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Research article

A novel application of *Paracoccus pantotrophus* for the decolorization of melanoidins from distillery effluent under static conditions

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ABSTRACT

Melanoidin is the hazardous byproduct formed during the production of ethanol in distilleries. In the present study, a highly effective melanoidin decolorizing bacterial isolate, SAG_1, was isolated from the effluent enriched soil of a distillery. This strain, identified as *Paracoccus pantotrophus*, was highly efficient to decolorize melanoidins up to 81.2 ± 2.43% in the presence of glucose and NH_4NO_3. The effects of autoclaved as well as living cells and inoculums size on decolorization activity were investigated. The results indicated that only living cell showed the decolorization activity i.e. 78.6 ± 2.62%, while, no activity has been observed using autoclaved cells. The inoculums size of 8% v/v, showed maximum activity of 62.9 ± 3.00%. The isolate SAG_1 was found to be more efficient in decolorizing the melanoidins from distillery effluent as compared to the reference culture *Pseudomonas putida*.

1. Introduction

Waste management is the major thrust area of research in the world, as these wastes creates many problems in the environment. The wastewater characteristics and type of pollutants varies significantly from industry to industry. Pollution from distilleries is one of the most alarming dangers that confront humankind today. The discharge of pollutants into the environment from distilleries creates a threat to all organisms which causes a big environmental hassle. In India, more than 40 billion liters of effluent has been produced annually by ~300 distilleries (Naik et al., 2010). Melanodins are the major colorant compound formed after heating treatment, if it is present in high quantity it leads to environmental contamination (Agnihotri, 2015). These are complex bio-polymer of amino-carbonyl compounds that can form stable complexes with metal cations (Chandra et al., 2008). The melanoidins play a major role in edaphic and aquatic pollution. It is the big cause of high BOD, COD, TDS, phenolics, sulfate, and phosphate values in distillery effluent. In soil, it inhibits seed germination by reducing alkalinity as well as manganese accessibility and ultimately effects agricultural crop production. It reduces penetration of sunlight in water bodies causing death of aquatic animals due to oxygen deficiency (Raghukumar et al., 2004; Kumar and Chandra, 2006; Bharagava et al., 2008).

Various physico-chemical techniques have earlier been developed for the elimination of this xenobiotic compound from the environment. These techniques are efficient but require high reagent dose and too expensive. Beside this, they generate large amount of sludge and form hazardous products (Sridevi et al., 2011). Conventional biological processes are not enough to treat these effluents. In addition to these techniques, bioremediation, an eco-friendly technique has been reported in which the removal of hazardous compounds from the environment occurred by the use of microorganisms (Boopathy, 2000). Various biological studies have earlier been carried out by a number of researchers using microorganisms that included *Proteus mirabilis*, *Bacillus* sp., *Raoultella planticola* and *Enterobacter sakazakii* (Yadav and Chandra, 2012), *Pseudomonas* sp. (Chavan et al., 2006), *Pseudomonas putida* (Ghosh et al., 2009), *Alcaligenes faecalis* (Santal et al., 2011), *Bacillus* (Krzywonos, 2012), *Bacillus badius* (Mehta et al., 2014), consortium of *Pediococcus acidilactici* and *Candida tropicalis* (Tiwari and Gaur, 2014), *Aspergillus oryzae* JSA-1 (Agnihotri, 2015), *Lactobacillus plantarum* (Krzywonos and Seruga, 2015), *Bacillus licheniformis*, *Bacillus* sp. and *Alcaligenes* sp. (Bharagava et al., 2009). Therefore, to overcome the physico-chemical treatment, the biological

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treatments using microbes are drawing attentions. We reported here a bacterial isolate *Paracoccus pantotrophus* strain SAG_1 having novel application for decolorization of melanoidins present in distillery effluent.

2. Materials and methods

2.1. Culture media

For the present study, 'King's B' broth media and Melanoidin pigment broth (MPB) media were used (Santal et al., 2011).

2.2. Wastewater collection and melanoidins preparation

The distillery effluent was collected from Haryana distillery Ltd., Yamunanagar, Haryana, India. The effluent was very dark brown in color. The synthetic melanoidins was prepared by using the method followed by Adams et al. (2003).

