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SUMMARY

Phanerochaete chrysosporium and *Geotrichum candidum* were obtained from the Microbial Type Culture Collection, IMTECH, Chandigarh and used for the decolorization studies of different dyes including triphenylmethane dyes. For the growth of *P. chrysosporium* a low cost medium was designed and most of the experiments were carried out with the cells grown in this medium. For the production of higher amount of cellmass, a 7 l agitated reactor was used. The rates of decolorization of different dyes by *P. chrysosporium* grown in simple and complex medium were compared. In another study decolorization of the several dyes (acid green 20, red HE-8B, malachite green, navy blue HE-2R, magenta and crystal violet) and industrial effluent from a textile and dyeing industry with the growing cells of *P. chrysosporium* in static and shake culture was observed. It has been observed that only the cells of both *P. chrysosporium* and *G. candidum* are responsible for decolorization. The culture supernatant did not manifest any extracellular dye decolorizing activity. The biotransformed products obtained after digestion of crystal violet with *G. candidum,* were extracted and checked by thin layer chromatography.

A number of soil and water samples were collected from the vicinity of effluent treatment plant of a textile and dyeing industry. Several organisms were screened for their ability to decolorize triphenylmethane group of dyes. The best strain was selected on the basis of dye decolorizing activity. The bacterial strain, that decolorized triphenylmethane group of dyes very rapidly, was identified as *Kurthia sp.* Under aerobic condition maximum 98% color was removed by this strain. The strain did not manifest extracellular activity. A number of triphenylmethane dyes such as magenta,

crystal violet, pararosaniline, brilliant green, malachite green and ethyl violet are used in this study. The rates of decolorization of magenta, crystal violet, pararosaniline, brilliant green and malachite green were found to be more than that of ethyl violet. After the decolorization of most of the dyes, viable cell concentration of *Kurthia sp.* has reduced significantly. In the case of ethyl violet, viable cell concentration was almost negligible after decolorization. Considerable efforts were made to enrich dye degradation culture by the "shake soil slurry method", however, no organism was found to utilize dye molecule as a source of energy. Three organisms were isolated from the active slurry of the enriched cultures and were used to decolorize different triphenylmethane dyes. The rates of growth and decolorization by the enriched culture were less compare to those by *Kurthia sp.* The types of biotransformed products formed were also same.

The strain *Kurthia sp.* was found to have a great potential for decolorization of triphenylmethane group of dyes compared to all other organisms tested. Hence, all the subsequent experiments were carried out with *Kurthia sp.* only. *Kurthia sp.* was maintained on nutrient agar plates containing 3.0 μg/ml crystal violet at 30^{0}C. Different parameters (carbon and nitrogen sources, growth factors, inoculum age and volume, metal ions and chemicals, surface active agents, initial culture pH and temperature) were optimized for the production of maximum cellmass and decolorizing activity. The organism was also grown in a stirred tank reactor and agitation and aeration rates were optimized. The initial culture pH of 8.5 and temperature of 30^{0}C were found to be optimum for the growth and decolorizing activity. The cell yield increased when malt extract (1%, w/v), molasses (5%, v/v), biopeptone (0.5%, w/v) and ammonium peroxodisulphate (0.2%, w/v) were used in the medium. The optimum pH and temperature for decolorizing activity was 8.5 and 40^{0}C, respectively. Different metal

ions (Zn^{2+}, Co^{2+}, Cu^{+} and Ag^{+}) inhibited the growth and decolorizing activity in 0.5 to 2.0 mM concentration range except Ca^{2+} which increased the decolorizing activity at 0.5 mM level. By using surface active agent (PPG) at the 0.1% (v/v) level, the cellmass increased significantly. The agitation (300 rpm) and aeration (0.75 vvm) rates on the growth and production of decolorizing activity by *Kurthia sp.* in an stirred tank reactor were optimized. Optimum biotransformation conditions of the different triphenylmethane dyes were determined in an aerated and agitated reactor. In the case of crystal violet and magenta, the optimum agitation and aeration rates were 300 rpm and 1.0 vvm, respectively. However, in the case of malachite green the optimum aeration rate was 0.5 vvm, while the optimum agitation rate was the same (300 rpm).

