PRODUCTION OF RECOMBINANT PORCINE CARBOXYPEPTIDASE B IN
Escherichia coli

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Carboxypeptidase B is a metalloprotease capable of hydrolyze lysine, arginine
and ornithine from the C-terminal end of polypeptides. The enzyme is used in
pharmaceutical and biotechnological industries to remove those C-terminal amino
acids residues. Our aim is to produce active carboxypeptidase B enzyme. The
vector pET28a, containing gene coding to porcine procarboxypeptidase B was
inserted into the Escherichia coli by heat shock method and high-producer clone
was selected. The pre-inoculum was grown in SOB medium at 37 ºC/ 250 rpm/
overnight. The fermentation was performed using culture medium for high density
cell fermentation and inoculated with 5% V/V from pre-inoculum cells. The initial
growth was done at 37 ºC/ 250 rpm for 2 h or when the optical density at 600 nm
reaches between 0,6 - 1,0, then the protein expression was induced by lactose-
containing medium. The induction medium was added three times with intervals of
an hour each. The induction was done at 26 ºC/ 250 rpm/ overnight, yielding 5
gDCW/L. So, the cells were harvested by centrifuged at 8000 rpm for 5 min and
lysed by high-pressure homogenization. The inclusion bodies were harvested and
solubilized in buffer containing 0,1M Tris/HCl, pH 8,0, 8 M urea and 0,05 M DTT.
The protein produced was purified using a pre-packed column HisTrap FF, and
refolding was carried out during the purification process with a decreasing linear
gradient of urea. The purified protein concentration was estimated in 15 µg/mL by
bicinchoninic acid assay using bovine serum albumin as standard. The production
of carboxypeptidase B using E. coli reached a high yield and purity. Analyses of
the protein structure and enzyme kinetic are still in development.

Key words: Carboxypeptidase B, enzyme, recombinant protein purification.
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