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Overexpression and Characterization of *Vibrio mimicus* metalloprotease (VMC)

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To investigate the biochemical properties of *V. mimicus* metalloprotease, which gene was isolated previously from *Vibrio mimicus* ATCC33653, overexpression and purification were attempted. The 1.9 kb of open reading frame was amplified by PCR from pVMC193 plasmid which ligated the *vmc* gene with pUC19 and introduced into *Escherichia coli* BL21 (DE3) using the overexpression vector, pET22b (+). The overexpressed metalloprotease (VMC) was purified with Ni-NTA column chromatography and characterized with various protease inhibitors, pHs, temperatures, and substrates. The purified VMC showed the proteolytic activity against gelatin, soluble and insoluble collagens, and synthetic peptides. Unlike the observations made with all metalloproteases originated from other *Vibrio* sp., the VMC did not hydrolysis the casein. The proteolytic activity was critically decreased when the VMC was treated with metal chelating reagents, such as EDTA, 2,2 -bipyridine and 1,10-phenanthroline. In particular, the 71 kDa VMC exhibited the hemagglutinating activity against human erythrocyte. As the purified VMC was treated with CuCl2 and NiCl2 for the chemical modification of metal binding, the proteolytic activity and hemagglutinating activity were profoundly influenced. The multi-alignment analysis made on the reported *Vibrio* metalloproteases showed the difference of amino acid sequence similarity between the two distinctive classes of *Vibrio* metalloproteases.