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Original Article

Response in Cadmium Tolerance of *Penicillium cyclopium* Westling *S*ubsequent to Exposure to Gamma Irradiation

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Abstract

Role of gamma irradiation in modulating 1.1 times more cadmium (Cd) tolerance in *Penicillium cyclopium* Westling has been detailed in this paper. Augmentation in metal tolerance was recognized by escalation in response to Cadmium and Cd removal efficacies than that of their un-irradiated group. FTIR spectra and electron microscopic photographs further strengthen the role of low absorbed dose of gamma in modulating Cd tolerance in *P.cyclopium*. Up regulated activities of anti-oxidatives in gamma exposed fungal groups might be the reason for enhanced Cd tolerance than that of their un-irradiated counter parts. This findings reveal a positive and eco-friendly step for heavy metal bioremediation and metal stressed lignocellulosic waste degradation.

Keywords: *P. cyclopium; Gamma Bioremediation; Antioxidative defense system*

Introduction

Cadmium is a non-essential element that negatively affect ecosystem. Due to its high mobility Cadmium affect plant health at very low concentration (Barceló and Poschenrieder, 1990). Due to cost and inadequate efficiencies of conventional heavy metal removal methods, an alternative for metal elimination was a prolonged research for the scientists. Recently microbial bioremediation is considered as a sustainable and ecofriendly tool to clear-out polluted situation (Tarekegen *et al.,* 2020) Having higher surface to volume ratio fungi are considered better prospective to be used in bioremediation than the other microbes and in turn fungi utilize the metal for its normal growth and metabolism (Abioye *et al.,*2018).

Current biotechnological research such as using physical and chemical mutagens significantly modified microbial output in different industrial and environmental field. Gamma being such a physical mutagen established its potential for microbial strain improvement (Dadachova *et al.,* 2007; Robertson *et al*., 2012) although higher doses of gamma used as an excellent tool for sterilization, food preservation and different food engineering process (Singh *et al*., 2016). Enhanced industrial enzyme activities in gamma exposed fungal strains than that of their un-irradiated counterparts were also reported by Iftikhar *et al. (*2010), Huma *et al.* (2012) and Aleem *et al.(*2018).

In this perspective this work is stipulated to appraise the role of gamma irradiation on Cd tolerance, change in metabolic enzyme activities in *Penicillium cyclopium* and simultaneously the depiction of anti-oxidative activities in gamma exposed *P. cyclopium* grown in Cd riched growth media.

Materials and Methods

Gamma exposure to the spore suspension

According to the method of Kava-Cordeiro *et al*.(1995) spore suspension of *Penicillium cyclopium* Westling (2ml suspension containing 5 x 10^5spores/ml)was prepared using Tween20 (0.02%v/v) and NaCl (0.85%w/v) solution and were exposed to 20-100 Gray of absorbed doses of gamma (absorbed dose of gamma calculated by Fricke Dosimetry) from a Co^{60} gamma source (GC 1200, BRIT). The fungal strain isolated from soil of garbage dump site of Dhapa, Kolkata identified following standard fungal identification key (Frisvad and Filtenborg 1989). All further experiments were done considering MIC (Minimum Inhibitory Concentration) and in close proximity to MIC.

Estimation of Colony Forming Unit (CFU) andand Cd removal efficacy

How significantly cadmium removed (from the liquid growth media) by gamma exposed fungal groups compared to their unirradiated counterparts were evaluated following the method of Srivastava and Thakur (2006) in AAS (Atomic Absorption Spectrophotometer; FI-HG-AAS Perkin Elmer Analyst 400).

Analysis of the functional groups involved in Cd adsorption

The efficient functional groups (present in fungal cell wall) responsible in metal adsorption were studied through FTIR (JASCO-6300 FTIR with a diffuse reflectance mode (DRS8000) attachment) following the methods of Xu *et al.* (2009).

Analysis of Morphological structures

Fungal samples(prepared according to the methods of Mishra and Malik (2013)were gold coated in IB_2 ION Coater for 30mins and observed in SEM (HITACHI S-530),keeping the constant voltageat 25 KeV.

Antioxidant activity

Super-oxide dismutase (SOD), Catalase (CAT), total Glutathione (GSH) level and antioxidant Metallothionein (MT) were studied as antioxidative response.SOD activity was measured following the method of Paoletti *et al.*, (1990). CAT activity was measured following the method of Aebi (1984).Total reduced glutathione was assayed according to the method of Moron *et al.,* (1979).Quantitative estimation of metallothionein protein was estimated according to the method of Viarengo *et al.*(1997) and modified according to the method of Pal *et al*. (2005).

