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Research Article

DECOLOURIZATION OF TEXTILE DYES BY MALAYSIAN *RHODOCOCCUS* STRAINS

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ABSTRACT
Coomassie Brilliant Blue, Crystal Violet and Safranin dyes which have been utilized extensively as biological stains and commercial textile dyes are recalcitrant chemical substances. Hence, the generation of voluminous effluents resulting from the textile industry has to be treated prior to discharge into the environment. Taking this into consideration, in the present study, twenty three locally isolated *Rhodococcus* strains were subjected to decolourization of Coomassie Brilliant Blue, Crystal Violet and Safranin dyes. However, all twenty three strains were unable to decolourize Safranin dye which may be due to the high toxicity of the compound. In contrast, the Malaysian isolates were able to successfully decolorize Coomassie Brilliant Blue and Crystal Violet dyes. Among the tested microorganisms, twelve locally isolated *Rhodococcus* strains demonstrated promising ability in removing the Coomassie Brilliant Blue dye which resulted in the formation of clear or colourless zone around the colonies of bacteria. One particular *Rhodococcus* strain, designated as *Rhodococcus* strain UCC 0009, exhibited excellent growth after 7 days of incubation and formed a clear zone with the biggest diameter of 7.6 ± 0.1cm. In addition, these strains were also evaluated for their potential in decolorizing Crystal Violet dye. Strain UCC 0009 showed evidence of good growth after 10 days of incubation with a clear zone measuring the largest diameter of 6.3 ± 0.3cm. Strain UCC 0009 was further subjected to degradation of 0.05g/L Coomassie Brilliant Bluedye under static and shaking condition which resulted in 87± 1% and 49± 2% degradation, respectively. These findings clearly indicated that the *Rhodococcus* strain UCC 0009 possessed tremendous capability in the decolourization of Coomassie Brilliant Blueand Crystal Violet dyes which can be utilized to develop a greener and cost−effective alternative in treating textile industrial wastes containing the tested dyes.

Keywords: *Bacterial Degradation, Coomassie Brilliant Blue, Crystal Violet, Decolourization, Rhodococcus, Safranin*

INTRODUCTION
There is a wide utilization of Crystal Violet in human and veterinary medicine as a biological stain and in various commercial textile processes as a dye (Chen *et al.,* 2007) whereas Safranin dye is commonly applied as a biological staining reagent and as a redox indicator in analytical chemistry. In addition, the triphenyl methane dye, Coomassie Brilliant Blue, was developed for application in the textile industry and the use of this dye is expanded in the area of analytical biochemistry whereby Coomassie Brilliant Blue dye is used as the reagent for staining proteins. These dyes are persistent in the environment suggesting the recalcitrant properties of the compounds thereby making them poorly metabolized by microorganisms (Chen *et al.,* 2007).
Considering the large application of Coomassie Brilliant Blue, Crystal Violet and Safranin dyes in the textile industry and as staining reagents, the decolourization of wastewaters containing these dyes prior to discharge is mandatory by environmental regulations in most countries (Chen *et al.,* 2008). There are many reports available on the use of physical or chemical treatment processes for colour removal from dye−containing effluents.
However, these methods have high operating costs and limited applicability and also produce large quantities of sludge (Parshetti, 2009). The development of biological methods through Green Chemistry approach is considered as an attractive alternative due to their low cost, eco-friendly nature and public

acceptability. *Rhodococcus* species in particular can be considered as a competent bioremediation organism with substantial commercial value owing to their many attractive properties (Kandelbauer, 2012).

Rhodococcus spp. possess many commercially interesting characteristics that actually or potentially make them useful in environmental and industrial bitechnology. *Rhodococcus* spp. can persist under harsh environmental conditions even under starvation conditions and they are able to compete successfully in complex bacterial populations.

Thus, rhodococci can be considered as promising candidates for inocula for bioremediation (Bell *et al.*, 1998).

Their natural occurrence in contaminated environments, broad catabolic versatility, physiological and ecological adaptations to extreme environmental conditions imply that they may play a significant role in bioremediation of textile dyes as reported previously (Ramesh, 2012).

The Culture Collection Unit, Institute of Bio−IT Selangor has an extensive collection of *Rhodococcus* strains which can be capitalized as biological tool to address the current increasing trend of textile dye industrial effluent pollution.

