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Decolonization of Direct Blue- 129 Using Molecularly Identified Soil Microorganisms

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Abstract

There are several industries which could threat our environment. Among them the textile factories by using different dyes disturbing more. Therefore, introducing the new techniques or, bacterial strains is interesting for the environmental –friendly researchers. Hence, the aim of this study is isolation of bacteria from soil samples with decolorization capability. For this purpose, 30 soil samples were collected from Marvdasht province, Iran. The samples were serially diluted and cultivated on nutrient agar media. The selected colonies were identified using biochemical tests and the percentage of decolorization was calculated. The optimum temperature and dye concentrations were evaluated and the best isolates were molecularly identified. The results obtained from this study indicated that five different strains were able to decolorize the direct blue 129. Among the isolates *Bacillus licheniformis* strain DSM 13 and *Pseudomonas aeruginosa* strain SNP0614 were the best one. The best temperature and dye concentration were 40 and 0.1g/l respectively. Overall, although different dyes using in this geographical area, identification of the native strains and spending them for wastewater treatment could help the dirty environment and reduce the amount of toxic materials.

Keywords: Direct blue 129, decolorizing, Bacillus, Pseudomonas.

Introduction

By increasing the human population in the worldwide, several industries such as: plastic, pulp and paper, pharmaceutical, petroleum, textile, food and dairy products made by human. Among the industries, textile industry could threat our environment due to using different types of colors. Although nature is full of different colors, the manmade activities are disturbing these colors by disposing the synthetic ones to the nature. Indeed, the people for achieving the primary materials in textile industries started using of chemical fibers instead of natural ones, and presence of such material in environment with different structures could threat our locations including: soil, surface and ground water (Prasad MP et al, 2013). Currently these industries are supporting economy of many developed as well as developing countries (Shah MP, 2014). In addtion, the disposal of untreated textile wastewater is a serious hazard. Because, first of all the dyes are the most visible pollutant in the wastewater and the second problem is most of them are potentially toxic to aquatic habitat and even carcinogenic for human life. Furthermore, presence of such color in the aquatic environment may effect on the light penetration and photosynthesis (Franciscon E, 2009). Nowadays, at least 3500 dyes are in practical use and among them Azo dyes contribute 84% of them. Although there are different methods for degrading the dyes, among them some aerobic bacterial strains have ability to use azo dyes as sole source of carbon and nitrogen (Coughlin MF, 2002). Therefore, the present study tried to isolate bacteria from soil samples with capability to degrade direct blue 129 and then the isolates were molecularly identified to achieve the best bacterial dye degrader in this geographical area.

Methods and Materials

Direct blue 129:
The dye is soluble in water with blue color which is mainly used for wool, silk and polyamide fiber dyeing and printing directly (Figure

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1). The selected dye was prepared from Keyhan faum Company, Tehran, Iran, and before starting the project its wave length was checked using uv.visible spectrophotometer.

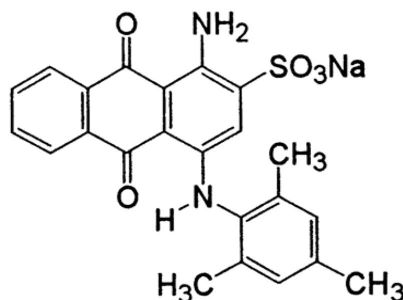

Fig. 1: Chemical structure of direct blue 129

Isolation, purification and maintenance of dye decolorizing bacteria

Totally, 30 soil samples were collected from different region of Marvdasht province, Fars, Iran. The samples were collected from 5cm depth of surface soil. After sieving, the soil samples were serially diluted up to 10^{-6}. Then 0.1 ml of each dilution were cultivated on nutrient agar media and incubated at 37º C for 24 hrs. Then out of 300 colonies 20 were selected based on different morphology and appearance. The well grown bacterial colonies were purified by streaking on nutrient agar slants and stored at 4°C for further analysis (Shah MP ., 2014).

Bacterial screening

Isolates were individually tested for their growth and decolorization ability on Zhou and Zimmermann medium (Yeast extract 5g/l, glucose 5g/l, 0.5 g/l (NH4)2SO4, 2.66 g/l KH2HPO4, 4.23 g/l Na2HPo4) containing 0.1g/l of dye. The flasks were inoculated with 5 ml of bacterial inoculums of each isolates and they were incubated at 37 ºC for 6 days and they were drawn every 24 hours intervals for observation. Then, 10 ml of the dye solution was filtered and centrifuged at 6000 rpm for 15 minutes. Decolorization was assessed by measuring absorbance of the supernatant with the help of spectrophotometer at 597 nm. The tests were evaluated in triplicate and the data were analyzed.

