

## Cloning, expression and purification of arylsulfatase from disk abalone (*Haliotis discus discus*) cDNA

Chamilani Nikapitiya and Jehee Lee\*  
Cheju National University

### Introduction

Sulfatases are members of a highly conserved gene family sharing extensive sequence homology and unique post translational modification. They are responsible for the hydrolysis of sulphate ester bonds in biological systems. In mammalian sulfatases the most important residue in the active site is a 2-amino-3-oxopropanoic acid, formylglycine (FGL). Arylsulfatases are distributed in a wide range of organisms from mammals to bacteria (Dierks et al., 1998). Moreover, it has been reported that purified arylsulfatase with novel enzymatic properties from *Streptomyces griseorubiginosus* has potential application to cancer therapy. Further, the enzyme showed high specificity against agar rather than other sulfated marine polysaccharide (Kim et al., 2004). However, there is little information available on mollusk arylsulfatases, especially on its biochemical properties. In this study, displayed the cloning and sequence characterization of a full length cDNA of disk abalone (*Haliotis discus discus*) arylsulfatase gene, including induced expression and purification of arylsulfatase using *E. coli* K12 TB1 system.

### Materials and methods

Arylsulfatase gene was selected and cloned from *H. discus discus* cDNA library. The full sequence of arylsulfatase was analyzed from the vector. The open reading frame (ORF) of the gene was determined by using DNAssisit (2.2) programme, and it was compared with coding sequence of the other organisms in public data base. Using a forward primer with *EcoRI* site and reverse primer with *XbaI* site, coding sequence was amplified by PCR. Purified PCR product was ligated into pMAL-c2X vector and transformed into *E. coli* K12(TB1) protein expression host cells. The protein was induced with IPTG at 25°C for 3 h and the expressed protein was purified using maltose binding column. Moreover, protein was analysed on SDS-PAGE to find out the degree of purification as well as the molecular weight of

the protein.

## Results and Summery

The full length of the arylsulfatase gene was 1557bp and ORF was 1446bp including 63bp signal sequence. The deduced amino acid, calculated molecular weight and predicted isoelectric point was 481aa, 54kDa and 5.8, respectively. Moreover, when amino acid sequence of abalone arylsulfatase gene compared with *Helix pomatia*, *Homo sapience*, *Mus musculus* and *Rattus norvegicus*, 146 similar amino acid residues were identified. Further, abalone arylsulfatase protein exhibited 45%, 43%, and 42% identities with *H. pomatia*, *H. sapience* and *M. musculus*, respectively. Molecular size of the purified protein band was approximately 96.5 kDa, however, molecular size of the maltose binding fusion protein was 42.5 kDa. Therefore, molecular weight of our purified protein was smiler to predicted protein size of 54 kDa. The purified arylsulfatase could be applied as an alternative method for the production of agarose by enzymatic conversion of agaropectin to agarose, and facilitate its application in the production of agarose commercially. Further studies are expected to determine a clinical aspects of the arylsulfatase.

## References

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