## Ubiquitous Urease: Expression, Purification and Biological Properties. <u>Kappaun, K</u>.<sup>1</sup>, Martinelli, A.H.S.<sup>1</sup>, Becker-Ritt, A.B.<sup>2</sup>, Pasquali, G.<sup>1,3</sup>, Carlini, C.R.<sup>1,2</sup>

<sup>1</sup>Graduate Program in Cellular and Molecular Biology – Center of Biotechnology; <sup>2</sup>Department of Biophysics, I.B; <sup>3</sup> Department of Molecular Biology and Biotechnology. UFRGS, Porto Alegre, RS.

Urease (urea amidohydrolase; EC 3.5.1.5) is a nickel-dependent metalloenzyme, that catalyzes the hydrolysis of urea to form ammonia and carbon dioxide. Ureases are produced fungi, plants, and bacteria. Soybean produces two isoenzymes: the embryo-specific urease is encoded by the Eu1 gene, being synthesized only in the developing embryo, and the ubiquitous urease, which is encoded by Eu4 gene and is present in small amounts in all plant tissues. The ubiquitous urease is responsible for recycling metabolically-derived urea while the role of the embryospecific urease remains unknown. Previous studies had suggested that the embryo-specific urease could be involved in plant defense. Since the ubiquitous urease is found in low amounts in plant tissues making difficult its purification, little is known about this protein. Protocols for the expression and partial purification of soluble soybean ubiquitous urease were established to obtain a protein fusioned with glutathione S-transferase (GST). The best expression conditions were obtained by induction with 0.1 mM IPTG during 16 h at 28°C. The recombinant urease was purified by affinity chromatography, cleaved with trombin and analyzed by SDS-PAGE. Assays for urease activity, Western blot, and Elisa were performed. Similar to the embryo-specific isoform, the recombinant soybean ubiquitous urease was also able to induce aggregation of rabbit platelets at 0.42 µM final concentration (CNPq, CAPES, FINEP).