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Molecular Cloning of *Arabidopsis thaliana AOX1a*: Heterologous Expression of Functionally Active Recombinant AtAOX1a in *E. coli*

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Abstract—*The alternative oxidase (AOX) is a non-protonmotive ubiquinol oxidase and it is localized on mitochondrial inner membrane towards the matrix side. Mitochondria of all higher plants, some fungi, protists and few animal species have AOX. AOX plays an important role in both abiotic and biotic stress tolerance of higher plants. It competes with complex III of cytochrome pathway and bypasses the oxidative phosphorylation at both complex III and IV. AOX1a is the most stress-responsive gene among all the AOX gene members, hence the characterization of AOX1a protein would provide biochemical basis for stress tolerance. However, it is difficult to obtain sufficient native mitochondrial membrane protein from a plant source. The model plant Arabidopsis thaliana provides an opportunity as a gene source of alternative oxidase. Functional expression of A. thaliana AOX1a (AtAOX1a) in Saccharomyces cerevisiae, has proved that the AtAOX1a is important in alleviating oxidative stress. In the present study, we over expressed and purified functionally active rAtAOX1a from E. coli BL21(DE3) pLysS cells. A. thaliana AOX1a cDNA was cloned into pET28a (+) vector and transformed to BL21(DE3)pLysS E. coli for heterologous expression. E. coli that are transformed with pET28a vector carrying AtAOX1a cDNA (E. coli/pAtAOX1a) were tested for cyanide-resistant and n-propyl gallate sensitive growth. E. coli/pAtAOX1a has shown cyanide-resistant growth, whereas n-propyl gallate has*

suppressed the growth of this recombinant E. coli. These results indicated that the expressed recombinant AtAOX1a (rAtAOX1a) is functionally active. Further, rAtAOX1a has been solubilized from E. coli membranes and purified to homogeneity in a stable and active form using cobalt resin column. MALDI-TOF/TOF and western blot analysis have confirmed that purified protein is rAtAOX1a.

Keywords*: Arabidopsis thaliana; Alternative oxidase; oxidative stress; Escherichia coli; heterologous expression; cyanide-resistant growth; Protein purification.*