Results

Current study establishes the potential of low absorbed doses of gamma in amending metal tolerance in fungi. Low doses of ionizing radiation could improve cadmium tolerance in *P. cyclopium* which is inferred through the significant escalation of number of CFUs, metal removal efficacies.

Acceleration in Cd tolerance and removal efficacies

Gamma irradiated *P. cyclopium* showed 1.1 times more Cd tolerance in terms of CFU (colony forming unit) and higher removal potential than that of their un-irradiated counterparts. Gamma exposed *P. cyclopium* could grow in 750ppm of Cd in growth media while the un-irradiated group could tolerate upto 650ppm.Prior exposed to gamma (20Gy-100Gy)and then grown in 300-650 ppm of Cd,*P. cyclopium* showed a radiation dose dependent enhancement of CFU up to a certain dose of gamma exposure. Effective dose showing maximum escalation in CFU was noticed at 80Gy against 300ppm of Cd and against 650ppm that effective dose was at 60Gy. 20-60Gy irradiated *P.cyclopium* grown in 300ppm of Cd showed 20%-73% increase in CFU (Fig 1a) while at 80Gy 1.13fold CFU was studied than that of their un-irradiated counterparts. A slight decrease in CFU was perceived in the group exposed to 100Gy in comparison with the group exposed to 80Gy, although it was still 93% more than un-irradiated counterparts. Further increase in metal concentration *i.e* against at 650ppm the effective dose manifesting maximum (1.2fold) CFU was noted at 60Gy (Fig 1a). In contrast to no growth of *P.cyclopium* at only 750 ppm Cd, gamma exposed group showed significant growth of the fungi having maximum CFU in the group exposed at 40Gy (absorbed dose) of gamma irradiation (Fig 1b). CFU analysis depicted an interesting data which corroborated the potential of gamma irradiation in augmenting Cd tolerance of *P.cyclopium*

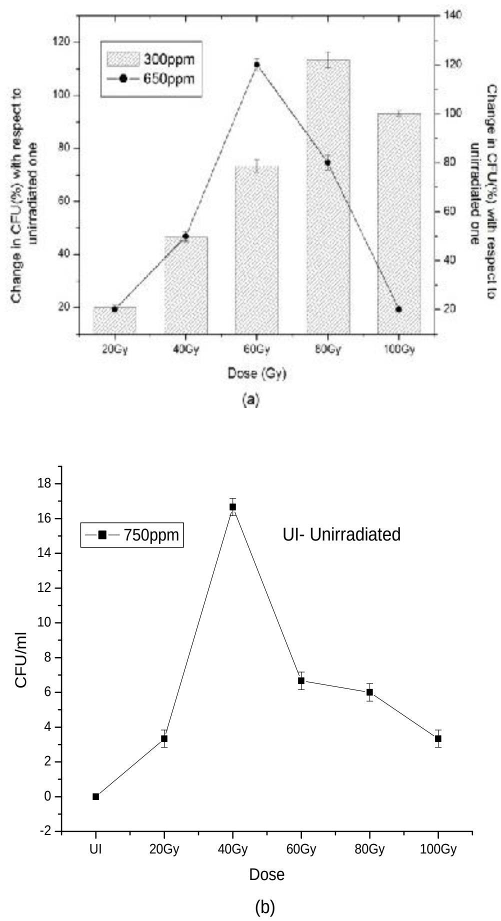

Fig.1 Colony Forming Unit of *P. cyclopium* with or without exposure to gamma irradiation grown in Cd riched media **(a)** 300ppm - 650ppm Cd **(b)** 750ppm Cd(n = 6. p≤0.05 was considered significant)