Therefore, in the present work, twenty three locally isolated *Rhodococcus* strains were screened for their potential in Coomassie Brilliant Blue, Crystal Violet and Safranin dyes decolourization. In addition, the effect of static and shaking condition on the removal of Coomassie Brilliant Blue was investigated to understand the role of oxygen in Coomassie Brilliant Blue dye decolourization.

MATERIALS AND METHODS

Chemicals

In the current study, all chemicals and media ingredients used were of analytical grade and procured either from Sigma (USA), Fisher Scientific (Singapore) or Merck (Germany).

Microorganism

Twenty threelocally isolated *Rhodococcus* strains were kindly provided by the Culture Collection Unit, Institute of Bio-IT Selangor, University Selangor**.**

Enrichment and Growth Condition of Microorganism

The respective *Rhodococcus* strains were cultured from beads source which was stored in deep freezer at −80°C onto nutrient agar plate and incubated at 30°C for further use. After 14 days of incubation, a loop full of enriched bacterial strains were streaked on nutrient agar plates and incubated at 30°C. Such serial transfer was performed to maintain strain stock.

Screening of Coomassie Brilliant Blue, Crystal Violet and Safranin Dye−Decolorizing Bacteria under Aerobic Conditions

Twenty three locally isolated *Rhodococcus* strains were screened for their potential in dye decolourization in this following investigation. An amount of 20.0g of nutrient agar, 4.0g of agar−agar and selected dye concentration (0.05g of Coomassie Brilliant Blue dye, 200µL of Crystal Violet dye and 0.05g of Safranin dye) were mixed in 1.0L of deionized water. The solution was then autoclaved at 121°C for 15min. The melted agar media was let to cool and subsequently poured approximately 25 to 35mL per sterile Petri dish.

The available *Rhodococcus* strains were then streaked on the plates in triplicate and incubated at 30°C for 14 days. The growth of the bacterial strains, colour changes and possible formation of colourlesszone around the colonies of the *Rhodcoccus* strains which signify the decolourization activities were monitored daily for 14 days. The fastest growing bacterial strain of *Rhododoccus* with the largest formation of colourless zone will be selected for further characterization and secondary screening.

Effect of Shaking and Static Condition

Decolourization of Coomassie Brilliant Blue dye by strain UCC 0009 was studied under continuous shaking and static conditions.

The seed culture was prepared by inoculating a loop of the bacterium *Rhodococcus* strain UCC 0009 into 50 mL medium consisting of 8.0 g nutrient broth in 1 L deionized water. The flask was then kept on an

incubator shaker at 30°C and agitated at 160rpm for 24hrs. This culture with an optical density ranging between 0.800 to 0.900 was used as a standard inoculum throughout this study.

For decolourization study under continuous shaking condition, 50mL medium consisting 0.8%w/v nutrient broth amended with 0.05g/L Coomassie Brilliant Blue was taken in 100mL Erlenmeyer flasks and inoculated with 5%v/v inoculum.

These mixtures were then incubated at 30°C for 12 days under shaking condition at 150 rpm in triplicate. For static condition, the above mentioned inoculated medium was taken in 50mL Erlenmeyer flask and incubated at the same temperature and incubation period at 0rpm.

Control experiments were established in identical conditions without the presence of bacterial cells strain UCC 0009.

Decolourization Assay

The amounts of residual Coomassie Brilliant Blue dye in the culture medium at continuous shaking condition and static condition were monitored daily. An amount of 2mL of sample was centrifuged at11000×*g* for 30 min at 4°C, respectively and supernatants were collected to determine the percentage decolourization. The decolorizing activity is expressed in terms of percent decolourization activity by calculating the decrease in absorbance for Coomassie Brilliant Blue dye at wave length 560nm. Decolourization activity (%) was calculated as [(A – B)/ A] × 100%.

A = initial absorbance

B = observed absorbance

Statistical Analysis

Each experiment was performed in triplicate. The standard error (SE) was calculated using Microsoft Excel version 2010 and the results were presented as mean ± SE values.

