

# Biodegradation and decolorization of azo dyes by adherent *Staphylococcus lentus* strain

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Received: 2 January 2016 / Accepted: 21 January 2016 / Published online: 15 February 2016  
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**Abstract** A *Staphylococcus lentus* strain, isolated from Red sea water, was tested for decolorization capacity of Congo red, Evans blue, and Eriochrome Black T azo dyes. Biodegradation ( $100 \text{ mg l}^{-1}$ ) of these dyes was studied within 24 h in Mineral Salt Medium solution containing 0.10 % (w/v) yeast extract and 7 mM of glucose at a pH of 7.2 and a temperature of 37 °C. Using a  $2.2 \times 10^6$  CFU/mL inoculum size, *S. lentus* was able to decolorize these azo dyes with different degree. The staphylococcal biomass achieves approximately 100 % decolorization of Congo red and Eriochrome Black T by the end of treatment. FTIR and UV–Vis analyses confirm biodegradation potential of the tested strain. Moreover, the phytotoxicity of the dye solutions resulting from this treatment shows lower toxic nature compared to untreated solution of the respective dyes.

**Keywords** Azo dyes · Biofilm · Decolorization · *Staphylococcus lentus* · Phytotoxicity

## Introduction

Environmental pollution is one of the most important problems of the modern world. Synthetic dyes are used in paper printing, leather, and textile processing industries (Tony et al. 2009). Azo dyes are widely used due to their covalent attachment to the fibers, brilliant colors, and minimal energy consumption. The textile industry discharges a large amount of colored wastewater causing a considerable environmental pollution (Sanmuga Priya et al. 2015).

These dyes are a diverse class of chemicals containing various sulfonate groups and other substituents which affect its rate of decolorization (de los Cobos-Vasconcelos et al. 2012). A number of conventional physico-chemical wastewater treatment processes such as chemical, electro-coagulation, adsorption, flocculation, ozonation, and reverse osmosis have been proposed for decolorization of colored effluent (Robinson et al. 2001; Jadhav and Govindwar 2006). However, various synthetic dyes may escape from these treatment processes and persist in the environment for a long time.

Biodegradation is a process by which different types of microorganisms use complex chemical molecule and convert them into a simpler one (Solís et al. 2012). Biological treatment of effluent using fungi (Parshetti et al. 2007), bacteria (Kalyani et al. 2009), and yeast (Waghmode et al. 2011) has been reported elsewhere. Ding et al. (2014) noticed that *Sphingomonas* had a high biodegradation potential against azo dye in anaerobic systems. In addition, Reactive Red 2 was shown to be degraded by *Pseudomonas* sp. cells (Kalyani et al. 2009). Recently, Cui et al. (2014) reported that *Klebsiella* sp. Y3 was capable to decolorize azo dyes (Methyl Red, Congo Red, Orange I, and Methyl Orange) under anaerobic conditions.

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Several microorganisms have developed enzyme systems for the efficient decolorization and metabolization of azo dye to colorless aromatic amines using azoreductases under both aerobic and anaerobic conditions, including *Clostridium* sp. (Rafii et al. 1990), *Enterobacter agglomerans* (Moutaouakkil et al. 2002), *Pseudomonas aeruginosa* (Nachiyar and Rajakumar 2005), *Sphingomonas paucimobilis* (Ayed et al. 2011), *Staphylococcus epidermidis* (Ayed et al. 2010), *Staphylococcus arlettae* (Elisan-gela et al. 2009), *Staphylococcus gallinarum* (Chen et al. 2011), *Staphylococcus cohnii* (Yan et al. 2012), *Derma-coccus abyssi* MT1 (Lang et al. 2014), *Acinetobacter baumannii* YNWH 226 (Ning et al. 2014), and *Staphylo-coccus aureus* (Pan et al. 2015).

In this study, *S. lentus*, isolated from Red sea water, was tested for its ability to decolorize three azo dyes (Congo red, Evans blue, and Eriochrome Black T). In addition, the phytotoxicity of the dye solutions before and after treatment was assessed.

## Materials and methods

### Dyes and chemicals

Congo red, Evans Blue, and Eriochrome Black T (Table 1) were purchased from Sigma (Chemical Company, MO, U.S.A.).