Data of AAS analysis reveal significant (p≤0.05) potential of gamma irradiation in amending Cd removal by *P. cyclopium*. 80Gy and 60Gy (effective dose showing maximum CFU against 300ppm and 650ppm respectively) exposed *P. cyclopium* when grown in 300ppm and 650ppm of cadmium respectively could remove 13% more cadmium than its un-irradiated counterparts. Interestingly 40Gy exposed *P. cyclopium* could remove 60% of Cd from when grown in 750ppm Cd, while un-irradiated one could not grow in such higher concentrations of Cd (Fig2). Inherent properties of microbes in interaction of metals have been reported by a host of researchers (Igiri *et al.,* 2018). In this study gamma induced escalation of Cd removal potential of irradiated fungal strains imitate the role of gamma in response of fungi towards heavy metals. Improvement in metal resistance. Impact of low doses of gamma irradiation on fungi have been reported earlier by Abbasi *et al*.(2016); de Queiroz Baptista *et al*.,(2015); Dadachova *et al*.(2007). Actually genetic manipulation of fungi is done to improve some specific factor and gamma has the potential to cause mutation to the genes of cells through DNA repair mechanism (Huma *et al*., 2012). In this study difference in gene expression due to exposure to gamma irradiation might be the cause behind higher Cd tolerance and removal by radiation exposed *P.cyclopium*. Such exposures may also enhance immune reactions of the organism and attenuate harmful effects of higher doses of radiation.

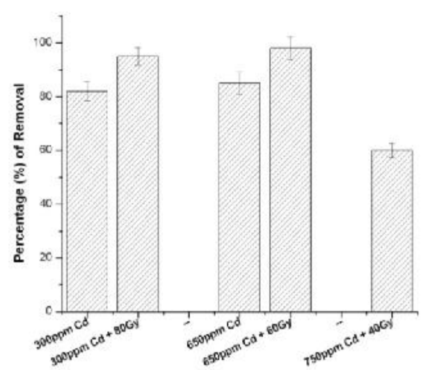

Fig. 2 Removal of Cd by *P. cyclopium* (with and without exposure to gamma irradiation) (n = 6.p≤ 0.05 was considered significant)

Depiction of FTIR spectrum and SEM image

Fig3 shows the FTIR spectra of different groups of *P. cyclopium* biomass. Spectral data analysis showed involvement of different functional groups (amide group [-NH], hydroxyl group [-OH], carboxylate group [-COO], carbonyl group [-CO]) in Cd adsorption. Significant shift in peak regions were found at 3394 Cm^{-1}, 2928 Cm^{-1}, 1548 Cm^{-1}, 1240Cm^{-1} regions both in gamma exposed and unexposed biomass of *P.cyclopium* grown in Cd supplemented media with respect to its control (Cd free biomass). The peak at 3394 Cm^{-1} region observed in the control *P.cyclopium* biomass (Fig 3a) was noted to be shifted to 3363 Cm^{-1} in Cd exposed biomass (Fig-3b) while in gamma irradiated and Cd treated group the same peak was shifted to 3385 Cm^{-1}(Fig 3c).

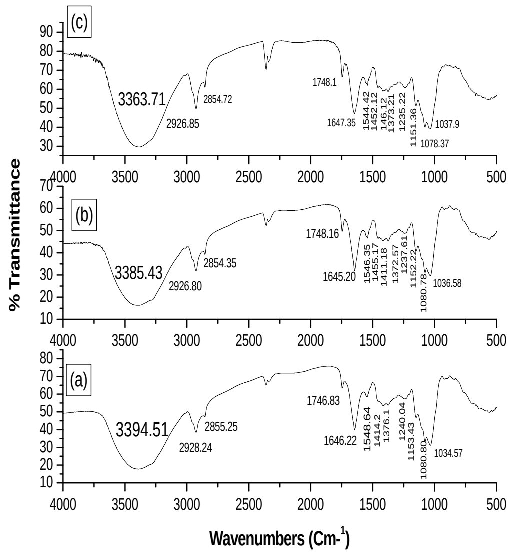

Fig. 3 FTIR spectrum showing effective functional groups (present in cell wall) responsible for Cd adsorption of *P.cyclopium* biomass
(a) Control biomass; (b) 300ppm Cd riched biomass (c) 80Gy gamma exposed biomass grown in 300ppm Cd riched media

Peaks in the regions 1548 Cm^{-1} and 1240Cm^{-1} were also noted to be shifted in the two experimental groups as compared to the normal control group. One new peak at 1450 Cm^{-1} region was noted in Cd treated biomass with or without gamma exposure, which was not observed in control one. Significant changes in the same functional groups in the gamma exposed fungi additionally treated with metals, confirms potential of gamma irradiation in modulating heavy metal tolerance in fungi involving important functional groups. Similar type of results have been reported earlier also (Manal *et al*., 2020; Rudakiya *et al*., 2018).

