DNA DAMAGE PROTECTIVE ACTIVITY OF THE CRUDE METABOLITES OF ENDOPHYTIC FUNGI ISOLATED FROM TWO ETHNO-PHARMACOLOGICALLY IMPORTANT MEDICINAL PLANTS OF THE KHASI HILLS OF MEGHALAYA, INDIA.

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Summary

Traditional medical practitioners of the Khasi Hills of Meghalaya, India, rely on herbs and plants for treating various ailments. These medicinal plants are found to be protected from human interferences in the sacred groves by the tribal population. Endophytic microorganisms residing in the plant tissues without causing any apparent harm to the host have been proven to produce metabolites with anti-cancerous and anti-oxidant properties. It is hypothesized that the metabolites produced by these endophytes may also protect DNA damage. The present study clearly indicates that the endophytic fungal metabolites were able to prevent UV and hydrogen peroxide induced DNA damage in the laboratory conditions.

Key Words: DNA damage, protective activity, medicinal plants, endophytic fungi, metabolites, Khasi Hills, Meghalaya.

Introduction

Oxidative stress is proposed to be one of the processes involved in aging by inducing DNA damage. DNA damage can be enhanced by exposure to various physical and chemical agents, diseases, dietary habits, life-style and advancing of age .The local communities residing in Meghalaya in the Eastern Himalayan range of India have traditionally used and relied on herbs and plants for treating various ailments (1). These medicinal plants are mostly found in the traditionally preserved "Sacred Forests", which have remain untouched for centuries due to religious beliefs of the indigenous people residing in those areas. These "Sacred Forests" represent the climax vegetation of the area and are home to many endemic and rare species of medicinal plants (2). These endemic plants have been used since time immemorial by the traditional medicine practitioners to treat various ailments like asthma, arthritis, diabetes, diarrhea, cancer, dysentery, hypertension, malaria, leucoderma, rheumatism, skin disease, jaundice, spondolytis, tuberculosis etc .*Osbeckia stellata* Buch.-Ham. ex D.Don. belonging to the family Melastomataceae, has been used traditionally by the local herbal medicine practitioners to treat cuts and wounds and as a remedy for toothaches (3). *Potentilla fulgens* L. of the Rosaceae family has been used traditionally as a folk remedy for a variety of ailments, including diabetes mellitus. Traditionally, pieces of tap roots of *P.fulgens* are chewed along with raw areca nut (*Arecha catechu* L.) and betel leaves (*Piper betel* L.) and extracts made from its roots are used to prepare herbal medicines for treating diabetes mellitus (4).

The current study was therefore undertaken to study the effect on DNA damage of the endophytic fungal metabolites isolated from two ethno-pharmacologically important medicinal plant species namely, *Osbeckia stellata* Buch. Ham. ex D.Don. and *Potentilla fulgens* L. used by the indigenous tribes of Khasi hills of Meghalaya.

Methods

1 ml of each of the fungal cultures in liquid culture medium (potato dextrose broth medium,Himedia laboratories Pvt.Limited ,Mumbai, India) were aseptically transferred under laminar flow to 1.5 ml microfuge tubes and centrifuged at 13000 rpm for 5 mins at 4ºC in an Haraeus Biofuge refrigerated ultra-centrifuge. The supernatant free from the culture media was transferred to a new 1.5 ml microfuge tubes and kept at 4ºC until, they were used for the electrophoresis experiment. 3 % H_2O_2 solution was prepared from a stock solution of 30 % H_2O_2 (S-d fine chem., Mumbai, India).

