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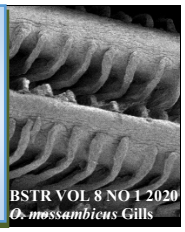

Decolourisation of Reactive Red 120 by a Heavy Metal-tolerant Bacterium Isolated from Juru River, Malaysia

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ABSTRACT

Application of dyes is prevalent in industries involved in textile and food manufacturing. Effluent discharge from these industries to neighbouring water bodies cause significant health concerns due to dye toxicity. To date, only very few bacteria are isolated with the ability to completely assimilate dyes. The main objective of this study is to isolate bacteria(s) with the ability to utilise reactive red dye 120. Local strains were isolated from contaminated sites in Northern Malaysia. Based on 16S rDNA gene sequence analysis, the best strain was identified as *Pseudomonas* sp. strain DRY011. Optimum RR120 decolourisation was observed at 200 ppm with 71.07% removal rate within 5 days and able to tolerate up to 500 ppm. The effect of heavy metals (silver, arsenic, cadmium, chromium, copper, mercury, lead and zinc) were investigated. Mercury, 1 ppm had the highest inhibition effect, followed by zinc and silver, with decolourisation of 12 % and 14.3 % respectively. Chromium had the least effect with 55.6% decolourisation and bacterial growth of 11.5 CFU/mL. The rest of the heavy metal had the least effect on the decolourisation rate. As a conclusion, the finding of microbial degrader able to utilise dye will become crucial bioremediation key in controlling the level of dye wastes in water bodies.

INTRODUCTION

Water is an invaluable resource. We use them as a source of hydroelectricity to powering technological advance aerospace vehicles as fuels. Although it provides no calories or nutrient value, it is vital for all living beings [1]. However, public awareness of its importance is rather despairing. Toxic intermediate in drinking water, the death of aquatic animals, even landslides due to a decrease in aquatic plants on riverbanks occurs as a disastrous consequence of its improper and mismanagement [2]. Pollution itself manifests in many forms. Fertilizers, sewage wastes, oxygen depleting compound, microbiological, suspended matter, chemical and oil spillage are known as a source in water pollution [3–5]. As most of the organic waste enzymatically breakdown by microbial activity, chemical-based pollutants resists to natural degradation [6]. These chemical pollutants can be categorized into two groups, organic and non-organic [7].

Azo dyes consist of both organic and non-organic. Compared to other chemical pollutants, dye pollution stood up the most as this contamination extremely visible to the naked eye due to their colour. While their breakdown amines are colourless, its toxic effect is far worse than the parent compound [8]. With ever-increasing in consumption, more than 10,000 types of dyes are available with 7 x 105 tons of them produced annually. As such, it fuels most of the global economy through leather and textiles industries, automotive manufactures, drugs and

cosmetics [9]. Countries with blooming capital industrialization such as India and China reported having vastly contaminated rivers due to textile industries [10,11]. The effluent containing hazardous waste, saturated in colour and organic chemical from the dyeing process are discharged in millions of gallons to a nearby stream, thus leading to pollution. As this coloured effluent serves no purpose, the waste usually disposed of the nearby drain which leads to rivers or sewage system during raining seasons. In Malaysia, the main source of drinking water comes from rivers and streams, with an annual rainfall rate of 2000-2500 mm [12]. Washed up contaminate from dye industries usually end up in our water system. Besides that, most of the dye consumable comes with heavy metals. It was found that both soil and plants exposed to dye effluents from Batik factories Kota Bharu, Kelantan contains traces of Cadmium (Cd), Lead (Pb), zinc (Zn), Copper (Cu) and Iron (Fe) [12].

To counter it, all possible solution was generated and applied as pollution already at the tipping point. Although advancements in technology provided a momentary solution, it also came with a new problem. Chemical treatment of the effluents employed to treat the waste. This chemical often expensive and requires a large amount of it, therefore most of the small-scale manufacture unable to bear the treatment cost. Therefore, the application of bioremediation cooped with current technology will further enhance these textile effluents while providing a cheap alternative.

MATERIALS AND METHODS

Experimental soil

Soil and water samples were collected at the end of November 2017 from Pulau Pinang located in Malaysia. This location was chosen due to their previous exposure to dye wastes. Since most of the textile and paint manufacturing industries located along Juru river which have been reported as a most polluted river in Malaysia, they are the prime locations for finding bacteria able to utilise and decolourise azo dye. Both water and soil samples were collected in sterilised 50mL falcon tubes and stored in the chiller until further use.

