Two-dimensional proteomic reference map of *Bradyrhizobium diazoefficiens* strain CPAC 7 (=SEMIA 5080).

Gomes, D.F.[1], Batista, J.S.S.[3*], Hungria, M.[2]
[1] Departamento de Genética, Universidade Federal do Paraná, Curitiba, Brasil; Fellowship from CNPq. [2] Embrapa Soja, Londrina, Brasil. [3] Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa, Brasil.
* douglasfgomes@yahoo.com.br

ABSTRACT
A two-dimensional gel electrophoresis profile was generated for *Bradyrhizobium diazoefficiens* CPAC 7 (=SEMIA 5080), a highly competitive strain against naturalized soil rhizobia and efficient in fixing nitrogen in symbiosis with soybean. We selected 150 spots and 124 proteins were effectively identified. The majority of the identified proteins were related to metabolic functions.

INTRODUCTION
Strain CPAC 7 was recently reclassified into a novel species named *Bradyrhizobium diazoefficiens* (Delamuta *et al.*, 2013). Due to its outstanding efficiency in fixing nitrogen, the strain is employed in soybean (*Glycine max* L.) commercial inoculants in Brazil, since 1992 (Hungria *et al.*, 2006). The genome sequencing of CPAC 7 is now in progress; so, the establishment of a proteomic reference map can add important protein-expression information into the genomic annotation process. In this study we present the two-dimensional proteomic reference map of CPAC 7 that will allow a comparative analysis with the published proteomic reference map of *B. diazoefficien*s, strain USDA 110 (Delmotte *et al.*, 2010).

MATERIAL AND METHODS
B. diazoefficiens CPAC 7 was grown in AG medium until exponential phase. Whole-cell proteins were extracted and separated by two-dimensional gel electrophoresis, using IPG-strips with pH range 4-7 (Gomes *et al.*, 2012). The experiment was performed in triplicate and 150 well-defined spots, present in all three gels were randomly selected for MALDI-TOF/TOF identification. Spectra generated were searched against the public database NCBInr, Proteobacteria, using the Mascot software v. 2.3 (Matrix Science).

RESULTS AND DISCUSSION
Well defined and reproducible 2D gel profiles were generated (Figure 1). Among 150 protein spots randomly selected to be analyzed by mass spectrometry, 124 proteins were successfully identified. In addition to Mascot identification, we also made a prediction of the subcellular location using the software PLSpread and PsortB.
Functional classification in clusters of orthologous groups (COG) was also performed, and proteins were distributed in 17 COG categories, belonging to four functional groups. Proteins related to metabolic functions were the majority, representing 39% of identified proteins (Figure 2). The first hit of most proteins was with USDA 110, defined as the type strain of *B. diazoefficiens.* In relation to the cellular location, 105 of the proteins are located in the cytoplasm.

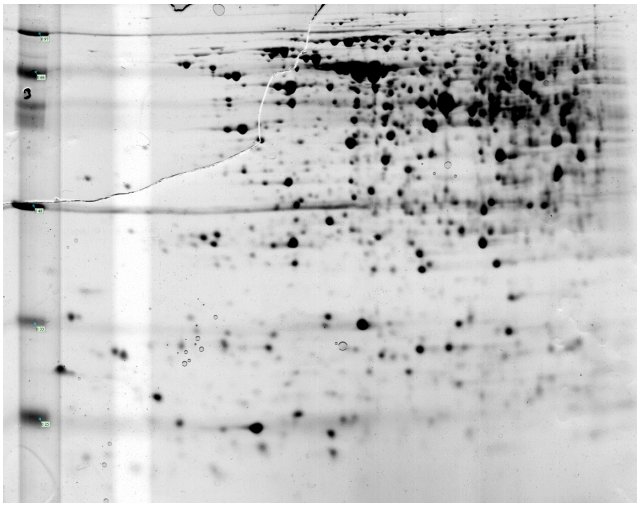

Figure 1. 2D gel profile of *B. diazoefficiens* CPAC 7

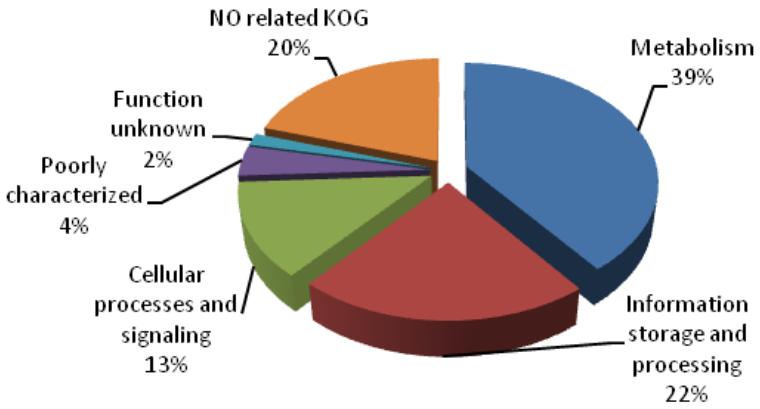

Figure 2. Distribution of the identified proteins into COG categories.

Among the proteins identified in our study, it is worth mentioning that ATP-dependent Clp protease, GTP-binding tyrosin phosphorylated protein and electron transfer flavoprotein, besides others, were up-regulated when *B. japonicum* strain CPAC 15 was grown in the presence of genistein (Batista and Hungria, 2012). This reference map will be useful to add an expression point-of-view in the oncoming genome sequencing of strain CPAC 7.

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