### Sample collection, bacteria isolation, and culture conditions

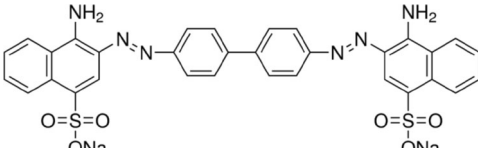
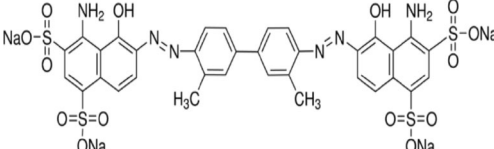
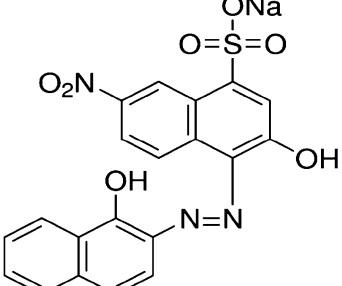
Fresh water sample (100 mL) was collected in a sterile glass container from Red Sea Coast, Yanbu el Bahr, El Madina Elmounaoura, KSA.

Water sample (25 mL) was added to halophilic broth (Microgen, Maharashtra, India) containing the respective dyes (100 mg l<sup>-1</sup>) and incubated at 37 °C. After 2 days of incubation, 1 mL of the culture was serially diluted and spread on halophilic agar (Microgen) plates containing 100 mg l<sup>-1</sup> of dyes. Then, colonies were screened for their ability to form a clear zone around the respective dyes (Ayed et al. 2009). Bacteria with positive reaction were isolated and subcultured in halophilic broth containing the respective dyes (100 mg l<sup>-1</sup>). Suspected colonies of *Staphylococci* were identified by their positive Gram stain, and catalase positive reaction (Oxoid, Basingstoke, UK). Species identification was performed using API 20 Staph strips (Bio-Merieux, France) according to the manufacturer's recommendation. The microorganism responsible for a significant degradation of the tested dye was found to be *S. lentus*.

### Adherence assay to measure biofilm production by *S. lentus* cells

Semi-quantitative adherence assay was performed on 96-well plates as described previously (Christensen et al.

**Table 1** Molecular formula and chemical structure of dyes used in this study

Dyes	Molecular formula	Structure	$\lambda_{\max}$ (nm)	Chemical class
Congo red	C <sub>32</sub> H <sub>22</sub> N <sub>6</sub> Na <sub>2</sub> O <sub>6</sub> S <sub>2</sub>		488	Azo dye
Evans blue	C <sub>34</sub> H <sub>24</sub> N <sub>6</sub> Na <sub>4</sub> O <sub>14</sub> S <sub>4</sub>		611	Azo dye
Eriochrome black T	C <sub>20</sub> H <sub>12</sub> N <sub>3</sub> NaO <sub>7</sub> S		503	Azo dye

1985). An overnight culture of *S. lentus* was diluted to 1:100 in Tryptic Soy Broth supplemented with 2 % (w/v) glucose. A total of 200  $\mu\text{L}$  of each cell suspension was transferred in a U-bottomed 96-well microtiter plate and incubated for 24 h at 37 °C. After incubation, bacterial culture was removed, washed three times with phosphate-buffered saline, and then dried in an inverted position. Adherent bacteria were fixed with 95 % ethanol and were colored with 100  $\mu\text{l}$  of 1 % (w/v) crystal violet solution for 5 min. The wells were rinsed three times with sterile distilled water and air dried for 2 h. The optical density (OD) of each well was measured at 570 nm using an automated Multiskan reader (ELX800, Biotek instrument, USA). Biofilm formation was interpreted as follows: highly positive ( $\text{OD}_{570} \geq 1$ ), low-grade positive ( $0.1 \leq \text{OD}_{570} < 1$ ), or negative ( $\text{OD}_{570} < 0.1$ ) (Knobloch et al. 2001).

### Organisms and culture conditions for biodegradation assay

Mineral salt medium (MSM) solution was prepared by mixing  $\text{K}_2\text{HPO}_4$  ( $1.36 \text{ g l}^{-1}$ ),  $\text{MnSO}_4$  ( $1.1 \text{ mg l}^{-1}$ ),  $(\text{NH}_4)_2\text{SO}_4$  ( $0.6 \text{ g l}^{-1}$ ),  $\text{NaCl}$  ( $0.5 \text{ g l}^{-1}$ ),  $\text{ZnSO}_4$  ( $0.2 \text{ mg l}^{-1}$ ),  $\text{CuSO}_4$  ( $0.2 \text{ mg l}^{-1}$ ),  $\text{FeSO}_4$  ( $0.14 \text{ mg l}^{-1}$ ),  $\text{MgSO}_4$  ( $0.1 \text{ g l}^{-1}$ ), and  $\text{CaCl}_2$  ( $0.02 \text{ g l}^{-1}$ ) in 1000 ml of distilled water (Moutaouakkil et al. 2002).