Cylindrical normal hyphae distorted into thinner with flocculations and pores after exposed to metal stress (Fig. 4a & 4b). However, prior exposed to gamma and then grown in metal enriched growth media, the fungal hyphae exhibited less structural deformities (Fig. 4c). Manal *et al.,* (2020) also noticed alteration of normal structure of the fungal hyphae exposed to the heavy metals. Upholding close to normal hyphal morphology in gamma exposed groups indicate potential of gamma in providing protection against metal-induced morphological alteration.

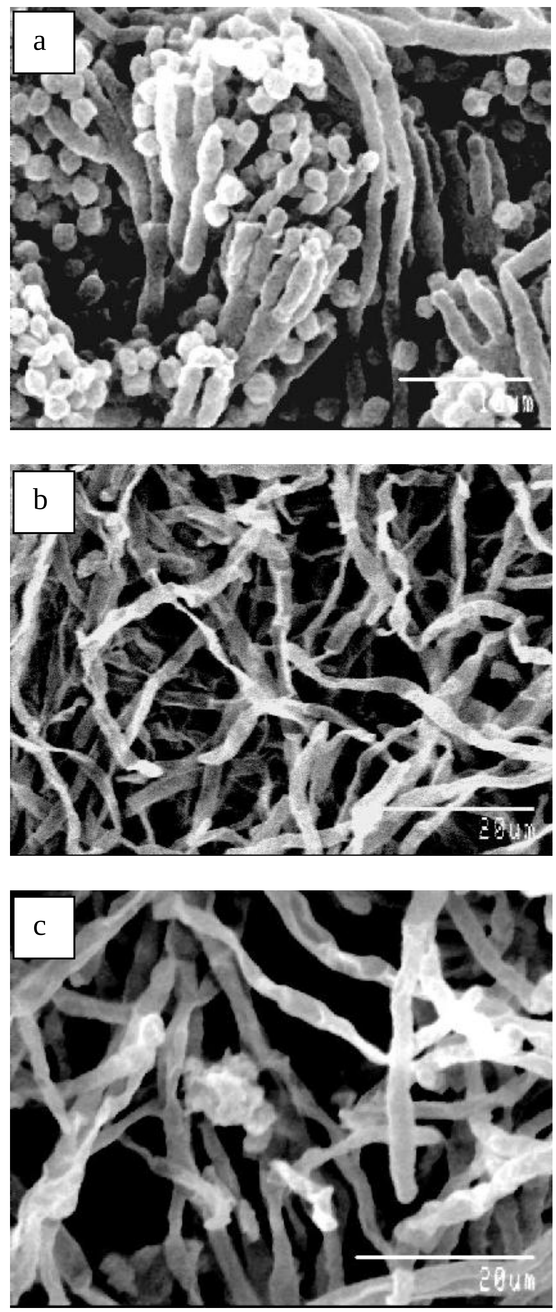

Fig. 4 Hyphal morphology (SEM) of *P.cyclopium* (1500-2000X)[a : Control *P.cyclopium,* b : 300ppm Cd treated

P.cyclopium, c: 80Gy exposed *P.cyclopium* grown in 300ppm Cd]

Change in Antioxidative activities

Any chemical or physiological stress (metal and gamma exposure) produce Reactive Oxygen Species (ROS) such as superoxide anion radical ($O_2\cdot^-$), hydrogen peroxide, and hydroxyl radical (OH·) (Fridovich, 1995) which cause harm for the cell damaging DNA, lipids and proteins. To combat the stress of ROS all living cells upregulate their non-enzymatic and enzymatic antioxidant defense mechanisms. In this study the escalation of anti-oxidative response in metal treated fungal strains proved their role in protecting fungi from the toxicity of heavy metals (Fig.5a). Further increase in antioxidant activities in gamma exposed group of fungi (grown in metal treated growth media) suggest potential of low doses of gamma in better tolerance of heavy metals in fungi (Fig. 5b & 5c).300ppm of Cd in growth media of *P.cyclopium* resulted in two times increase in SOD and CAT activity and 1.8times more GSH level as compared to control group of the fungi without having any metal treatment. A higher concentration of Cd in growth media (650ppm) manifested further increase in all the three parameters.80Gy exposed *P.cyclopium* grown in 300ppm Cd supplemented growth media manifested 2fold increase in activity of both SOD and CAT and 1.6 times GSH level (Fig5b). 60Gy exposed *P.cyclopium* grown in 650ppm of Cd showed higher stimulation in activities of these markers as compared to that of its un-irradiated counterparts when grown under same metal stress. Upregulation of antioxidative enzymes and anti-oxidants after being treated with heavy metals in fungal strains were reported by a group of researchers (Huang *et. al.,* 2017; Krumova *et al*., 2011). Similar result was also noticed by Robertson et al. (2012) in *W. dermatitidis* cells exposed to radiation. In short, this validates ionizing radiation activates DNA repair mechanism and anti-oxidative defense systems as mentioned by Huma et al., (2012). MTs have been shown to be associated in intracellular sequestrations of heavy metals and balancing oxidative stress along with other numerous cellular functions.

