Environ Chem Lett (2017) 15:531–536
DOI 10.1007/s10311-017-0627-1

ORIGINAL PAPER

Bacillus algicola decolourises more than 95% of some textile azo dyes

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Received: 4 January 2017 / Accepted: 27 March 2017 / Published online: 3 April 2017

Abstract Textile effluents in natural waters pose environmental health problems if not treated to safe limits. Various bacterial species have the potential to degrade dyes. Here we studied the ability of *Bacillus algicola* to decolourise red, blue and yellow azo dyes. *B. algicola* was isolated from soil samples taken from a sanitary landfill site. Isolation and screening were performed using mineral salt medium. Dye-decolourising isolates were assessed in their capacity to decolourise dyes. Experiments were conducted at pH 6, 7 and 8, and 25, 35 and 45 °C. Phytotoxicity of the dyes and biodegradation products was assessed by seed germination tests. Results show that *B. algicola* gave the highest decolourisation at pH 8.0 and 25 °C in the presence of yeast extract as media supplement. *B. algicola* degraded the red and blue azo dyes by over 95%. The phytotoxicity results indicated that biodegradation products of the red and blue azo dyes were not toxic. Biodegradation products of the yellow dye were, however, toxic and considerably hindered germination. From these results, we infer that *B. algicola* has good potential for degrading and decolourising the red and blue test azo dyes.

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Keywords Azo dyes · *Bacillus algicola* · Biodegradation · Phytotoxicity

Introduction

Textile effluents pose environmental health problems (Sultan 2016) and hence need treatment them before discharge. Wastewater treatment involves both chemical and physical methods such as flocculation, ozonation, filtration and chemical oxidation Sultan 2016). However, these remediation methods produce secondary pollution and are expensive (Varadarajan and Venkatachalam 2016). Therefore, research has been oriented towards the more cost-effective and efficient bioremediation approach whereby certain species of algae, fungi and bacteria are used to degrade dye effluents (Yadav and Yadav 2015). Bacteria of the genus *Bacillus, Pseudomonas, Providencia, Staphylococcus, Micrococcus* and *Enterococcus* have been frequently used for bioremediation of textile wastewaters because of lower cost, simpler cultivation, faster growth rate and higher decolourisation efficiency (Ong et al. 2005; Solis et al. 2012). Different strains of *Bacillus* have been tested, and their respective degradation performance has been encouraging. Padmanaban et al. (2016) have tested *Bacillus cohnii* RAPT1 and reported it could significantly decolourise Reactive Red 120 dye under the optimum conditions. Das et al. (2016) assessed *Bacillus pumilus* HKG212 and demonstrated considerable decolourisation of Remazol Navy Blue dye solutions under anaerobic conditions. However, data on the ability of the *Bacillus algicola* strain to degrade and decolourise azo dyes are very scarce.

Accordingly, this study was conducted to isolate the *B. algicola* strain from a local landfill site and thereafter assess its potential to degrade three test azo dyes under

different pH and temperatures. Phytotoxicity tests using cucumber (*Cucumis sativus*) were also conducted to elucidate the toxicity of the test dyes and their respective biodegradation products.

Experimental

Dyes used and landfill soil samples

Three reactive azo dyes of (blue, yellow and red) were obtained from a local textile industry and used in this study. The wavelengths of maximum absorbance (λ_{max}) were 597, 572 and 453 nm for the blue, red and yellow azo dye, respectively. Soil samples were collected from a local sanitary landfill site found in the south-east region of the country. About 25 g of soil samples with decaying clothing and other materials containing dyes were taken from a depth of 7–10 cm, using cleaned and 70% alcohol-disinfected shovels. They were preserved in sterile polythene bags before analysis. Additionally, 5 g of soil were aseptically diluted tenfold in sterile water and shaken at 40 revolutions per minute for 24 h to determine the pH of the culture medium.

