RESEARCH ARTICLE

Antioxidant Efficacy in Selected Estuarine Actinomycetes

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ABSTRACT
To evaluate the antioxidant efficacy in selected estuarine Actinomycetes sp. Marine soil samples were collected from different stations in Pulicat back water lagoon. Samples were also collected from different stations in Muttukadu back water lagoon and Ennore estuary, Tamilnadu, India. The crude extract was obtained by removing solvents using rotary evaporator, dissolved in water and lyophilized to a powder form. Potential DNA damage inhibition by marine actinobacteria extracts was tested by photolysing H₂O₂ by UV radiation in presence of pBR322 plasmid DNA and performing agarose gel electrophoresis with the irradiated DNA. The electrophoretic pattern of positive control comprising untreated and non-irradiated plasmid DNA, negative control untreated UV irradiated plasmid DNA and treated with EA extracts of isolated Actinomycetes sp with UV irradiated plasmid DNA revealed. In conclusion, the ethyl acetate extract of the isolates were subjected to DNA damage inhibition test which resulted DNA protection against UV irradiation generated oxidative stress in plasmid DNA.

Key words: Actinomycetes, Estuary, Metabolites, DNA, Antioxidant.

1. INTRODUCTION
Marine Actinomycetes have emerged as a rich source of novel compounds. Actinomycetes are potent sources for the production of antibiotics and other secondary metabolites. Each strain has the potential to produce 10-20 metabolites [1, 2]. The marine environment exhibits different characteristics when compared to the terrestrial environment and they have a potential for new enzyme inhibitors and antioxidants. Reports state that actinomycetes are powerful producers of antioxidants and enzyme inhibitors [3, 4].

Marine organisms are exposed to high levels of reactive oxygen species (ROS) through accodation of photosynthesis, symbion oxygen production, intense sunlight leading UV induced free radical production, so it could be expected that micro-organisms which were highly exposed to reactive oxygen species (ROS) should have effective antioxidant mechanisms. Many of these marine organisms contain powerful and novel antioxidant compounds [5].

During normal cell metabolism, the reactive oxygen species occurs both in animals and plants. The intemperance of ROS leads to oxidative stress, ensuing a oxidative DNA damage which is implicated in the pathogenesis of abundant disorders, e.g. cardiovascular, atherosclerosis, repression injury, catracto-to-genesis, hematodiarthritis, inflammatory disorders and cancer [6, 7]. Many synthetic antioxidant butilatedhydroxylanisula (DHA), butilateahydroxytoluene (DHT) and propylgalate (PG) have been used to retard the oxidative process; however, the use of synthetic antioxidants should be strict regulated due to potential health hazards [8, 9]. Due to important role of free oxygen radicals in various diseases, it has prompted to investigate for novel and potent antioxidant compounds from marine Actinomycetes.

The studies of the isolated Actinomycetes species of the estuarine regions of the East coast, Tamilnadu. In continuation of further studies
the above isolated actinomycetessp were tested for antioxidant activity.

2. MATERIALS AND METHODS

2.1. Collections of soil samples

Marine soil samples were collected from different stations in Pulicat back water lagoon between 13°33’ to 13°66’ North latitude and 80°23’ to 80°25’ East longitudes. Samples were also collected from different stations in Muttukaduback water lagoon between 13°59’ to 13°16’ North latitude and 80°15’ to 80°04’ East longitude and Ennore estuary 13°14’ N, 80°22, E, Tamilnadu, India. Samples were collected from 6-10 cm depth and transported to the laboratory in sterile polythene bags and stored for further study.

2.2. Selected estuarineactinomycetes antioxidant efficacy with reference to DNA damage.

The potential strain of identified marine actinomycetes from Pulicat estuary, Muttukadu estuary and Ennore estuary was inoculated in Kuster’s broth and incubated for 7 days at 25ºC. It was centrifuged for 15 minutes at 10,000 rpm and the supernatant collected was mixed with an equal volume of ethyl acetate and kept for overnight in rotary shaker (100 rpm). The crude extract was obtained by removing solvents using rotary evaporator, dissolved in water and lyophilized to a powder form [10]. The stock concentration was prepared as 100 mg/ml.

