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EVALUATION OF BIOLOGICAL RESPONSE OF RADIORESISTANT BACTERIUM INM-3 AGAINST THE OXIDATIVE STRESS

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ABSTRACT In the present study, RKIP006.G isolated from a radioresistant bacterium Bacillus sp. INM-1 was evaluated for its protection efficacy against of oxidative stress and a possible mechanism of resistance to radiation was studied for novel bacteria INM-3 isolated from rocky soil taken from Qutub Meenar, Delhi in India. Isolation of bacteria INM-3 from soil, protection efficacy of RKIP006.G against hydrogen peroxide induced oxidative stress in bacteria INM-3, Radiation induced stress in bacteria INM-3, Protein estimation, and SDS-PAGE analysis methods were used to know the differential expressed protein which involved in protection activity of bacteria INM-3 in the presence of RKIP006.G. The results suggest that anti oxidative activity of bacteria INM-3 was induced by RKIP006.G and it was protected against oxidative stress induced by hydrogen peroxide. Further, observed that the isolated INM-3 bacteria have radio resistance characteristic against high dose of gamma radiation.

KEYWORDS : Oxidative stress, Hydrogen peroxide, RKIP006.G, Radiorésistance

INTRODUCTION

Oxidative stress defined as the imbalance between proxidant and antioxidant [1]. Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defences, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids [2]. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage [3, 4]. Bacteria INM-3 isolated from different soil samples of rocky soil area from Qutub Meenar, Delhi in India exhibiting high tolerance to radiation. The study of mode of protection and adaptation of bacteria INM-3 against high levels of oxidative stress, represented by high dose of gamma radiation, gives us an insight to the enzyme regulation system of involved in response to oxidative stress for this strain.

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used are highly purified. The chemicals such as agar, nutrient broth, Bovine Albumin Fraction-V (Cat No.RM105-5G), acryl amide (Cat No.1.10784.0100), Tris base (Cat 648310) and Glycine (Cat 357002) was procured from E Merck Germany. Ammonium per sulphate (Cat No. T829027) was purchased from SRL, India. H2O2, and Commassie brilliant blue G-250 were procured from Sigma-Aldrich chemical USA. Ethanol, acetate, methanol, were obtained from Ranbaxy-Ranchem chemical, India.
Isolation of bacteria INM-3

Soil sample was serially diluted at different concentrations (10-1 to 10-7) and poured on the agar plates and incubated for 24 h at 37◦C. The bacterial colonies appeared on the agar plates were streaked on the separate plates to get the pure culture of the bacterial populations. The bacteria obtained were again grown in the broth medium of nutrient broth. The culture was further allowed to grow in the fresh medium. The bacterial culture was maintained and isolated bacterial pellet stored at 4˚C for future used [5].

Bacteria INM-3 treated with Hydrogen peroxide

Nutrient broth was prepared by the inoculation of bacteria in nutrient broth media. Broth was divided into four parts. First was control (untreated sample), second was H2O2 (5%) treated for this added H2O2 in broth and incubated for 5 min. Third was RKIP006.G pre treatment, for this added RKIP006.G (1mg/ml or 2mg/ml)) in broth and incubated for 30 min. After this step, added H2O2 (5%) and again incubated for 5 min. Fourth was RKIP006.G post treatment, added H2O2 (5%) in broth and incubated for 5 min. After this step, added RKIP006.G (1mg/ml or 2mg/ml) and again incubated for 30 min. The plating was done to grow the colony and counting of colony done through naked eye after 24 h incubation. Further grow the all sample in broth with similar treatment. After incubation centrifuged at 1000 xg, for 5 min at 27˚C. Obtained bacterial pellet was dissolved in 500µl PBS and sonicate for 2 min at 4 pulses. After sonication, again centrifuged at 6000 xg for 20 min at 4˚C temperature and took out the supernatant and stored at 4˚C temperature further experiment.

Irradiation of bacteria INM-3

The INM-3 bacterium were irradiated at low dose (5kGy) to high dose (30kGy) at Central Radiation Facility, Institute of Nuclear Medicine Allied Sciences, Delhi. Samples were exposed with 60Co radiation using Gamma Cell-5000 (Board of Radioisotope technology, Mumbai, India) at the dose rate of 1.11kGy/h and all the radiation Safety measures were strictly followed during experimentation [6].

Protein estimation

Total soluble protein in the bacterial cell lysate was estimated by Bradford method [7].

SDS-PAGE of bacterial proteins

Sodium dodecyl sulphate polyacrylamide gel Electrophoresis was performed for bacterial extract protein at room temperature with 10% gels and Tris glycine Buffer (pH8) at 125 V for 90 min. To obtain the molecular weight of the enzymes, protein bands were stained by 0.05% Commassie Brilliant Blue R [8].