Isolation and screening of dye-decolourising bacteria isolates

Isolation and screening were done using mineral salt medium, with and also without yeast extract with 100 mg/L of dye was added. The composition of the mineral salt medium was as follows (g/L): NaCl, 2.0; $MgSO_4 \cdot 7H_2O$, 0.4; $CaCl_2 \cdot 2H_2O$, 0.2; KH_2PO_4, 0.5; K_2HPO_4, 0.5; $(NH_4)_2SO_4$, 0.5; yeast extract, 2.0; agar (for solid medium only), 15. Deionised water was made up to 1.0 L and the pH of the medium was adjusted to 7.4 ± 0.1. Mineral salt medium agar plates were inoculated with 0.1 mL of dilutions 10^{-3} up to 10^{-6} of the mother sample and incubated at 30 °C for four days. Morphologically different and prominent dye-degrading isolates were streaked again on nutrient agar plates and incubated at 35 °C to check for purity. Dye-degrading isolates were then stored at 4 °C for further screening. Each dye-decolourising bacterial isolate was sub-cultured in 5 mL of nutrient broth for 24 h at 35 °C. 0.1 mL of this culture was then used to inoculate each of the blue, red and yellow dye.

Biodecolourisation ability of isolates

Selected dye-decolourising isolates were further tested for their ability to decolourise the test dyes. 10-mL nutrient broth was inoculated with a loopful of each isolate and incubated at 35 °C for 24 h. The absorbance of the 24 h broth cultures was measured at 600 nm to check for uniformity of cell density. 10 mL of the broth culture was then transferred to 100 mL of mineral salt medium containing 100 ppm of azo dye and incubated at 35 °C for 48 and 72 h. After incubation, aliquots of 7 mL of mineral salt medium broth culture were centrifuged (Hettich Zentrifugen Universal 320 R at 9000 rev/min for 15 min) to generate cell free supernatant extracts of dye biodegradation products. All assays were done in triplicates, and uninoculated broths were used as abiotic controls. The decolourising ability was calculated as follows:

$$\text{Decolourisation } (\%) = \frac{\text{Absorbance before degradation} - \text{Final absorbance after degradation}}{\text{Absorbance before degradation}} \times 100$$

Optimum pH and temperature

One set of decolourisation assays was carried out at three different pH (6, 7 and 8), and another set was run at three different temperatures (25, 35 and 45 °C) keeping the pH of the mineral salt medium at 7.4 in this set. All culture tubes were incubated for 24 h. The decolourisation assay was then run under the predetermined optimal conditions of pH and temperature over 4 days, using the most effective dye-decolourising isolate.

Identification of dye-decolourising isolates

Identification of the most efficient dye-decolourising isolate was done using Gram stain, biochemical tests and 16S rRNA gene sequencing after DNA extraction of the pure culture as per Cheng and Jiang (2006) and PCR amplification. The 16S rRNA gene of the isolate was purified and sequenced using Sanger dideoxy sequencing by Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa). The gene was compared with known nucleotide sequences using Basic Local Alignment Search Tool (BLAST) accessed at http://www.ncbi.nlm.nih.gov/BLAST.

Phytotoxicity assessment

The toxicity of the test dyes and their respective biodegradation products was assessed through cucumber (*Cucumis sativus*) seed germination tests 28 °C and under sunlight. Cucumber seeds were sown in trays and watered with 3 mL of each test solution daily for a week. For the parent test dyes, a 100 ppm aqueous solution was used while for their respective biodegraded products, 3 mL of the 96 h mineral salt medium culture broth was used. Ten seeds were grown per treatment. Control sets were carried out using sterile water. The percentage germination, shoot length and root length were recorded after 7 days. Single-factor ANOVA tests were conducted in Minitab® 17 to determine the effect of treatments.

