Purification and Crystallographic Studies of Chlorocatechol 1,2-Dioxygenase From *Pseudomonas Putida*

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The increasing demand of industrialized products and decades of modern agriculture practices are the main factors responsible for the environment contamination. The accumulation of organic pollutants, mainly the polycyclic aromatic hydrocarbon (PAH), generate compounds such as catechol, chlorocatechol and their derivatives, substances that are among the most hazardous ones because of their carcinogenesis potential and recalcitrant properties to degradation. A modern and efficient biotechnological strategy for the elimination of these compounds is called bioremediation and it is based on the use of living microorganisms, or theirs enzymes to reduce or eliminate these toxic chemical products. The target of our study is the chlorocatechol 1,2-dioxygenase from *Pseudomonas putida* (Pp 1,2-CCD), a dioxygenase belonging to the intradiol class which shows high specificity to catechol, chlorocatechol and halogenated substrates derived from substituted catechol. Recombinant Pp 1,2-CCD was produced in *Escherichia coli* BL21(DE3) as a intein-tag fusion protein. The protein expression and purification protocols have been optimized in order to successfully crystallize Pp 1,2-CCD using both hanging and sitting drop vapor diffusion methods. Crystals have been obtained using polyethylene glycol as a precipitant agent. The brown crystals appeared after three days and display a rectangular shape. In order to solve the structure, the crystals will be taken for X-ray diffraction experiments at MX-1 beam line located at LNLS. Our results can be further explored to potentialize the biotechnological use of Pp 1,2-CCD as a bioremediator.

Key words: chlorocatechol 1,2-dioxygenase, bioremediation, crystallography of proteins