For the large scale production of *Kurthia sp.* 150 l fermenter was run with a working volume of 90 l. The cells obtained after centrifugation were used for the biotransformation of different triphenylmethane dyes. The cells were washed thrice with 0.05 M phosphate buffer, pH 7.0 and resuspended in the same buffer. Crystal violet (1 mg/ml) was added intermittently to the cells at room temperature ($25^{0}C$) with constant stirring till it is colorless. The reaction mixture was centrifuged. Biotransformed products of crystal violet were checked in supernatant and pellets. The supernatant was extracted with chloroform at different pHs ranging from 2.0 -11.0. No organic matter was found in the supernatant. The products did not come out of the cells and the digested cells were extracted with methylene chloride. The impure concentrated product was loaded on silica gel (mesh size 60 - 120) column, pre-equilibrated with hexane. Elution was done by ethyl acetate and hexane gradient. Fractions containing products were concentrated by rotavapour and dissolved in minimum amount of hexane. The product was repurified by loading on silica gel (mesh size 230 - 400)

column pre-equilibrated with hexane and eluted with the same solvent system. The UV-Visible absorption spectra of the transformed product of crystal violet by *Kurthia sp.* was observed to be similar that of an authentic sample of leucocrystal violet. The purified product has a melting point 177^{0}C, which was found to the same as reported for leucocrystal violet. Its structure was further supported by appearance of molecular ion (M^+) peak at 374 in mass spectral data. Its structure was confirmed by NMR spectral data. Its NMR spectral data was identical with those of authentic sample of leucocrystal violet. Finally the structure was confirmed by comparing IR spectra with the authentic compound. Biotransformed products of malachite green and magenta were isolated and characterized in the same way as that of crystal violet. The identification of the biotransfomed products were carried out by ^{1}H and ^{13}C NMR, MS and IR spectra and compared with the authentic compounds.

The acute toxicity of the crystal violet, leucocrystal violet, malachite green, magenta, brilliant green, ethyl violet, pararosaniline and biotransformed product of crystal violet were found out based on the toxic effects of the test substances on cell killing and growth inhibition. The cell concentration increased with time in both cases (with and without crystal violet), however the rate of increase of cell growth in the presence of crystal violet, is less compared to control. The value of IC_{50} for crystal violet was estimated 1.146 ppm. Upto 600 ppm, leucocrystal violet had shown no inhibition effect on yeast cell growth. In our report, the biotransformed product, leucocrystal violet, having high chronic toxicity, was found to be cell bound. So the strain, *Kurthia sp.* can be used as decolorizing agent and for removal of toxicity from the effluent containing triphenylmethane dyes.

The acute toxicity of other triphenylmethane dyes was determined by the same procedure as described above. The IC_{50} values of malachite green, magenta, brilliant green, ethyl violet and pararosaniline were 1.502, 30.002, 10.021, 0.7847, and 8.738 ppm, respectively. The reduction in COD values of different triphenylmethane dyes digested with *Kurthia sp.* was significantly high. The COD values of synthetic and textile dye-stuff effluent were also reduced after treatment with *Kurthia sp.*

Kurthia sp. does not exhibit extracellular decolorizing activity. Cells are responsible for the decolorization of dyes. It can decolorize the triphenylmethane dyes in a very short time compared to any other organisms reported so far. It was observed that some dyes are comparatively resistant to decolorization whereas others are readily decolorized by *Kurthia sp.* Among all the dyes used, pararosaniline has the simplest structure and it was decolorized (100 %) very rapidly, whereas a very less decolorization was noticed with ethyl violet at the same time. The specific decolorizing activity of *Kurthia sp.* was more than any other organisms reported so far. It is also evident that 1 g cellmass (dw) of *Kurthia sp.* can declorize 32.73 mg crystal violet in 6 h while 2.04 mg crystal violet was decolorized by 1 g cellmass of *Pseudomonas pseudomallei* 13NA in 120 h. Considering all the above facts, it is evident that *Kurthia sp.* may be a promising bacteria to depollute the textile and dye-stuff effluent containing triphenylmethane dyes.