2.3. Isolation of bacterial cultures

A modified enrichment culture technique was used for the isolation of bacteria from the effluent enriched soil (Mohana et al., 2007; Santal et al., 2011) while *P. putida* (MTCC No. 2445) was kindly provided by the Institute of Microbial Technology, Chandigarh, India and used as a reference culture. In this technique King' B broth media having distillery effluent instead of distilled water was inoculated with contaminated soil and incubated for seven days. After four days of incubation fresh media was inoculated in the old incubated media so that only melanoidin degrading bacteria can survive.

2.4. Screening of efficient isolates

Selection of isolates was carried out by primary and secondary screening. For primary screening each isolate was inoculated in the broth media having distillery effluent and was kept in the incubator for seven days, each day the visual change in color of media was observed. The isolates showed visual decolorization in primary screening was used for further study. For secondary screening, decolorization assay was performed using MPB media. To study the decolorization yield, the sample was centrifuged and then color intensity was measured at 475 nm with UV–VIS spectrophotometer. Decolorization activity was the difference between initial and final absorbance divided by initial absorbance multiplied by 100 (Santal et al., 2011; Seruga and Krzywonos, 2015).

2.5. Optimization of physico-chemical parameters

To enhance the distillery effluent decolorization, various growth parameters of the isolate SAG_1 and the reference culture *P. putida* were studied. These physico-chemical parameters were pH, temperature, different distillery effluent concentrations (5–100%, v/v), different carbon and nitrogen sources and their combined effect (Santal et al., 2011).

2.6. Decolorization by living and dead cells

To check whether the decolorization was due to biological or non-biological activity, appropriate inoculums (1.0 ml) of living and autoclaved cells of both the cultures were inoculated into 50 ml melanoidin pigment broth media separately as per method followed by Jirianuntipon et al. (2008) and Sirianuntapiboon and Prasertsong (2008).

2.7. Effect of shaking and non-shaking conditions on decolorization activity

To check whether the maximum decolorization activity under static and non-static conditions of the isolate, each flask having MPB media were cultivated with 1.0 ml inoculums of isolate SAG_1 and *P. putida* respectively. Flasks were incubated both in shaking (180 rpm) and in non-shaking conditions. About 4 ml sample was taken out every day and OD was recorded after centrifugation at 10,000 rpm for 15 min.

2.8. Effect of different concentrations of inoculum size

To see the effect of inoculum sizes on melanoidin decolorization, different concentration (1.0–10.0%, v/v) of inoculums were used. For the preparation of inoculums, flask was poured with 50 ml of broth media and autoclaved. The broth was inoculated with a loop full of 24 h old bacterial culture and placed in an incubator. Different concentrations (1.0–10.0%, v/v) from 24 h old broth culture was inoculated in MPB media and kept under incubation for 5 days. After interval of 24 h, 4 ml of inoculums was taken and decolorization activity was observed on each day.

2.9. Biochemical characterization of isolate

The morphological and biochemical characterization of the isolate SAG_1 was done by MTCC (Microbial Type Culture Collection and Gene Bank), Institute of Microbial Technology (IMTECH), Chandigarh, India.

2.10. Phylogenetic analysis

The molecular characterization of the isolate SAG_1 was done by using 16S rDNA sequencing as per the method followed by Santal et al. (2011).

2.11. Statistical analysis

The experiments were conducted in triplicates. All the data collected after experiments were analyzed by using MS Excel.

3. Results and discussion

3.1. Isolation and screening of efficient bacterial isolate

The bacterial isolate SAG_1 showed 62.5 ± 2.75% decolorization while reference culture exhibited 47.5 ± 2.25% decolorization activity at 1.0% (w/v) concentration of melanoidin during screening studies. In one of the earlier study by Sankaran et al. (2015) *Pseudomonas* sp. was isolated using king's B media from the wastewater contaminated soil.

3.2. Optimization of physico-chemical parameters

Various growth parameters studied during optimization of cultural conditions resulted that at optimum temperature 37 °C maximum decolorization activity of isolate SAG_1 was 66.1 ± 2.1% while *P. putida* exhibited 60.4 ± 2.25% (Fig. 1a). It was also observed that with the further increase in temperature, the activity of isolate was completely inhibited. Similarly, according to Mohana et al. (2007) the temperature from 20 °C to 37 °C favors the decolorization activity but further increase in temperature inhibited the decolorization activity. However, the optimum temperature for the maximum decolorization activity was reported 35 °C (Yadav and Chandra, 2012; Krzywonos, 2012; Boopathy and Senthilkumar,