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HETEROLOGUOS EXPRESSION OF A PEROXIRREDOXIN OF *Bacillus pumilus* WITH ESTERASE PROMISCUOUS ACTIVITY

Carolina Peña-Montes, Nelson Salazar-Cabrera, Gabriela Jacinto Alcocer and Amelia Farrés; National Autonomous University of Mexico, Faculty of Chemistry, Food and Biotechnology Department, Mexico City; carpem72@yahoo.com

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Introduction. Peroxiredoxins (Prx) are a family of proteins that catalyze the reduction of H_2O_2 to H_2O using a thiol as the other reactant. Their importance in providing protection against oxidative stress and in signal transduction has been clearer in the past few years (1). Many, enzymes can promiscuously catalyze reactions, or act on substrates, other than those for which they evolved. In 2011, Ayala Esquivel identified the peroxiredoxin YkuU in crude extracts of *Bacillus pumilus* GMA1 with a molecular weight of 20.6 kDa which had promiscuous esterase activity (2).
The present study was aimed to clone and express the enzyme YkuU in *Escherichia coli* in order to corroborate their promiscuous activity.

Methods. Primers were designed for the gene sequence encoding the Prx YkuU reported in genome database of *B. pumilus* (3). The *ykuU* gene was amplified by PCR, cloned in pet 20b+ vector, sequenced and finally overexpressed in cells of *Escherichia coli (E. coli*) BL21. Protein expression was induced by IPTG and peroxirredoxin YkuU was visualized in SDS-PAGE and zymograms (4,5). Esterase activity was evaluated according to Karpuchova (2005) and peroxirredoxin activity was evaluated according to Jiang (2005) (6).

Results. The *ykuU* gene has the expected size of 540 bp which encoded a protein of 180 amino acids. The nucleotide sequence showed 96% identity with that reported in the genome of *B. pumilus* for the same gene. The deduced amino acid sequence of *ykuU* gene showed a difference of 3 amino acids at the C-terminal region with respect to the sequence reported in the genome of *B. pumilus*.
After induction on plates, two recombinant clones showed a promiscuous esterase activity, which was observed as red colonies. Esterase activity on α-naftil acetate (α-NA) was observed in intracellular extracts of recombinant clones after induction on liquid cultures of transformants**.** However "in situ" activity has not been detected after renaturation of SDS-PAGE gels, while it has been observed on native gels. A similar behavior was displayed by wild YkuU enzyme in extracellular extracts of *B. pumilus* GMA1.
Prx activity was also detected in intracellular extracts of induced recombinant clones. Prx activity was visualized as a single band of 20.6 kDa, after renaturation of SDS-PAGE which correspond to calculated molecular weight.

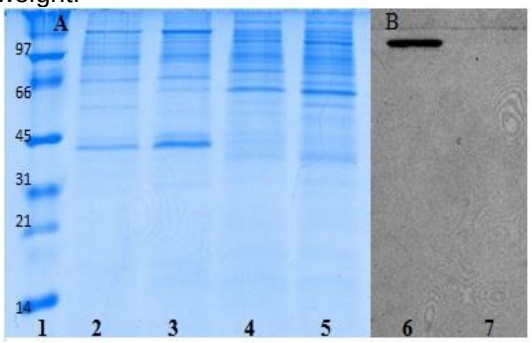

Fig.1 Protein pattern after silver staining (A) and zymogram (B) of intracellular extract (IE) of recombinant clones expressing YkuU enzyme in *E. coli* after renaturation of YkuU. 1, low molecular weight marker; (2,4) IE of non-induced clones ; (3,5); (6,7) IE of clones after 3 induction hours.

Conclusions. Promiscuous esterase activity of Prx was corroborated in two recombinant induced clones. On SDS-PAGE gel, a single band of 20.6 kDa with Prx activity was detected after renaturation. Native gels showed one band of esterase activity with a high molecular weight in the intracellular extract of recombinant clone 5, which may correspond to enzyme aggregates as a similar behavior has been also observed for wild enzyme.

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