Results and discussion

Biodecolourisation capacity and isolation of dye-decolourising isolates

Abundant bacterial and fungal growth was noted only on mineral salt medium supplemented with yeast extract. A total of 90 morphologically distinct bacterial CFUs were selected for further screening. These dye-decolourising isolates were purified on nutrient agar and again spread plated on blue, red and yellow mineral salt medium agar plates to confirm their dye-degrading ability. Only 16, 15 and 19 isolates could degrade the red, yellow and blue test dye, respectively. Only nine isolates could degrade all three test dyes and they retained for further analysis.

Binding of the azo dyes to the cell wall of the dye-decolourising isolates was indeed visible after centrifugation as the bacterial pellets were red, blue or yellow. However, enzymatic degradation appeared to the predominant mechanism for the test dye decolourisation (Pandey et al. 2007). Besides having to acclimatise to the dye, bacteria also have mobile genetic elements. Elbanna et al. (2010) suggested the genes required for azo dye degradation by *Lactobacillus spp*. were located on a 3 kb plasmid and the loss of the plasmid led to incapacity of the bacteria to decolourise the azo dye. Since textile effluents contain complex dye molecules with many functional groups, the azo dye-degrading bacteria should have high decolourising ability for a wide range of dyes.

Table 1 summarises the extent of decolourisation of the three azo dyes in mineral salt medium broth by each of the nine selected dye-decolourising isolates after 48 h of incubation at 35 °C. The control refers to the respective uninoculated culture broth. Increasing the incubation time increased the extent of decolourisation in all cases. The isolates were capable of degrading all three dyes with varying efficiency. None of the isolates could degrade the yellow dye efficiently even after 72 h. Of the nine preselected dye-decolourising isolates, isolate 82 showed the highest degrading efficiency of the red, blue and yellow test dyes. This isolate was retained for identification and subsequent parameter optimisation and toxicity assays.

The phenotypic features of isolate 82 indicated it was rod-shaped, Gram-positive and not a strict anaerobe which

Table 1 Extent of decolourisation of the three test azo dyes in mineral salt medium by dye-decolourising isolates after 48 h at 35 °C

Dye-decolourising isolate	Percentage decolourisation (number of samples analysed = 3, mean ± SD)		
	Blue azo dye (λ_{max} = 597 nm)	Red azo dye (λ_{max} = 572 nm)	Yellow azo dye (λ_{max} = 453 nm)
Control	0	0	0
18	91.06 ± 2.11	73.28 ± 39.58	20.44 ± 3.50
20	26.75 ± 9.33	94.70 ± 0.78	8.34 ± 2.78
26	35.61 ± 1.99	25.67 ± 3.58	10.47 ± 3.48
28	32.09 ± 1.50	15.59 ± 0.30	6.78 ± 0.56
30	89.28 ± 1.07	20.40 ± 4.41	14.90 ± 8.25
31	31.67 ± 2.28	14.35 ± 6.50	2.47 ± 2.60
44	38.00 ± 4.80	14.51 ± 3.27	6.14 ± 1.80
52	36.66 ± 7.04	17.80 ± 4.16	6.14 ± 1.65
82	90.85 ± 1.98	94.97 ± 0.81	20.17 ± 5.41

The extents of decolourisation of the red, blue and yellow azo dyes were the most when isolate 18 and isolate 82 were tested. These results indicate that the extents of microbial degradation of the three dyes by isolate 18 and isolate 82 were the most pronounced. Irrespective of the isolate used, the yellow dye was the least decolourised