Decolorization assay

Decolorization assay was measured in the terms of percentage decolorization using UV-Spectrophotometer and it was calculated from the following equation.

% Decolorization= (Initial OD-Final OD*100)/Initial OD

Phenotypical and biochemical identification of the selected isolates

The selected isolates were examined for their morphological properties, such as: shape, cell arrangement and staining properties using gram, spore and acid fast staining. Furthermore, physiological and biochemical characteristics of the isolates were evaluated by catalase, oxidase, hydrolysis of starch, nitrate reduction and O/F test and they were identified up to genus based on the tests (Zahmati S et al ,2013).

Optimization

The dye degradation using the selected organisms was optimized under different conditions and parameters. The Dye concentration for optimization was 0.1, 0.5 and 1g/l and the temperature was 20, 30 and 40º C for 6 days. The extent of degradation of the dye samples under the optimization conditions were determined by measuring the absorbance at 597nm using UV-Vis spectrophotometer (Prasad MP et al., 2013 and Prasad and Bhaskara, 2010).

Molecular characterization of selected isolate

Molecular identification of the selected isolates was evaluated using 16SrRNA polymerase chain reaction. For this purpose the isolates were cultivated on Luria Bertani Broth medium and incubated at 25 ˚C for 24h. Then DNA was extracted according to Cinna Gen extraction kit instruction (Cinna Gene, Tehran, Iran). The PCR process was done using two set of primers including: HRK1 (5' ACTCCTACGGGAGGCAGCAG 3'); and HRK2 (5'TGACGGGCGGTGTGTACAAG 3') which amplify 1400bp fragment and the reaction mixture consisted of 10 pmol of each primer, 5 ul from colony suspension as template DNA and 12.5 µl of Master mix. The PCR amplification was performed by Thermal Cycler using the following program: Denaturing at 94°C for 3 minutes, followed by 30 cycles of 60 seconds of denaturing at 94°C, 60 seconds of annealing at 58°C and 1 minutes of elongation at 72°C with a final extension at 72°C for 10 minutes for first set. The 16S rRNA sequences were initially analyzed at NCBI server (http://www.ncbi.nlm.nih.org) using BLAST tool and corresponding sequences were down loaded (Greisen K et al .1994).

Statistical Analysis

The experiments were performed repeatedly and the samples were analyzed in the replicates of three. The results obtained from each set of data have been expressed in terms of mean (average) and standard error by using Microsoft Excel (version Windows 2010).

Results

As shown in Fig 2. Among 20 different isolates, five isolates had capability to degrade the direct blue 129 with different percentage of decolorization. Out of selected bacteria two isolates were most effective. The first one was gram positive, spore former, bacillus shape, non-acid fast with catalase, oxidase, starch and citrate positive results, which confirmed as genus of Bacillus, and the second one was gram negative, catalase positive and oxidase negative with TSI: Alk/Alk, which is identified as Pseudomonas.

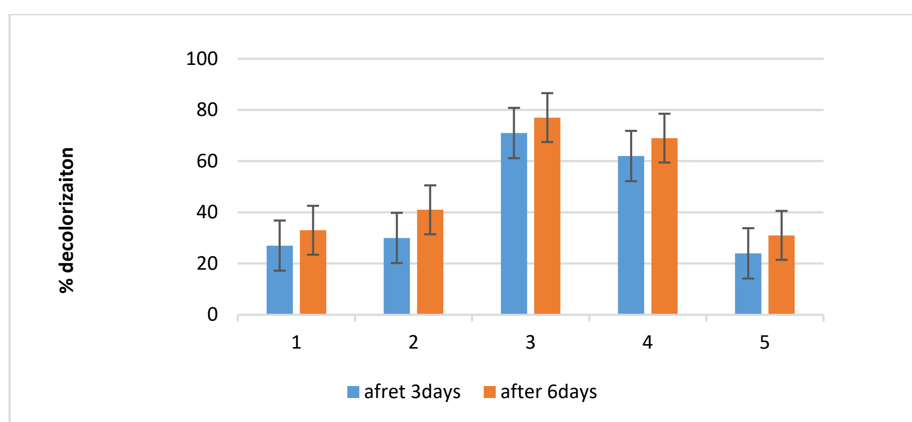

Fig. 2: Effect of different isolates on decolorization of direct blue 129

In addition, the results obtained from this study indicated that the best temperature and dye concentration was 40ºC and 0.1g/l respectively. Furthermore, by increasing the initial dye concentrations, the decolorization extent over the same time interval decreased (Figs 3 and 4).