Dyes were prepared in MSM, filter sterilized through 0.22  $\mu\text{m}$  filter, and stored at +4 °C in the dark until use. Glucose (7 mM) and yeast extract (0.1 % w/v) was prepared in MSM and sterilized.

The pH of MSM was adjusted to 7.2, supplemented with 7 mM of glucose, 0.1 % (w/v) of yeast extract, 1 mL of  $2.2 \times 10^6$  CFU/mL, and 100  $\text{mg l}^{-1}$  of azo dyes (agitation: 150 rpm, T: 37 °C, and pH: 7.2). The yeast extract, glucose, and dyes were added to sterilize MSM.

Color removal was determined spectrophotometrically (UV-1800 SHIMADZU, Japan) based on the maximum absorbance of UV–Visible spectrum (Jadhav et al. 2008) using the following equation:

$$\text{Color removal (\%)} = (A_i - A_t) \times 100 / A_i,$$

where  $A_i$  is the initial absorbance and  $A_t$  is the absorbance at incubation time  $t$ .

The efficiency of dye removal was expressed as the percentage ratio of decolorized dye concentration to that of the initial one.

### Fourier transform infrared spectroscopy

The FTIR spectra of the samples before and after decolorisation were done using Fourier transform infrared spectroscopy (Nicolet iS 10 Thermoscientific). The samples were dried and mixed with spectroscopically pure KBr

(1:20 w/w) and then pressed to obtain IR-transparent pellets. The FTIR was first calibrated for background signal scanning with a control sample of a pure KBr, and then the sample was scanned. The spectra were collected within a scanning range of 400–4000  $\text{cm}^{-1}$ .

### Phytotoxicity of the degraded dyes

The phytotoxicity studies were performed in laboratory at room temperature, in order to assess the toxicity of dye solution before and after biodegradation using *Triticum aestivum* and *Sorghum bicolor* seeds (10 seeds of each plate) according to Moutaouakkil et al. (2002). Each day, 2 mL of initial solution (dyes) after biodegradation ( $100 \text{ mg l}^{-1}$ ) was added to the seeds. The MSM solution served as a control. After 7 days, germination (%) and length of shoot and root (cm) were recorded.

### Statistical analysis

Each analysis was performed using the SPSS 17.0 statistics package for Windows. The differences in germination (percentage) and length of shoot and root (cm) under different treatments were investigated with the Friedman test, followed by the Wilcoxon signed-rank test.  $P$  values <0.05 were considered significant.

## Results

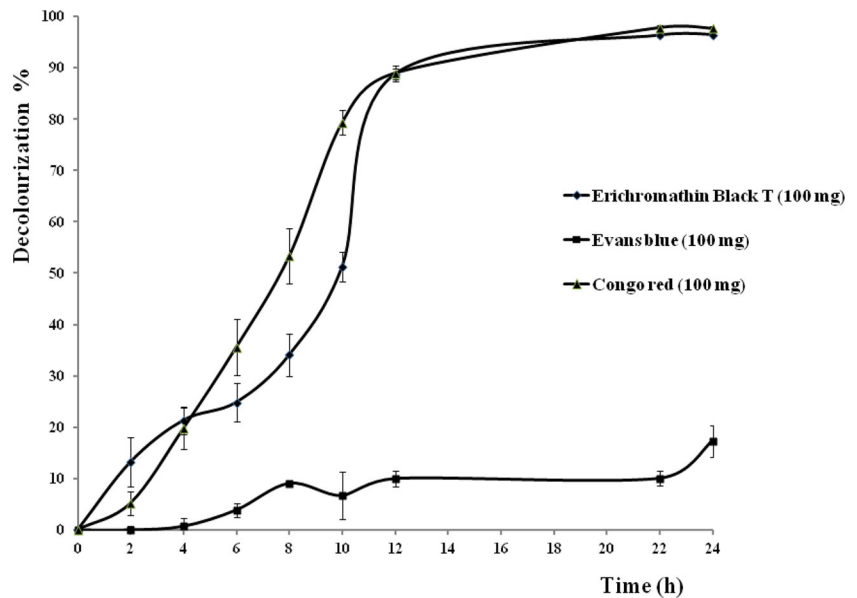
### Semi-quantitative adherence assay

One adherent halotolerant *S. lentus* strain was isolated from Red Sea water displaying a high decolorization effect against three azo dyes: Congo red, Evans blue, and Eriochrome Black T ( $100 \text{ mg l}^{-1}$ ). The semi-quantitative adherence assay shows the ability of *S. lentus* to adhere ( $\text{OD}_{570} = 1.2$ ) to abiotic surface such as polystyrene plate.

