TITLE: PRODUÇÃO DE MMP-2 RECOMBINANTE HUMANA EM *ESCHERICHIA COLI*

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ABSTRACT:

Extracellular matrix metalloproteinases (MMPs) are the main group of enzymes involved in extracellular matrix degradation. Currently 23 metalloproteinases genes have been identified in humans, among them MMP-2, which is described as elevated in cardiovascular diseases. To express MMP-2, a previously characterized clone was used which contains the complete human recombinant MMP-2 sequence inserted into the multiple cloning sites of the pet5A plasmid vector. Transformation into *E. coli* bacteria (plys) was performed by electroporation (100 ng of 200-ohm pulsed DNA. Then, the bacteria underwent growth for one hour in LB medium in shaker at 37 ° C at 170 RPM. 1h, the bacteria were centrifuged and the pellet plated on LB agar containing 100 µg / mL ampicillin. Ten ampicillin-resistant clones, named rhMMP-2, were generated. These clones were expanded for induction of expression using 50 µM Isopropyl-Beta-D-Thiogalactopyranoside (IPTG) at 18 ° C for 18 h in shaker at 170 RPM. Expression confirmation was performed by gel zymography activity assay. The best activity clone was subjected to expression in 500 ml of LB medium for further column chromatographic purification. The bacterium was centrifuged, lysed by sonication and the collected supernatant. Purification was performed by gelatin affinity liquid chromatography using a Gelatin-Sepharose resin in a 12 cm column; the passage of the sample into the column was performed with the following washes: a wash with 60 mL of TNC buffer, 20 mL of the sample (pre-sonicated) a wash 60 mL of Triton X-114 (0.1% v / v) to remove the LPS from the sample, another wash with 60 ml of TNC buffer and finally 60 ml of DMSO (5% v / v). Protein activity was assayed by gel zymography.

Keywords: Matrix 2 metalloproteinase ; gelatine ; transformation