Table 2 Effects of initial pH of mineral salt medium and temperature of incubation on the percentage decolourisation of blue, red and yellow azo dyes by bacterial isolate 82 (*Bacillus algicola*)

pH	Percentage decolourisation (%) at 35 °C after 24 h		
	Blue/597 nm	Red/572 nm	Yellow/453 nm
Data set 1			
6	2.57 ± 0.26	10.45 ± 0.55	7.38 ± 0.50
7	9.76 ± 0.06	20.06 ± 2.36	8.85 ± 1.40
8	27.52 ± 4.50	21.01 ± 1.22	10.27 ± 1.72
Temperature (°C)	Percentage decolourisation (%) at pH 7.4 after 24 h		
	Blue/597 nm	Red/572 nm	Yellow/453 nm
Data set 2			
25	35.13 ± 3.18	18.05 ± 0.80	14.40 ± 0.55
35	16.43 ± 0.81	15.50 ± 1.17	7.54 ± 2.07
45	11.67 ± 2.76	14.39 ± 0.21	3.95 ± 1.11
Time (hours)	Percentage decolourisation (%) at optimal temperature of 25 °C and optimal initial pH of 8.0		
	Blue/597 nm	Red/572 nm	Yellow/453 nm
Data set 3			
24	32.07 ± 2.63	20.04 ± 0.48	15.61 ± 1.93
48	80.30 ± 3.07	85.02 ± 2.50	21.40 ± 2.37
72	88.83 ± 1.25	91.09 ± 1.35	24.32 ± 3.01
96	96.19 ± 1.79	96.34 ± 1.78	26.63 ± 2.05

The extent of decolourisation was significantly larger in a weakly alkaline medium (pH 8), at a low mesophilic temperature of 25 °C and for longer incubation times. The data given here are average and standard deviation for samples analysed in triplicates

had an ability to hydrolyse starch and could also bring about catalase and urease activity. Isolate 82 also demonstrated citrate, glucose, sucrose and maltose utilisation ability. These phenotypic features were presumptive of the genus *Bacillus*. The PCR-amplified 16S rRNA gene sequence of isolate 82 was slightly more than 500 bp in size when run on 1% agarose gel. Sanger dideoxy sequencing confirmed that the gene sequence was precisely 538 bp in size. BLAST analysis confirmed that isolate 82 was of the genus *Bacillus* with 99% similarity with *Bacillus algicola* strains (GenBank Ac. No.: KF933697, JX501684, JX005838). Other workers namely Dawkar et al. (2008), Anjaneya et al. (2011), Deng et al. (2008), Prasad and Rao (2013) Mahbub et al. (2015) have reported the merit of the *Bacillus* genus in decolourising azo dyes but that of *B. algicola* is scantily reported in the literature.

Optimum pH, temperature and incubation time

Table 2 gives the results for the effect of initial culture medium pH on the mean percentage decolourisation of the

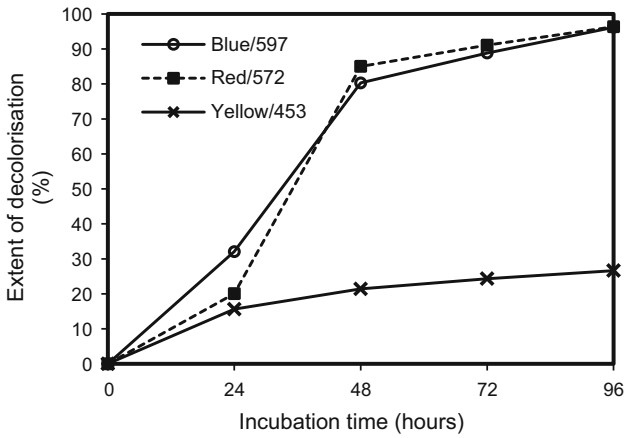

Fig. 1 Extent of decolourisation with incubation time of the *red*, *blue* and *yellow* test azo dyes in mineral salt medium by *Bacillus algicola* under the experimentally determined optimal temperature and initial pH conditions of 25 °C and pH 8, respectively. The data points and error bars are means and standard deviation values for samples tested in triplicate. After an initial lag phase of 24 h, the extent of decolourisation of the *red* and *blue* dye shot from around 15–30% to reach above 90% over the next 24 h rate of incubation time

