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Short Communication

Decolorization and degradation of malachite green by *Aspergillus flavus* and *Alternaria solani*

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Bioremediation using a variety of microbes for the degradation of xenobiotics seems a green solution to the problem of environmental pollution. Microbes have been gifted by nature with the ability of degrading a wide spectrum of environmental pollutants. Different fungi have the potentials to degrade complex and recalcitrant organic compounds into simpler fragments; sometimes achieving complete mineralization. In this work, we have investigated the decolorization and degradation of a triphenylmethane dye, malachite green by two fungal microorganisms, *Aspergillus flavus* and *Alternaria solani*. Both the species were able to decolorize different concentrations of malachite green (10 to 50 µM) almost completely (> 96 %) within 6 days.

Key words: *Alternaria solani*, *Aspergillus flavus*, Decolorization, degradation, malachite green.

INTRODUCTION

Removal of dyes from textile waste effluents has been carried out by physical, chemical and biological methods, such as flocculation, membrane filtration, electrochemical techniques, ozonation, coagulation, adsorption and fungal discoloration (Fu and Tiraraghavan, 2004). Fungal bioremediation is becoming an attractive option for removal of dyes from industrial effluents as microorganisms are nature's tools for cleaning the environment. Without fungi and their decomposing activities, the world would have become a heap of dead bodies of plants and animals.

Removal of dyes from industrial waste waters is of global concern because dyes cause many problems in aqueous environments. Dyes may significantly affect photosynthetic activity in aquatic life because of reduced light penetration and may also be toxic to some aquatic life due to the presence of aromatics, metals, chlorides, etc. (Daneshvar et al., 2007). Malachite Green (MG) is a triphenylmethane dye, which is most widely used for coloring purposes, amongst all other dyes of its category (Gupta et al., 2004). MG is highly toxic to human beings as it affects the immune and reproductive systems and possesses carcinogenic properties (Rao, 1995). From environmental point of view, there is concern about the fate of MG and its reduced form, leuco malachite green in aquatic and terrestrial ecosystems since they occur as contaminants and are potential human health hazards.

In recent years, there has been an intensive research on fungal decolorization of dye waste waters. It is turning into a promising alternative to replace or supplement present treatment processes (Ramya et al., 2007). Biological processes have potential to convert or degrade the pollutant into water, carbon dioxide and various salts of inorganic nature. In this work, we have investigated the decolorization and degradation abilities of two fungal species, *Aspergillus flavus* and *Alternaria solani* for malachite green.

MATERIALS AND METHODS

Fungal cultures

Pure cultures of *A. flavus* and *A. solani* were grown in Petri dishes for 3 days using Czapek Dox Agar medium. This medium contains 30 g sucrose, 3 g $NaNO_3$, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, 0.01g $FeSO_4.7H_2O$, 1 g K_2HPO_4 and 15 g agar per liter. Final pH of the medium is 7.3 ± 0.2 at 25°C. After 3 days, fresh cultures were grown in liquid medium (the same medium but without agar) in 250 mL Erlenmeyer flasks again for 3 days.

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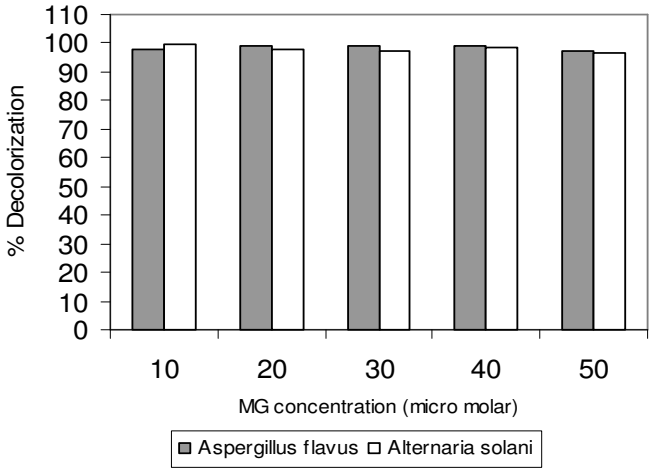

Figure 1. Efect of dye concentration on % decolorization of malachite green. Incubation period: 6 days.