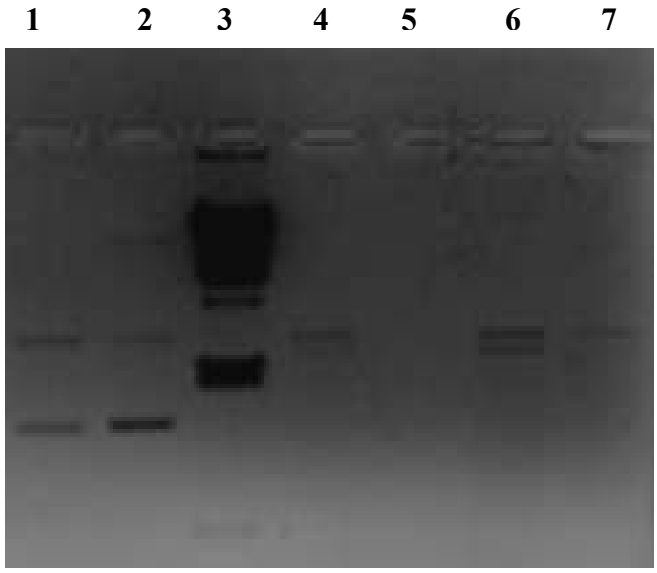
Plasmid DNA was oxidized with H_2O_2 + UV treatment and electrophoresis was carried out on a 1 % agarose gel along with the treatments and control DNA as per the standard electrophoresis procedure (5), in a Bangalore Genei horizontal slab gel electrophoresis unit at 100 volts. The DNA bands were stained with ethidium bromide and fluorescent profile was photographed by Kodak, Gel logic 100 gel documentation system.

Results

Plasmid DNA was oxidized with H_2O_2 + UV treatment and checked on 1 % agarose gel. After UV irradiation OH° radicals generated from UV photolysis of H_2O_2 caused DNA strand scission. Results showed that faster moving supercoiled DNA nicked to form linear and open circular DNA on 1 % agarose gel (lane 1 and 2, Figure 1). Lane 3 was loaded with λ DNA digested with Hind III restriction enzyme, lane 4 was loaded with untreated DNA and lane 5 was left as blank (negative control). Lane 3 and 4, therefore , served as positive controls for the experiment since, the fragments generated by the induced DNA damage with UV and H_2O_2 were different in size and migration pattern in the gel when compared to the fragments generated as a result of restriction digestion by a restriction endonuclease such as *Hind* III.The other positive control used was untreated DNA control in lane 4, and this was done to ensure that the untreated plasmid DNA used for the experiment did not produce any major visually distinguishable fragment artifacts during the electrophoretic procedure. Addition of the crude

endophytic fungal metabolites had a protective effect on this DNA damage (Lane 6 and 7, Figure 1).

Figure 1: The electrophoretic profile of the DNA damage experiment on 1% agarose gel.



Lane 1: Plasmid DNA+ H_2O_2 (3%)

Lane 2: Plasmid DNA+ H_2O_2(3%) + U.V. (30 min)

Lane 3: λ DNA digested with Hind III

Lane 4: Untreated DNA control

Lane 5: Left as blank (negative control)

Lane 6: Plasmid DNA+ H_2O_2(3%) + U.V (30 min) + *O. stellata* endophyte extract (4 µl).

Lane 7: Plasmid DNA+ H_2O_2(3%) + U.V (30 min) + *P.fulgens* endophyte extract (4 µl).

Table 1: Description of the medicinal plants and the endophytic fungal isolates obtained from them.

Plant	Family	Habitat and location of the plants	Part of the plant from which isolation was done	No: of endophytic fungi isolated	Morphological identification of the endophytic fungal isolates
Osbeckia stellata Buch. Ham. ex D.Don	Melastomataceae	*Pinus Kesiya* forest undergrowth (Altitude 1500 m above M.S.L) Shillong, Meghalaya, India.	Stem & Roots	1	*Mortierella hyalina* (Harz) W.Gams.
Potentilla fulgens L.	Rosaceae	Shillong Peak grassland area. (Altitude 1700 m above M.S.L) Shillong, Meghalaya, India.	Roots	1	*Talaromyces flavus* (Klocker) Stolk & Samson

Discussion

Majority of the oxidative damage in the biological system is caused by the OH° (6).These OH° bound on the DNA would lead to strand breakage, de-oxy sugar fragmentation and base modification. In fact, pyrimidine dimers formed due to UV exposure of scDNA can be corrected by synthesis of the enzyme photolyase that cleaves this pyrimidine dimer. This is followed by ligation, which rectifies the damage. In the present study, the possible role of the endophytic fungal metabolites may involve the activation of the locus called phr (photo-reactivation) locus by visible light, which results in the synthesis of the enzyme followed by ligation (7).The results of the present study indicate that the crude endophytic metabolites prevent DNA damage under the test conditions. Efforts are currently on to isolate and characterize the active component of the metabolites responsible for the phenomenon using LC-MS and gas chromatography.

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