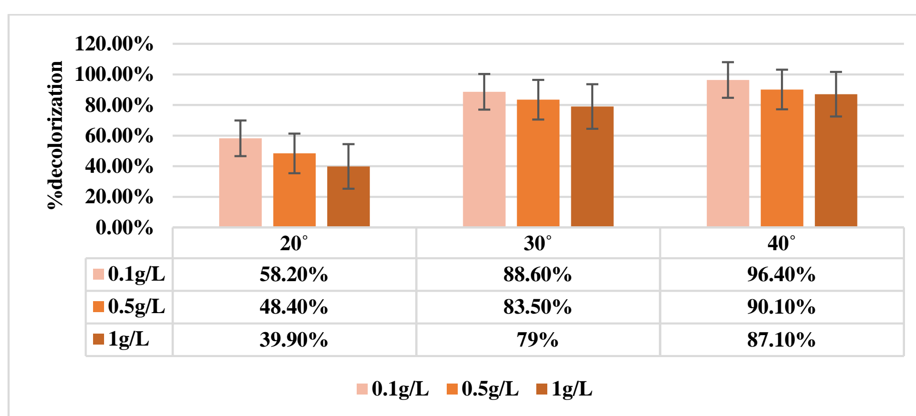

Fig. 3: Direct blue 129 degradation under different conditions Using Pseudomonas

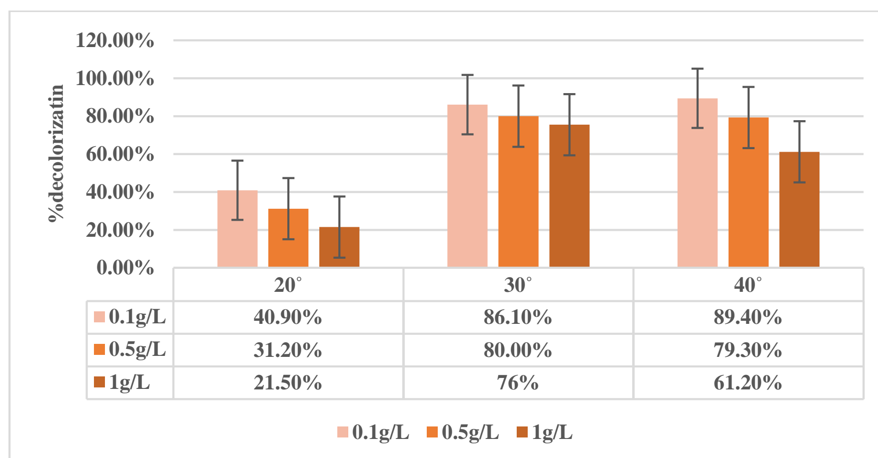

Fig. 4: Direct blue 129 degradation under different conditions Using Bacillus

According to results obtained from molecular identification *Bacillus licheniformis* strain DSM 13 with accession No: NR_118996.1 and *Pseudomonas aeruginosa* strain SNP0614 with 99% identity were the most effective strains which could degrade direct blue 129.

Discussion

In deed several researchers isolated different decolorizing bacteria from the wastewater of textile industries (Lu and et al, 2009), activated sludge (Wang and et al , 2009) and polluted soil with dye (Leena and Selva, 2008; Mabrouk and Yusef , 2003). They were believed that the isolated strains had natural adaptation to the different dye concentrations which is parallel to our study. Furthermore, our results showed that the Direct blue 129 as an Azo dyes with azo and sulfonic groups (Rieger and et al , 2002), could be degrade by different types of microorganisms. Although scientific showed that *Micrococcus* sp. illustrated highest decolorization rate on Reactive Yellow 42 and Reactive Red 52 within 24h under aerobic condition without forming any toxic end products (Olukanni and et al , 2009), our research did not evaluate the last product which could be check in further study. In addition, Singh and his colleague showed that *Staphylococcus hominis* RMLRT03 strain had ability to decolorize textile dye Acid Orange up to 600 mg l^{-1} which, was isolated from textile effluent contaminated soil of Tanda, Ambedkar Nagar, Uttar Pradesh (India). Additionally, they showed that activity under natural environmental conditions (pH, Temperature, carbon and Nitrogen sources and shaking) with high percentage of decolorizing indicates that the bacterial strain has practical applications (Singh RP et al ,2014). Besides, Vasileva and his colleague showed that some bacterial strains are capable to decolorize the azo dyes under anoxic batch reactor, while our organism were able to decolorize the specific Direct blue 129 under aerobic conditions within 6 days with percentage decolorization 89.40% for *Bacillus licheniformis* and 96.40% for *Pseudomonas aeruginosa*.. Therefore, based on our results the isolated strains has practical applications for treatment of polluted wastewater with dyes.

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