

Characterization of macromolecular oxidative damage in native *Agrobacterium isolate*

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As a part of systematic study of nature genetic engineer mediated transformation of plants. Experiments were designed to simulate the conditions of rhizospheric environment and related molecular changes in a native soil isolate agrobacterium (Doon GE1). Agrobacterium feels oxidative stress at and above 0.15% H₂O₂ or low pH values. Further, agrobacteria were evaluated for oxidative stress mediated damage in DNA and proteins macromolecules.

Key words : Agrobacterium, Oxidative damage, DNA/Proteins

INTRODUCTION

Agrobacterium comes under the rizobiaceae family. It is a gram negative rod shaped soil bacteria of 0.6 -1.0 μm width and 1.5 -3.0 μm length and occurs singly or in pairs. It is a causative agent of crown gall disease on dicotyledonous plants. Agrobacterium is frequently exposed to reactive oxygen species from the environment (rizosphere) and from those generated by aerobic respiration as a result of the partial reduction of molecular oxygen. The toxicity of this ROS arises from its ability to oxidize and leach iron from iron sulphure clusture (4 Fe -4s) containing proteins and there by inactivating enzymes such as aconitase, 6-phosphogluconate, dehydratase, fumarase and dihydroxy acid dehydratase of common biosynthetic pathways. Clusture (4 fe-4s)²⁺ is utilised as a lewis acid to catalyse substrate dehydration. The solvent exposure of the clusture allows it to be accessible to o²⁻ for its univalantly oxidation. The resultant (4fe-4s)³⁺ clusture is unstable and disintegrate into (4fe-4s)⁺ form, which is catalytically inactive with loss of a ferrous iron atom to the cytosol. The iron accumulates in the cytosol and could participate in the 'fenton reaction', leading to increased production of the highly deleterious hydroxyl radicals which can cause damage to macromolecules including DNA, Protein and lipids (Saenkham *et al.*, 2007).

In response to reactive oxygen, agrobacterium differs from most other bacteria having Fe-superoxide dismutase (fe SOD) iso-metalloenzyme, catalyzing the dismutation of superoxide anions to form H₂O₂, a substrate of catalase and molecular oxygen. Chromosome gene Kat A and Cat E encode catalase which is known to detoxify H₂O₂, a

major component of oxidative stress, imposed on a cell. Kat A plays the 1^o role and Cat E acts synergistically in protecting agrobacterium from H₂O₂ toxicity during all phases of growth. Therefore, in the present study, 'in vitro' experiments were designed, in terms of oxidative superoxide anion stress, to simulate the conditions of rhizospheric environment during agrobacterial infection.

MATERIALS AND METHODS

Soil samples were collected from the fields of S.B.S.P.G.I. campus, Balawala Dehradun for the isolation and characterization of agrobacterium as per the standard identification tests and finally acridine induced plasmid curing was performed on the L.B. media containing 0.1% kanamycin. Toxic effects of the H₂O₂ and low pH were seen on the different growth stages by plate sensitivity and oxidative damage of macromolecules (DNA and Proteins) was assessed electrophoretically.

Isolation of genomic DNA from bacterial cell was done by taking 2ml of bacterial cell suspension, centrifuged at 5000 rpm for 10 minutes, added .2ml of extraction buffer and .2 ml of SDS buffer for rupturing the bacterial cells then centrifuged for 5 minutes at 5000 rpm at 4°C. 0.2ml of SDS buffer was used to dissolve the pellet. Allowed to stand at room temperature for 10 minutes. Deprotonise the solution by adding .2ml of chloroform : isomyl alcohol and then immulsify by gentle inversion. Centrifuged at 10,000 rpm for 10 minutes and pipetted out the upper layer for adding the equal amount of chilled isopropanol. Incubation was done for 30 minutes at -20°C, again centrifuged at 10,000 rpm for 10 min. .2ml of chilled ethanol was added on the supernatant