### Decolorization rate by *S. lentus*

*S. lentus* was tested for its ability to decolorize three azo dyes under aerobic conditions. According to Fig. 1, the maximum percentage of decolorization and the degradation time varied according to the structure of the tested dyes. Almost 100 % of Congo red and Eriochrome black T (97.63 and 96.43 %, respectively) were decolorized within 24-h incubation period, whereas the decolorization of Evans blue was low after the same time. Meanwhile, the degradation of Evans blue reached 19.7 % after 24-h incubation period. The degradation reached 50 % after 8 and 10 h for Congo red and Eriochrome black T, respectively.

**Fig. 1** Decolorization of 100 mg l<sup>-1</sup> of Congo red, Evans blue, and Eriochrome black T by *Staphylococcus lentus*. [bacterium]<sub>0</sub> = 2.2 × 10<sup>6</sup> CFU/ml, temperature = 37 °C, pH = 7.2, shaking (150 rpm)



### UV–Vis characterization

Decolorization of the dyes was followed by the comparison of changes in their absorption spectrum in the range of 250–800 nm using UV–Vis spectroscopic analysis. UV–Vis spectrum of azo dyes (Congo red, Evans blue, and Eriochrome black T) is presented in Fig. 2A, B, and C, respectively.

For untreated dyes, as shown in Fig. 2A, Congo red presented one absorbance peak at 488 nm. Concerning Evans blue (Fig. 2B), the spectrum presented one absorbance peak at 611 nm. However, in Fig. 2C we noticed a large peak at 503 nm of Eriochrome black T. The treated samples after 24-h incubation period showed a decrease in optical density.

### FTIR characterization

The FTIR spectra of the three studied azo dyes (Congo red, Evans blue, and Eriochrome black T) before (A) and after (B) decolorization by *S. lentus* are presented in Figs. 3, 4, and 5, respectively. Figure 3A shows that the spectra of untreated Congo red indicate a broad band stretching vibration at 3319, 2855, 1652, 1615, 1600, 1556, 1450, 1405, 1078, 1035, 713, 698, and 683 cm<sup>-1</sup>.

The FTIR spectra of decolorized sample (Fig. 3B) showed a significant change in the position of peaks. Wavenumbers resulted in the degradation spectrum of Congo red showing bands at 3335, 2993, 2380, 1652, 1635, 1615, 1675, 1405, 1078, 1035, 713, 698, and 683 cm<sup>-1</sup>.

Figure 4 shows FTIR spectrum of untreated and treated Evans blue dye. The spectrum indicates peaks at 3315, 2890, 2325, 1617, 1456, 1405, 1238, 1077, 1036, 915, 778, 739, and 683 cm<sup>-1</sup>. FTIR spectrum of treated Evans blue

(Fig. 4B) shows a significant change in the position of peaks and disappearance of some peaks.