Screening of decolouriser

In order to isolate high-performance azo dye degraders, Reactive red 120 (RR120) was first chosen as the target for screening azo-dye degrading bacteria as those dye commonly used in Pinang state. The mixed bacterial cultures from the soil and water sample were acclimated for 1 month. Initially, 5 g of each soil sample and 5 mL water sample was added into 1 L media azo dye (50 ppm) and 10 g/L nutrient broth. The sample was cultured in 2 L conical flask and aeration was introduced via a BB-800 aquarium air pump fitted with chrome tech MCE 0.45 µm syringe filter followed by incubation at room temperature for seven days. To prevent the growth of fungus, cyclohexamide was introduced into each culture medium during the acclimatization process.

Identification of isolate

Gram staining was done to determine the bacteria species based on physiology differences. The genomic DNA of the selected strains were extracted with innuPREP extraction kit, following the manufacturer's recommended procedure. The PCR reaction conditions included initial 4 min denaturation at 95°C, 30 cycles for 1 min denaturation at 95°C, primer hybridisation at 52°C for 1 min, elongation at 72°C for 1 min with 1 final cycle for 7 min extension step at 72°C.

The identification of 16S rDNA for each strain was done using BLASTn. Those 20 multiple alignments of 16S rDNA sequence with 90% and more in similarity were retrieved from Genbank. Construct of the phylogenetic tree was done using MEGA version 10.0.6.

Effect of Reactive Red dye 120 concentration

The effect of different dye concentration (RR120) was studied. The concentration of 50, 100, 200, 300, 400 and 500 was examined. Dye was supplemented as sole carbon source. Culture media with following composition in 1 L of dH_2O: : yeast (1), KH_2PO_4 (1.33), K_2HPO_4 (2.34), $MgSO_4.7H_2O$ (0.2), NH_4SO_4 (1), NaCl (0.5), while the trace element in mg/L CaCl (11.9), $NiCl_2$ (11.8), $CrCl_2$(6.3), $CuSO_4$(15.7), $FeCl_3$ (0.97), $CaCl_2.2H_20$ (0.78) and $MnCl_2.H_2O$ (10.0).

Effect of heavy metal on decolourisation of Reactive Red 120

A total of eight different types of heavy metals were used as its toxicity differs from one another. They were lead (Pb), silver (Ag), zinc (Zn), chromium (Cr), mercury (Hg), cobalt (Co), arsenic (As) and cadmium (Cd). Each heavy metal was supplemented at 1 ppm in 100 mL sterilised culture medium containing 200 ppm RR120.

Data analysis

RR120 dye removal was quantified using spectrophotometer analysis. All the reading in this study was taken on day 5.

RESULTS AND DISCUSSION

Isolate identification

A total of 12 different strains isolated from textile waste contaminated soils. Amongst the strain, isolate DRY011 were able to decolourise RR120 when supplemented the lower amount of yeast extract. The phylogenetic tree in **Fig. 1.** presents the result of 16s rDNA for isolate DRY011 which was constructed with 19 sequences which resulted in highly similar in identity. Based on the cladogram, isolate DRY011 falls under Pseudomonas spp., however, it doesn't closely attach with any known species in the clade. Therefore, isolate DRY011 identified as *Pseudomonas* sp. strain DRY011.

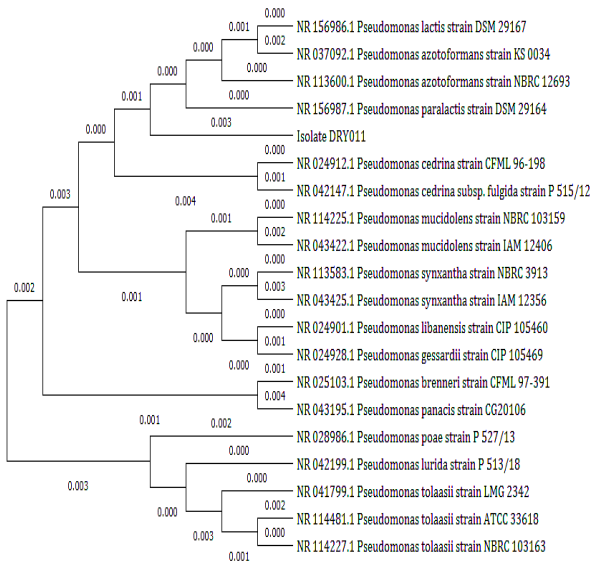

Fig. 1. The phylogenetic tree was generated based on the neighbour-joining method.

