Characterization of *Xylella fastidiosa* Alkyl Hydroperoxide Reductase (AhpR) System

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Xylella fastidiosa is a phytopathogenic organism responsible for important plant diseases such as CVC in citrus and Pierce disease in vine. In 2000, the *X.fastidiosa* genome was sequenced in Brazil and has motivated researches with this microorganism. The present work describes the characterization of alkylhydroperoxide reductase system (AhpR) from *X.fastidiosa*, one of the main eubacteria antioxidant defenses against hydroperoxides. This system possesses a protein from the peroxiredoxin family, AhpC (alkylhydroperoxide subunit C), and a flavoenzyme disulfide reductase, AhpF (alkyhydroperoxide subunit F). In a previous work, we described the cloning of the genes of these proteins; their expression in *Escherichia coli;* and their purification by nickel affinity chromatography. Furthermore, we described that AhpC presented DTT-dependent peroxide reductase activity as assessed by the ferrous oxidation in xylenol orange assay, against cumene hydroperoxide, hydrogen peroxide, and *tert*-butyl hydroperoxide. Here we show that AhpF possesses NADH-oxidase activity restricted to its flavin group and independent of cysteines, by means of oxygen consumption. By using N-*ethylmaleimide* (NEM), an irreversibly alkylating agent of cysteine residues, we confirmed this activity as being exclusively dependent on the flavin group, but not on the dithiol/disulfide redox center. Furthermore, we indirectly proved that AhpF is able to reduce AhpC efficiently using NADH (preferentially) or NADPH as an electron donor source. Therefore, in this work, AhpR system of *X.fastidiosa* was reconstituted *in vitro*, successfully. Since the oxidative burst is one of the main plant defenses against pathogens, the characterization of AhpR system from *X.fastidiosa* presented here might be of interest for possible agricultural pest controls.

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