RESULTS AND DISCUSSION

Identification of the Most Competent Rhodococcus Strain Capable of Decolourizing Coomassie Brilliant Blue, Crystal Violet and Safranin Dyes under Aerobic Conditions

Interestingly, all tested *Rhodococcus* strains were able to grow on Coomassie Brilliant Bluedye as shown in Table 1. Eleven strains namely UCC 0001, UCC 0002, UCC 0007, UCC 0008, UCC 0012, UCC 0014, UCC 0015, UCC 0016, UCC 0018, UCC 0022 and UCC 0023 exhibited poor growth and no colour changes were noted throughout the incubation period of 14 days.

However, these strains were able to form colourless zones around the colonies of the bacterial strains though the diameter of the clear zones were not prominent recording a mere 2.0 ± 0.3cm on average after 14 days of cultivation time.

This observation clearly indicated that these *Rhodococcus* strains were unsuccessful to carry out the decolourization of the Coomassie Brilliant Blue dye due to the toxicity of the dye even though the strains were able to proliferate on the colorant. In addition, these strains recorded a lengthened period of 14 days to grow which can be attributed to the high toxic level of the dye which proved to impede the growth of the *Rhodococcus* strains.

Moderate growth was observed for strains UCC 0003, UCC 0004, UCC 0005, UCC 0006, UCC 0010, UCC 0011, UCC 0013, UCC 0017, UCC 0019, UCC 0020 and UCC 0021. After 14 days of incubation period, these strains were able to decolourize the Coomassie Brilliant Blue dye completely and formations of clear zones around the colonies of the bacterial strains were also noticeable.

Strains UCC 0004 and UCC 0005 in particular were able to form clear zones measuring to 6.0± 0.4cm and 6.0 ± 0.1cm, respectively after 9 days of cultivation time which confirmed the ability of these *Rhodococcus* strains to decolourize the highly toxic Coomassie Brilliant Bluedye. Among all the tested *Rhodococcus* strains, strain UCC 0009 emerged as the most potential bacterium in decolourizing Coomassie Brilliant Bluedye despite its noxious properties.

Rhodococcus strain UCC 0009 exhibited excellent growth after 7days of incubation and formed a clear zone with the biggest diameter of 7.6 ± 0.1cm (Table 1) which evidently proved the capability of the bacterium to be utilized as a biological tool for decolourization of Coomassie Brilliant Blue dye.

Table 1: Decolourization of Coomassie Brilliant Blue Dye by *Rhodococcus* Strains

Strain	Color	Growth	Day	Diameter (cm)
UCC0001	No change	*	14	2.0 ± 0.1
UCC0002	No change	*	14	2.0 ± 0.0
UCC0003	Blue to colorless	**	10	4.0 ± 0.1
UCC0004	Blue to colorless	**	9	6.0 ± 0.4
UCC0005	Blue to colorless	**	9	6.0 ±0.1
UCC0006	Blue to colorless	**	10	5.0 ±0.2
UCC0007	No change	*	14	2.0 ± 0.3
UCC0008	No change	*	14	2.0 ± 0.1
UCC0009	Blue to colorless	***	7	7.6 ± 0.1
UCC0010	Blue to colorless	**	10	4.0 ± 0.1
UCC0011	Blue to colorless	**	10	4.0 ± 0.2
UCC0012	No change	*	14	2.0 ± 0.2
UCC0013	Blue to colorless	**	12	3.0 ± 0.1
UCC0014	No change	*	14	2.0 ± 0.0
UCC0015	No change	*	14	2.0 ± 0.1
UCC0016	No change	*	14	2.0 ± 0.2
UCC0017	Blue to colorless	**	10	4.0 ± 0.1
UCC0018	No change	*	14	2.0 ± 0.1
UCC0019	Blue to colorless	**	10	4.0 ± 0.5
UCC0020	Blue to colorless	**	10	3.0 ± 0.3
UCC0021	Blue to colorless	**	9	5.6 ± 0.1
UCC0022	No change	*	14	2.0 ± 0.1
UCC0023	No change	*	14	2.0 ± 0.1

*Diameter of colourless zone represents means of triplicate samples ± standard errors.
Growth indicator: *poor; **moderate/fair and ***good