Effect of Reactive Red 120 concentration

Fig. 2. illustrates the effects of reactive red 120 concentration on its decolourisation and bacterial growth rate by Pseudomonas sp. strain DRY011. An array of 50 to 500 ppm of RR120 was utilised in this study. 200 ppm of RR120 showed highest decolourisation rate at 71.07% with bacterial growth of 11.41 CFU/mL, followed by 150 ppm at 65.3% removal with 11.1 CFU/mL bacterial growth. Dye decolourisation and bacterial growth showed a dose increase pattern up to 200 ppm. Beyond 200 ppm of RR120, showed and increases in decolourisation and bacterial growth rate, as a higher concentration of RR120 becomes toxic for the bacteria. *Pseudomonas* sp. able to tolerate up to 500 ppm RR120 concentration with 16.67 % removal. No decolourisation and bacterial growth observed at 600 ppm (data not shown). Although based on the graph, 300 ppm above shows a decrease in decolourisation rate, however, there is no significant differences ($p > 0.05$) when compared with 400 and 500 ppm.

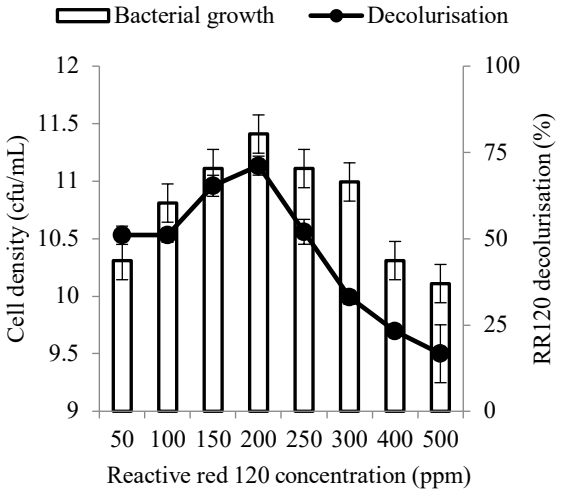

Fig. 2. Effects of different RR120 concentration on decolourisation rate by resting cells of *Pseudomonas* sp. strain DRY011. Data represent mean ± SD.

Effect of heavy metals

The effect of heavy metals was studied on *Pseudomonas* sp. strain DRY011 to investigate its effect in removing reactive red 120 dye (**Fig. 3.).** Based on **Fig. 3.** chromium had the least effect with 55.6% decolourisation and bacterial growth of 11.5 CFU/mL, followed by lead with 49.6 % decolourisation and bacterial growth of 10.94 CFU/mL. Both chromium and lead showed no significant differences ($p>0.05$) in term of decolourisation effect. Meanwhile, mercury significantly inhibits the decolourisation rate by 0.7% with bacterial growth of 10.1 CFU/m, followed by zinc and silver, with decolourisation of 12 % and 14.3 % respectively. In control, with no heavy metal, a depletion of 78.6 % RR120 with a bacterial growth of 11.42 CFU/mL was recorded. Metals like lead are believed to have the lowest toxicity meanwhile mercury have a high inhibitory effect [13]. Certain metal elements such as zinc are crucial for some cellular functions in bacteria, however at higher concentration exhibits cytotoxicity.

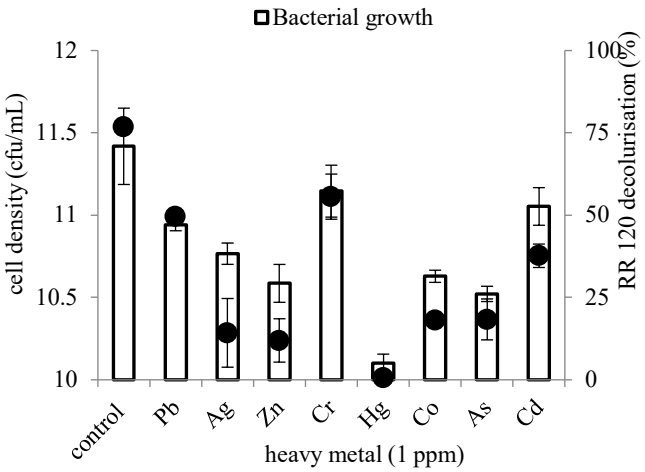

Fig. 3. Effects of different heavy metal on decolourisation of 200 ppm reactive red 120 by resting cells of *Pseudomonas* sp. strain DRY011. Data represent mean ± SD.

CONCLUSION

This study is the first report on the isolation of soil bacterial strain *Pseudomonas* sp. from textile contaminated soil in Malaysia that possesses the capacity to utilise reactive red dye as sole carbon source. In addition, this strain able to tolerate both heavy metal and high concentration of dye providing a good alternate solution for dye waste treatment.

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