three azo dyes by *B. algicola* after 24 h at 35 °C. Under alkaline conditions, *B. algicola* showed the highest decolourisation (27.5%) for the red dye. A decrease in pH from 8.0 to 6.0 resulted in a 90, 50 and 28% decline in decolourisation efficiency of the blue, red and yellow dye, respectively. Under this set of conditions, pH 8 was optimum for the decolourisation of the three dyes. Table 2 also reports the results for the effect of temperature on the decolourisation of the test dyes. The decolourisation was highest at 25 °C at 35.1% for the red dye, 18.0% for blue dye and 14.4% for yellow dye. Increasing the incubation temperature from 25 to 45 °C resulted in lower decolourisation of dyes. Under the present conditions, the optimal incubation temperature was at the lower end of mesophilic range (25 °C).

Table 2 equally reports the decolourisation of the azo dyes by *B. algicola* at 25 °C and pH 8.0, over 4 days of incubation. Under these conditions, the blue and red dyes were decolourised up to 96.2 and 96.3%, respectively, whereas the yellow dye was degraded by only 26.6% (Fig. 1), possibly due to more recalcitrance. According to Hussain et al. (2013), pH is an important factor affecting microbial growth and biodegradation of azo dyes since pH affects the charges on amino acids of enzymes. Temperatures affects decolourisation of azo dyes, and beyond an optimum temperature, bacterial growth and enzymatic activity are hampered and account for lower decolourisation. The optimum conditions in this work agree with the results of Ivanova et al. (2004).

Table 3 Germination, root length and shoot length of cucumber (*Cucumis sativus*) seedlings obtained from phytotoxicity tests of azo dyes before and after degradation by *Bacillus algicola*

Watering solution	Germination (%)	Root length (cm)	Shoot length (cm)
Distilled water (control)	90	13.7 ± 2.0	6.1 ± 0.9
Red azo dye	0	–	–
Blue azo dye	50	4.8 ± 1.1	3.5 ± 0.7
Yellow azo dye	50	6.9 ± 1.3	3.4 ± 0.6
Biodegraded yellow azo dye	50	6.4 ± 1.6	3.1 ± 0.4
Biodegraded red azo dye	80	13.8 ± 1.7	5.9 ± 1.2
Biodegraded blue azo dye	80	12.5 ± 1.7	5.8 ± 0.9

It is observed that the root length and shoot length of the seedlings in the case of the biodegraded red and blue azo dyes are significantly greater (at a 95% confidence interval from the ANOVA test) than those recorded from the other watering solutions. The red dye has been highly inhibitory to seed germination. The data given here for root and shoot lengths are average and standard deviation for ten samples

Phytotoxicity assessment

Table 3 reports the results of the germination tests. The parent azo dyes demonstrated much higher toxicity towards seed germination relative to the control and dye degradation products. In the case of the biodegraded red and blue dyes, there was a noticeably better germination and there was no significant difference among the mean root and shoot lengths of the seedlings treated with the extracts of these two degraded dye and control. In contrast, germination with extracts from the degraded yellow dye was severely inhibited.

Conclusion

Azo dye-degrading bacteria were isolated from a sanitary landfill site, and the most effective one bringing about degradation of test azo dyes was identified as *Bacillus algicola*. Decolourisation of the red and blue test azo dye was occurred in yeast extract supplemented mineral salt agar. The optimum pH and temperature for biodegradation were 8 and 25 °C, respectively, and under these conditions *B. algicola* was able to significantly degrade the red and blue azo dyes. Phytotoxicity tests showed that the breakdown products were not toxic in the case of the red and blue dyes. The results of this study hence indicate the promising ability of the *B. algicola* in producing significant microbial degradation of azo dyes which are otherwise recalcitrant.

Acknowledgements We thank the technical staff of the Department of Agricultural and Food Science, Faculty of Agriculture, University of Mauritius. We also acknowledge the support received at the landfill. We equally thank the anonymous reviewers for their helpful comments. This work received no grant or financial support.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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