2.3. DNA damage inhibition efficiency

Potential DNA damage inhibition by marine actinobacteria extracts was tested by phtholysing H₂O₂ by UV radiation in presence of pBR322 plasmid DNA and performing agarose gel electrophoresis with the irradiated DNA [11]. 1 µL aliquots of pBR322 (200 µg/mL) were taken in three polyethylene micro centrifuge tubes. 50 µg of each extract was separately added to two tubes. The remaining tube was left untreated as the irradiated control (CR). 4 µL of 3% H₂O₂ was added to all the tubes which were then placed directly on the surface of a UV transilluminator (300 nm). The samples were irradiated for 10 min at room temperature. After irradiation, 4 µL of tracking dye (0.25% bromophenol blue, 0.25 % xylene cyanol FF and 30 % glycerol) was added. The samples in all tubes were then analyzed by gel electrophoresis on a 1 % agarose gel in TBE buffer (pH 8). Untreated non-irradiated pBR322 plasmid (C) was run along with the extract treated UV-irradiated samples (methanolic extract treated = SM and aqueous extract treated = SA) and untreated UV-irradiated (CR) plasmid DNA. The gel was stained in ethidium bromide (1µg/mL; 30 min) and photographed on Lourmat Gel Imaging System (Vilbar, France).

3. RESULTS

The DNA damage efficiency was tested in the ethyl acetate extract of all the Actinomycetes sp isolated from the three estuarine regions of East coast, Tamilnadu. The electrophoretic pattern of positive control comprising untreated and non-irradiated plasmid DNA, negative control untreated UV irradiated plasmid DNA, and treated with EA extracts of isolated Actinomycetes sp with UV irradiated plasmid DNA revealed (Fig 1, 2 & 3), that the DNA was intact in Actinomycetes extract treated lanes and the electrophoretic pattern in the untreated UV-irradiated lane revealed DNA damage.

The electrophoretic pattern of Streptomycessp. RM 17 untreated DNA was shown in lane 1. The UV-induced photolysis with H₂O₂ induced oxidative damage on was shown in lane 2. The effect of EA extract on irradiated DNA was shown in lane 3. Electrophoretic pattern on Streptomycessp. RM 42 untreated DNA was shown in lane 4, UV-induced photolysis with H₂O₂ induced oxidative damage on was shown in lane 5 and the effect of EA extract on irradiated DNA was shown in lane 6.

The untreated plasmid DNA ARJLS, Nov, 2015, Vol. 1, Issue, 2
showed bands on agarose gel electrophoresis. UV-photolysis of H\(_2\)O\(_2\) in its respective lane, damages the entire DNA. However EA extract (50 μg) partially protected the DNA damage induced by UV-photolysis of H\(_2\)O\(_2\). The faster moving band represented the native form of super coiled circular DNA and the slower moving band corresponded to the open circular form. Antioxidants are found to play an important role in protecting DNA from various ROS mediated damages and may be useful in the treatment of human diseases where oxygen free-radical production is particularly implicated.

4. DISCUSSION

The DNA damage inhibition potential was tested by photolysis H\(_2\)O\(_2\) by UV radiation in the presence of plasmid (P\(_{BR}^{322}\)) DNA followed by performing Agrose gel electrophoresis to study the DNA pattern in the presence of UV radiation photolysis of H\(_2\)O\(_2\) generates hydroxyl OH radical which causes DNA damage by binding with DNA strand. It was reported that the end products formed by ROS reaction attaches to the DNA and releases metagenic adducts [12]. The results of the present study clearly envisages that the aliquot treated with the Actinomycetes sp extracts inhibited the DNA damage which was otherwise resulted in the aliquots without period of the extracts of Actinomycetes sp. The DNA damage inhibition pattern is compared with positive control where the aliquot was not irradiated and treated with extracts of Actinomycetes sp. the extracts of Pulicat estuarine region had potential DNA damage inhibition efficiency when compared to that of Ennore and Muttukadu estuaries. Similar results detecting the antioxidant DNA damage inhibition efficiency in marine Actinomycetes was recorded elsewhere [13, 14].

These results throw light on the presence of potential antioxidant compounds in the Actinomycetes sp isolated from the three estuarine regions Pulicat, Ennore and Muttukadu of Tamilnadu coastal line. These compounds might play an important role in protecting DNA from various ROS mediated damage and might be useful in the treatment of various diseases is particularly implicated. It was reported that the end
products formed as result of ROS reaction attaches to the DNA and it releases mutagenic adducts. Antioxidants are found to play an important role in protecting DNA from various ROS mediated damages and may be useful in the treatment of human diseases where oxygen free-radical production is particularly implicated.

In conclusion, the ethyl acetate extract of the isolates were subjected to DNA damage inhibition test which resulted DNA protection against UV irradiation generated oxidative stress in plasmid DNA.

REFERENCE