Five isolates namely *Rhodococcus* strain UCC 0001, UCC 0002, UCC 0007, UCC 0008, UCC 0019 and UCC 20 were unable to grow on nutrient agar supplemented with 200µL Crystal Violet in 1L media solution even after 14 days of incubation as shown in Table 2 suggesting that these strains were unable to withstand the high toxicity of the dye. Poor growth was detected for strains UCC 0003, UCC 0004, UCC 0005, UCC 0006, UCC 0010, UCC 0013, UCC 0014, UCC 0015, UCC 0016, 0017, UCC 0018, UCC 0021, UCC 0022 and UCC 0023 with an average clear zone diameter measuring between 1.0 ± 0.1cm to 2.5 ± 0.1cm after an incubation of 14 days. These strains were also incapable in decolourizing Crystal Violet dye completely. Three strains designated as UCC 0009, UCC 0011 and UCC 0012 demonstrated good growth which was accompanied by the formation of large colourless zones around the colonies of the bacterial strains after 10 days of incubation. Among these three isolates, strain UCC 0009 recorded the biggest diameter of 6.3 ± 0.3cm of clear zone and the violet colour of the plate containing this strain turned colourless after 10 days of incubation (Table 2). This observation was also spotted with strain UCC 0009 incubated with 0.5g/L Coomassie Brilliant Bluedye whereby the blue colorant disappeared almost completely after 7 days of incubation period.

When all twenty three strains of locally isolated *Rhodococcus* were subjected to decolourization of Safranin dye, it was found that all strains were unable to decolourize the dye which may be due to the high toxicity of the compound. Since *Rhodococcus* strain UCC 0009 emerged as the most competent biological tool in degrading Coomassie Brilliant Bluedye within a short period of 7 days compared to

that of 10 days for Safranin as evidenced in Table 1 and Table 2, further characterization and secondary screening were carried out to investigate the decolourization Coomassie Brilliant Blue dye by strain UCC 0009.

Table 2: Decolourization of Crystal Violet Dye by *Rhodococcus* Strains

Strain	Color	Growth	Day	Diameter (cm)
UCC0001	No change	No growth	Not applicable	0.0 ± 0.0
UCC0002	No change	No growth	Not applicable	0.0 ± 0.0
UCC0003	No change	*	14	2.1 ± 0.1
UCC0004	No change	*	14	1.9 ± 0.1
UCC0005	No change	*	14	2.1 ± 0.3
UCC0006	No change	*	14	2.0 ± 0.1
UCC0007	No change	No growth	Not applicable	0.0 ± 0.0
UCC0008	No change	No growth	Not applicable	0.0 ± 0.0
UCC0009	Violet to colorless	***	10	6.3 ± 0.3
UCC0010	No change	*	14	1.2 ± 0.2
UCC0011	Violet to colorless	***	10	5.5 ± 0.2
UCC0012	Violet to colorless	***	10	5.2 ± 0.2
UCC0013	No change	*	14	2.1 ± 0.1
UCC0014	No change	*	14	2.5 ± 0.1
UCC0015	No change	*	14	2.3 ± 0.5
UCC0016	No change	*	14	1.9 ± 0.0
UCC0017	No change	*	14	2.4 ± 0.1
UCC0018	No change	*	14	1.8 ± 0.1
UCC0019	No change	No growth	Not applicable	0.0 ± 0.0
UCC0020	No change	No growth	Not applicable	1.0 ± 0.1
UCC0021	No change	*	14	1.6 ± 0.2
UCC0022	No change	*	14	1.3 ± 0.1
UCC0023	No change	*	14	1.1 ± 0.3

*Diameter of colourless zone represents means of triplicate samples ± standard errors.
Growth indicator: *poor; **moderate/fair and ***good

Effect of Shaking and Static Conditions on the Decolourization of Coomassie Brilliant Blue Dye by Rhodococcus Strain UCC 0009

Oxygen has a significant effect on the physiological characteristics of the cells during the growth stage of the bacterium. The presence of oxygen can either favour or inhibit the microbial degradation of azo dyes (Pearce *et al.*, 2003). Figure 1 shows the percentage of degradation of Coomassie Brilliant Blue dye by *Rhododocccus* strain UCC 0009 when tested under static and shaking conditions.

