDG was used according to the manufacturer's instructions. Amplification reactions were conducted in triplicate using a 7500 Real Time System thermocycler (Applied Biosystems). We used 16S rRNA (GeneID: 1055154) as reference gene for normalization. Relative gene expression was determined by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Statistical analysis of the data was performed using the REST 2009 v.2 software, which enables the calculation of *P* values for each sample group and 95% confidence intervals.

RESULTS AND DISCUSSION
Based on an unpublished *B. diazoefficiens* CPAC 7 proteomic reference map of our group, we selected 18 hypothetical proteins identified and performed a preliminary functional inference using bioinformatics tools and based on the presence of function domains/motifs (Table 1).
Nine genes classified as hypothetical proteins that did not fit into any functional category of COGnitor (http://www.ncbi.nlm.nih.gov/COG/old/xognitor.html), assigned as "NO related KOG", were selected and analyzed by RT-qPCR. The analysis was performed to monitor the relative expression of these genes in response to genistein. Of the nine genes, six were up-regulated (Figure 1). Curiously, all genes coding for

periplasmic proteins (bll0565, blr7534, bll5307 and blr0227) were differentially expressed in response to the flavonoid.

Table 1. Identified hypothetical proteins of *B. diazoefficiens* CPAC 7.

Hypothetical protein	Cellular location	Functional inference
BJ6T_08050	Cytoplasmic	Thioredoxin-like protein
Bll7551	Periplasmic	
Blr5678	Cytoplasmic	L-aminopeptidase, probably related to arginine biosynthesis
Bll5131	Extracellular	ATP/GTP binding site, small transmembrane domain
Blr2961	Cytoplasmic	Fumarylacetoacetase
Blr3798	Cytoplasmic	Demethylmenaquinone methyltransferase
Blr5067	Cytoplasmic	
Blr0227	Periplasmic	
Blr2191	Periplasmic	Histidine phosphotransferase
Bll4565	Cytoplasmic	Ribonuclease, high similarity with Blr3798
Bll5307	Periplasmic	
Blr2761	Cytoplasmic	Universal stress protein UspA
Blr7436	Cytoplasmic	
Bll4752	Cytoplasmic	Predicted transcriptional regulator containing the HTH domain
Bll0565	Periplasmic	
Bll5663	Cytoplasmic	ATPase
Blr3064	Cytoplasmic	Succinyl-diaminopimelate desuccinylase
Blr7534	Periplasmic	

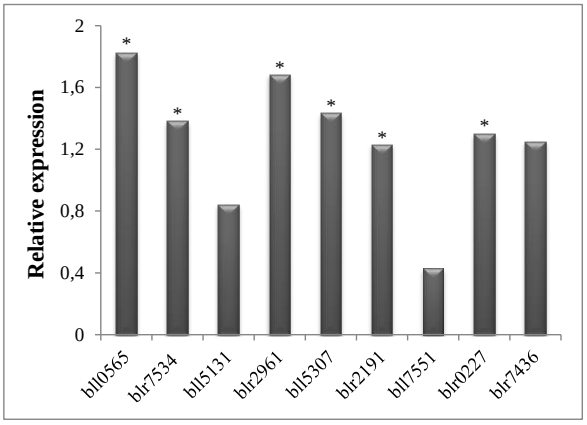

Figure 1. Gene expression analysis by RT-qPCR of nine hypothetical proteins.

The differential expression of these genes in response to genistein suggests that their respective proteins are probably involved in symbiosis establishment process.

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