RESULTS

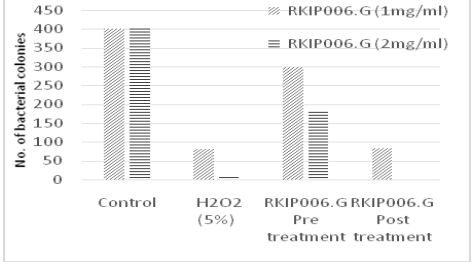

Figure 1: Effect of different concentrations of RKIP006.G on hydrogen peroxide (5%) mediated oxidative stress in bacteria INM-3.

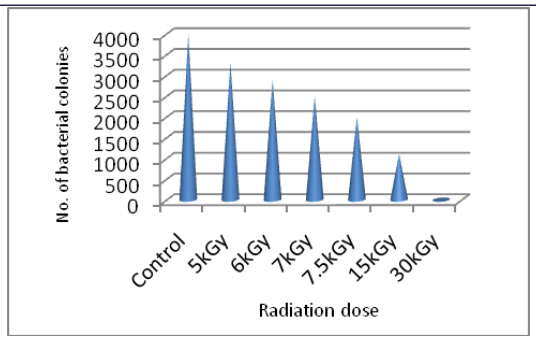

Figure 2: Radioresistant activity of bacteria INM-3 at different dose (5kGy to 30kGy) of gamma radiation.

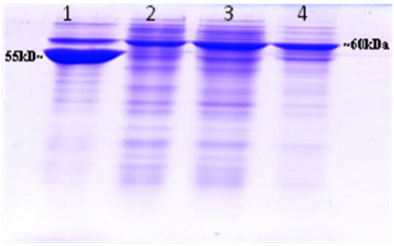

Figure 3: SDS-PAGE Analysis of INM3- bacterial lysate: Lane-1 was loaded with control (w/o treatment), Lane-2 was loaded with H2O2 (5%) treated lysate, Lane-3 was loaded with pre treatment RKIP006.G, and Lane-4 was loaded with post treatment of RKIP006.G and the gel was stained with Commassie brilliant blue.

Protection efficacy of RKIP006.G against hydrogen peroxide induced oxidative stress in bacteria INM-3

It shows decrease in bacterial growth due to H2O2 (5%) treatment. However, RKIP006.G treatment to bacteria demonstrates protection from H2O2 damage. It was also observed that 1mg/ml concentration of RKIP006.G was more effective when compared to 2mg/ml concentration of RKIP006.G (Fig 1). In addition, pre treatment with RKIP006.G showed more potential in shielding the bacterial growth in comparison to post treatment with RKIP006.G against H2O2 induced oxidative stress.

Radioresistant characteristics of bacteria INM-3 against of oxidative stress Bacteria INM-3 was showed the radioresistant activity against stress mediated by gamma radiation. The no. of bacterial colonies were decreased due to low dose (5kGy) to high dose (30kGy) of radiation treatment. But this bacterium was survived in high dose of radiation treatment in the absence of RKIP006.G (Fig 2).

SDS-PAGE analysis of differential expressed bacterial proteins

The SDS-PAGE analysis was carried out to evaluate the differential expression of protein in bacteria INM-3 lysate induced by RKIP006.G (1mg/ml). As shown in fig 3, protein electrophoresis showed the induced expression of ~ 60 kDa protein band in pre and post treated bacterium INM-3 with RKIP006.G as well as in H2O2 (5%).This ~ 60 kDa protein band may be catalase protein which expressed to tolerate the oxidative stress. One high intense band of ~ 55kDa was observed in control but highly reduced its expression in all treated sample.

DISCUSSION

A number of bacteria are capable of living under extreme environmental conditions, each having their own enzymatic defense mechanism to overcome abiotic stress [9, 10]. Bacteria INM-3 was used which has not been characterized in lab. It exhibited tolerance to very high dose of radiation and concentrations of hydrogen peroxide. RKIP006.G was evaluated for its in vitro protection efficacy against of oxidative stress [11, 12]. RKIP006.G was mediated antioxivity against of oxidative stress in bacteria INM-3. In present study we found that, 1mg/ml concentration of RKIP006.G was more effective as compared to 2mg/ml concentration of RKIP006.G for this bacterium. Protein electrophoresis showed the protein band in pre treated and post treated sample at ~ 60 kDa. This protein might be catalase [10]. After this, bacteria INM-3 was treated with low dose (5kGy) to high dose (30kGy) of radiation and this bacterium was protected to itself in the absence of RKIP006.G. This protein may be the reason of survivability of bacteria INM-3 in the presence of high dose of radiation. The ability of bacteria INM-3 to defend itself against of high dose of radiation was suggested in an earlier study.

CONCLUSION

RKIP006.G is a secondary metabolite isolated from radioresistant bacterium Bacillus sp. INM-1. In the present investigation, RKIP006.G was evaluated for its protection efficacy against oxidative stress. This is a preliminary study, further detailed investigation will require its over expressive proteins and organic molecules which may contribute to protection from oxidative stress will be carried out.

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