The locally isolated *Rhodococcus* strain UCC009 is capable of degrading the Coomassie Brilliant Blue dye efficiently. This is confirmed in the apparent change of color in the tested nutrient broth medium amended with 0.05g/L Coomassie Brilliant Blue dye. An increase in the optical density of the organism after the degradation of the dye was also observed. *Rhodococcus* strain UCC0009 strain showed 87 ± 1% decolourization of Coomassie Brilliant Bluedye under static condition compared to that of 49 ± 2% decolourization when shaking condition was applied as depicted in Figure 1. Both Coomassie Brilliant

Bluedye removal under static and shaking conditions were observed at a wavelength of 580nm. Similar results using *Acinetobacter calcoaceticus* was recorded which showed maximum decolourization of azo dye Amaranth under static condition (Ghodake *et al.,* 2011). Several researchers reported efficient dye decolourization under static culture conditions as compared to that of shaking (aerobic) condition. The mechanism of bacterial degradation of dyes to their corresponding amines is initiated by a reduction of azo linkage with the aid of low specificity cytoplasmic azoreductase. Azoreductase mediated degradation of azo dyes is inhibited by the presence of oxygen because oxygen was a preferable terminal electron acceptor over the azo groups in the oxidation of reduced electron carriers such as NADH. Under shaking conditions, the presence of oxygen deprives the azoreductase from receiving electrons required for azo bond cleavage, whereas under static conditions, these electrons are readily available to the enzyme from NADH to decolorize the azo dyes (Chang, 2000).

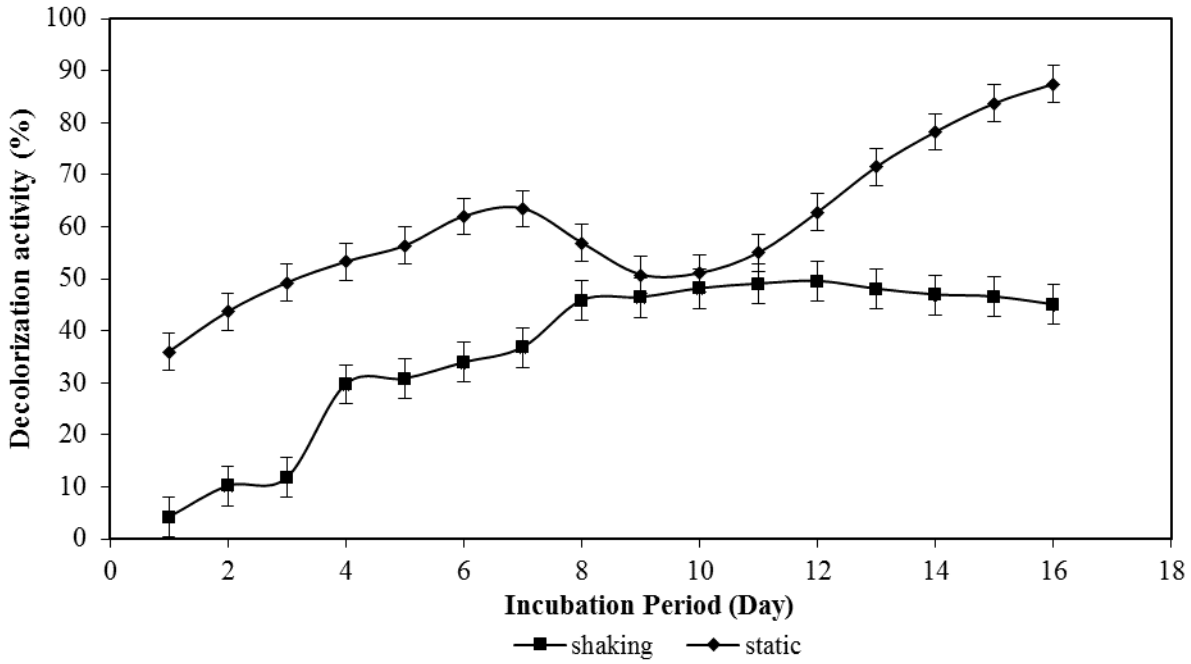

Figure 1: Percentage of Coomassie Brilliant Blue Dye Decolourization by *Rhodococcus* Strain UCC 0009 under Static and Shaking Condition. Error Bars Represent Standard Error between 3 Determinations

Conclusion

Collectively, the results obtained from this study indicated that the actinomycete *Rhodococcus* strain UCC 0009 has a promising potential in removing textile dyes. The data obtained from the present study can be utilized to design experimental approach to maximize dye removal efficiency. A practical system could be established by capitalizing on *Rhodococcus* strain UCC 0009 for the treatment of wastewaters containing the tested dyes.

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