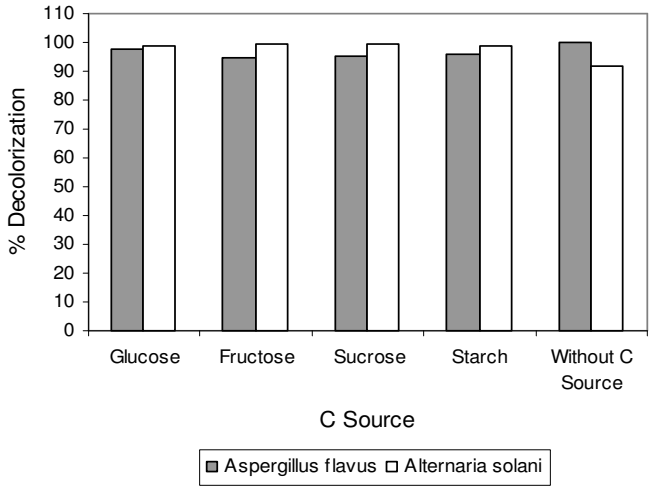

Figure 2. Efect of C source on % decolorization of malachite green. Incubation period: 6 days; MG concentration: 30 µM.

Decolorization study

For decolorization and degradation study, 250 mL Erlenmeyer flasks containing 125 mL solutions of malachite green prepared in the same growth medium were inoculated with the fungal biomass grown in the liquid medium. These flasks were kept in a static incubator at 37.5°C providing micro-aerobic conditions. After a treatment of 6 days, the decolorized solutions were filtered through 0.20 µm filters and centrifuged at 4400 rpm for 5 min. The supernatants were analyzed spectrophotometrically using UV-Visible spectrophotometer (UV-1700, Shimadzu).

To know about the effect of dye concentration on % decolorization, different initial dye concentrations i.e., 10, 20, 30, 40 and 50 µM solutions were used. Similarly in order to know about the effect of C source on % decolorization, different C sources, that is, glucose, fructose and starch were used instead of sucrose in the decolorization medium. In one case, no C source was used in the decolorization medium in order to know whether the microorganisms can use the dye as sole source of C and energy or not.

Each experiment was conducted in duplicate and mean values were taken.

RESULTS

The results of this study are given in Figures 1 and 2.

DISCUSSION

Microbial decolorization of malachite green has been investigated by a few authors. Youssef et al. (2008) have studied the decolorization of malachite green by *Acremonium kiliense*. According to them 95.4% MG was decolorized within 72 h when the concentration of the dye was 5 mg L^{-1} but decolorization was only 35.48% when the dye concentration was doubled. They have attributed this trend to be due to inhibition of fungal growth at high dye concentration. Biodegradation of malachite green by *Kocuria rosea* MTCC 1532 has been studied by Parshetti et al. (2006). According to them 50 mg L^{-1} MG was completely decolorized under static anoxic conditions within 5 h by bacteria *K. rosea* MTCC 1532; however decolorization was not observed at shaking condition. Ren et al. (2006) have investigated the decolorization of different dyes including malachite green by *Aeromonas hydrophila*. According to them 50 mg L^{-1} MG was 90% decolorized within 10 h under aerobic culture conditions. Decolorization of malachite green by *Citrobacter* sp. has been studied by An et al. (2002). According to their results, 100 µM MG was 80 % decolorized within 1 h.

The present study has revealed that *A. flavus* and *A. solani* are able to decolorize 50 µM malachite green by 97.43 and 96.91% respectively within 6 days. Dye concentration in the range of 10 to 50 µM had no significant effect on % decolorization in case of both the species. It is also clear that C source in the decolorization medium has no significant effect on % decolorization in both cases.

Use of malachite green as sole source of C

Both the microorganisms were able to use the dye as sole source of C and energy. When the dye was used as the sole source of C in the decolorization medium, *A. flavus* decolorized a 30 µM solution by 99.78% and *A. solani* by 91.72% within 6 days. This shows that both the species are able to use the dye for their growth.

Mechanism of decolorization

The UV-Visible spectral study of the decolorized solutions showed that the λ_{max} of the dye has changed significantly. In case of *A. flavus*, the original peak (at 617 nm) completely disappeared and another new single peak appeared at 387 nm, which shows that the mechanism of

decolorization is biodegradation. In case of *A. solani*, peaks were recorded at 352, 617 and 618 nm from which it can be inferred that decolorization occurred through both biodegradation and biosorption.

Conclusion

It may be urged that both *A. flavus* and *A. solani* are good potential candidates for the decolorization of malachite green and possibly other dyes. The ability of *A. solani* to decolorize acid violet 19 has already been established (Ali and Muhammad, 2008). Further studies in this domain are valuable to use these microorganisms for the decolorization of real dye waste waters (which are complex mixtures of many dyes) and to know about the nature and fate of the degradation products.

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