This study portrays 48% more MT in *P.cyclopium* when grown in Cd supplemented media (300ppm) while prior exposed to 80Gy and then grown in same Cd enriched media (300ppm) showed 23% more MT content when compared to their un-irradiated but Cd stressed counterparts (Table 1).

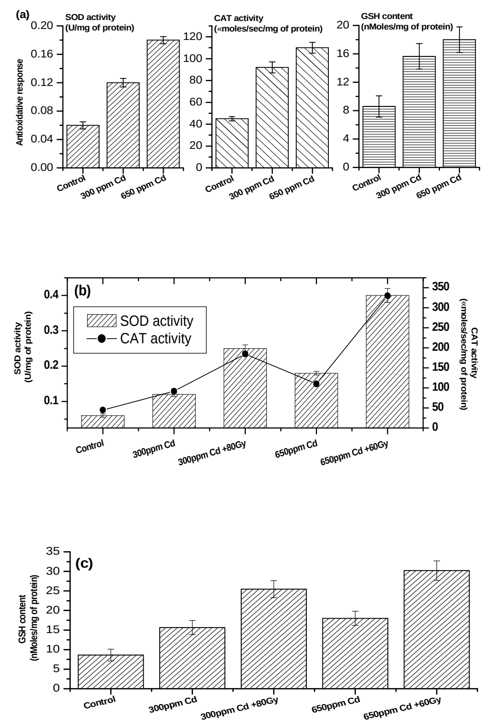

Fig. 5 Antioxidative response of *Penicillium cyclopium* **(**n = 6. p≤0.05 was considered significant.)

Sample	Total Metallothionein (µmole/mg of protein)
Control P. cyclopium	10.35×10^{-5}
300ppm Cd exposed *P.cyclopium*	15.36×10^{-5}
80Gy exposed *P.cyclopium grown in* 300ppmCd	18.85×10^{-5}
80Gy exposed *P.cyclopium*	13.65×10^{-5}

Table 1. Metallothionein (µmole/mg of protein) in *P.cyclopium*

Lorenzo-Gutiérrez *et al.,* (2019) reported same type of response in *Fusarium oxysporum* when exposed to heavy metals like Cd, Zn. Expression of metallothionein in gamma exposed fungi has not reported till now although gamma radiation induced expression of metallothionein in *Plantago ovate* for sk was reported by Saha *et al., (*2013). Data of correlation study shows positive correlation between antioxidative enzymes and marker proteins (Table 2).

	SOD	CAT	GSH	MT
SOD				
CAT	0.88**			
GSH	0.91*	0.89**		
MT	0.95*	0.85**	0.81**	

Table 2. Values of Correlation matrix (Pearson) between SOD, CAT, GSH, and MT of *Penicillium cyclopium* Westling exposed to absorbed doses of gamma (20–100 Gy) and grown in Cd-enriched growth media (Correlations were significant at * P< 0.0001; ** P<0.001)

Precisely the recent work reveals the role of low doses of gamma radiation in augmenting cadmium tolerance in *P. cyclopium* and upregulation of antioxidative defense system might be the responsible factors for being more metal tolerant after being exposed to gamma. The higher efficiency of the radiation exposed fungi to remove heavy metals from the media highlights the possibility of utilizing low absorbed doses of ionising radiation to improve the bioremediation potential of the fungi.

Conclusion

Potential of gamma irradiation for enhancing Cd removal of *P.cyclopium* than both of their un-irradiated counterparts were evaluated in this study. Up-regulated activities of anti-oxidants and metabolic enzymes activities supported them to improve cadmium removal efficacies than that of their un-irradiated counterparts.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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