PROTECTING ROLE OF POLYAMINES ON DNA UNDER THE COPPER STRESS; DETERMINED BY RAPD-PCR METHODS IN *ULVA LACTUCA*

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Abstract
RAPD (Random Amplified Polymorphic DNA) analysis is used for the detection of DNA alternations after treatment of many genotoxical agents. Copper is essential to living organisms, but at elevated concentrations, copper may become toxic for living systems. In this study, the effects of copper were evaluated at the molecular levels in a marine green algae *Ulva lactuca* L. In addition, the protection role of polyamines against DNA mutations and strand breaks against the copper treatment searched by RAPD analysis. The main changes observed in the RADP profiles have been resulted both in appearance or disappearance of different bands and variation of their intensity.
Keywords : Algae, Genetics, Metals.

Introduction
Copper is essential for living systems, because of its role in electron transport system, respiration, growth, etc. However, many studies have also reported that copper induces toxicity. For instance, the binding of copper to DNA bases unwinds the double helix and DNA damage can be generated. Also in another study it was shown that the binding of copper to DNA is necessary for the generation of double- strand breaks, 8- hydrodeoxyguanosine and intrastrand crosslinks in Fenton reactions.
Plant cells can be protected against the oxidative damage by a broad spectrum of radical-scavenger systems including antioxidant enzymes and a number of biologically active substance that may prevent the free radical induced cellular damage ([5]). To date, polyamines (PA) have been reported as efficient antioxidants in many experimental systems and various kind of environmental stresses ([8]). In addition, the biological activity of PAs is attributed to the cationic nature of these molecules. Furthermore, PA protects DNA damage by neutralizing charge and conformational changes of DNA ([3]). However, the PA role in the protection systems still remains unclear.
RAPD (Random Amplified Polymorphic DNA-Polymerase Chain Reaction) polymorphisms can occur due to base substitutions at the primer binding site sor to indels in the regions between the sites. The main purpose of this research is to investigate whether the protective role of polyamines on DNA mutation rate under copper stress in *U. lactuca* L. To test the above hypothesis, we employed molecular techniques and from our findings it seems that copper stress indeed causes damages to DNA and the damages caused to DNA could be conveniently and rapidly assessed by RAPD-PCR technique.

Materials and Methods
U. lactuca L. samples were collected from Izmir Bay and kept in ice during transportation to the laboratory. After cleaning with sterile distilled water the thallus divided into 5 pieces.
Samples were incubated for two hours with solutions of $CuSO_4$ (30 mM) and compared with control samples soaked in sea water medium. In a separate trial, thalli were treated with CuSO4 30 mM and polyamines putrescine, spermidine and spermine (1 mM) for 2h. The light density measured by luxmeter and reported as 210 lux.
After incubation DNA isolated from *U. lactuca* L. samples by CTAB method.
RAPD-PCR was performed by 10 RAPD primers after checking the DNAs by agarose gel electrophoresis. PCR bands were checked again by agarose gel electrophoresis after PCR. The PCR analysis performed three times as control.

Results and Discussion
The effects of polyamines on the protection of DNA strand breaks by $CuSO_4$ treatment searched by RAPD primers. There are band differenties between samples which are treated with different solitions; sea water medium, sea water medium with $CuSO_4$, sea water medium with $CuSO_4$ and spermine, sea water medium and $CuSO_4$ putrescine, sea water medium with $CuSO_4$ and spermidine, respectively.
In the case of ROS generated by transition metals, most of the studies about the influence of polyamines on DNA damage have been based on the effect that these polyacations produce on DNA strand breakage rather than in the changes elicited on DNA bases. However, Pedreño et al. (2005) found that at higher metal concentrations spermine stimulated DNA damage by increasing the formation of single and double strand breaks and even causing the disappearance of the supercoiled, open circular and linear forms of the ΦX174 DNA. Similarly, Mozdzan et al. (2006) showed that spermidin and spermine did not protect DNA and spermine even enchanced the DNA degradation by Cu^{+2}-H_2O_2 oxidizing system. On the other hand, spermidine and spermine were excellent protected to DNA from Cu^{+2}-H_2O_2-ascorbic acid and Fe^{+2}- H_2O_2-ascorbic acid-induced damage. Our results were showed that DNA damage occurs in *U. lactuca* L. after copper treatment.
The main changes observed in the RADP profiles have been resulted both in appearance or disappearance of different bands and variation of their intensity. The differentiation in band intensity and appearance or disappearance of some bands may correlate with level of photoproducts in DNA template and DNA structural changes such as deletions, insertions or breaks after copper treatment as genotoxic agent. When compairing the band variation between the samples incubated with only CuSO4, incubated with CuSO4 and polyamines putrescine, spermidine and spermine, and non-treated sample, it's seen that non-treated and spd treated samples didn't give any bands on agarose gel electrophoresis. But CuSO4 treated sample gave several clear bands. When we checked the polyamine treated samples spm and put treated samples have band variations among each other. Also intensity of the bands are variable. The RAPD analysis by other RAPD primers with these samples gave similar band profiles. Non of the samples gave same band patterns. On the bases of the results, it is conceivable, polyamines have some roles on protecting DNA base alternations against copper. Obviously, sensitivity of the RAPD assay depends on the mutation levels and it needs further investigations. We assume that this study may be help for the further searches on protective roles of polyamines on DNA strand breaks against genotoxic agents.

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