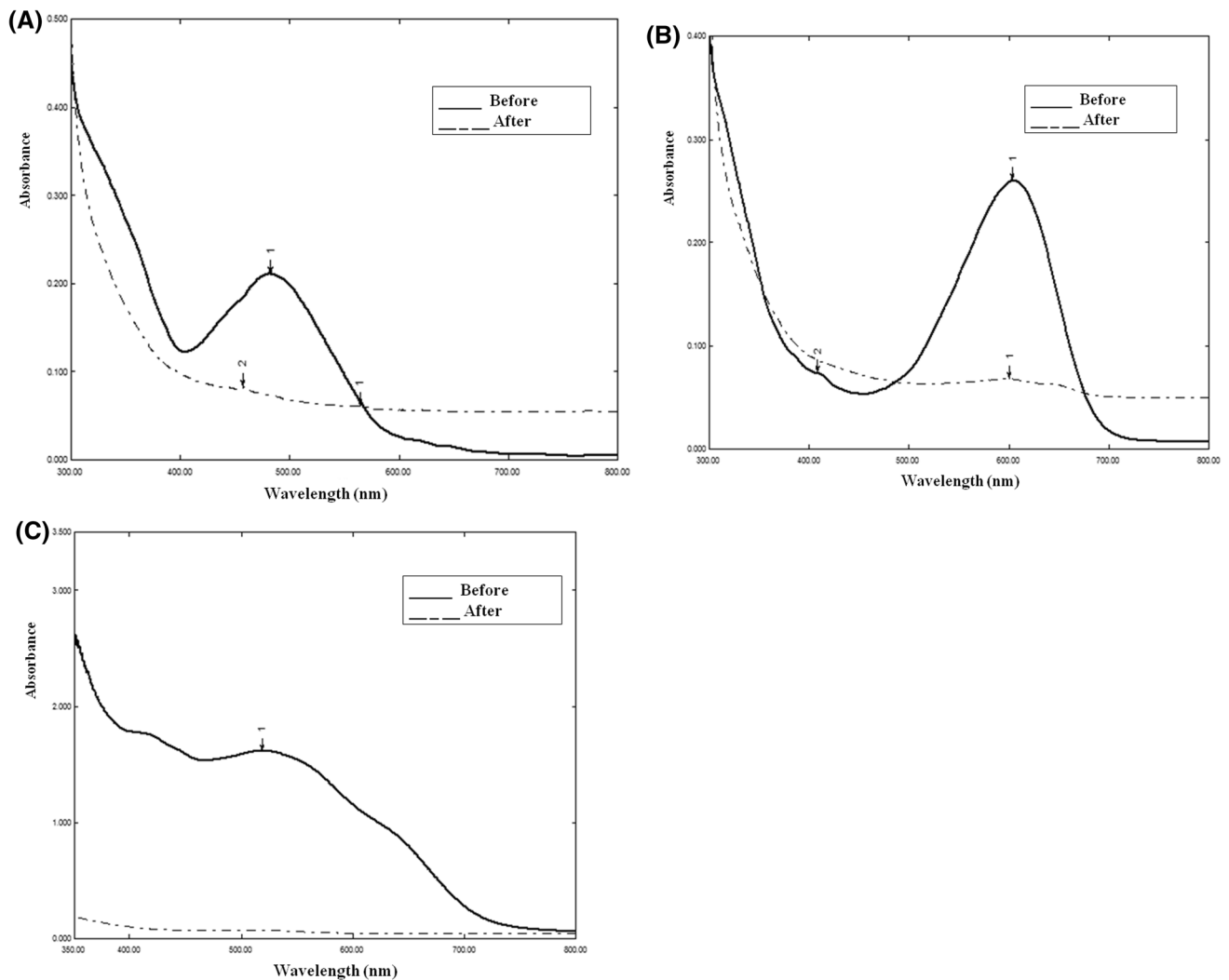
Figure 5 indicates FTIR spectrum of untreated and treated Eriochrome black T. The spectrum shows bands at 3343, 2925, 2850, 2359, 1633, 1417, 1230, 1077, and 1035 cm<sup>-1</sup>. FTIR spectrum after microbial degradation shows a significant change in the position of peaks and disappearance of some peaks as compared to untreated dye spectrum. The spectrum of treated dye (Fig. 5B) shows disappearance of peaks at 3277, 2927, 2850, and 2359 cm<sup>-1</sup> as well as 1592, 1409, 1225, 1075, and 1033 cm<sup>-1</sup>.

### Phytotoxicity studies

Data presented in Table 2 showed that the mean of shoot and root lengths of *Triticum aestivum* and *Sorghum bicolor* reaches 100 % germination in MSM. While, when treated with 100 mg l<sup>-1</sup> concentration of Congo red, Evans blue, and Eriochrome Black T, the germination rate of seed was inhibited (90, 80, and 90 %, respectively). In addition, the plumule and radical lengths were affected (Table 2). Meanwhile, treating seeds with 100 mg l<sup>-1</sup> of degraded product had minor effect on the plant, as the germination percentage reaches 100 % and the shoot and root lengths grew more rapidly ( $P < 0.05$ ) in comparison with non-degraded dye solution (Table 2).

### Discussion

The use of *Staphylococci* for degradation of azo dyes has been reported elsewhere. *S. arlettae* causes >90 % decoloration of Reactive Yellow 107 and Reactive Red in the



**Fig. 2** UV-Vis spectra of the azo dyes (100 mg) before (*straight line*) and after (*dashed line*) treatments: **(A)** Congo red, **(B)** Evans blue, and **(C)** Eriochrome black T. [bacterium]<sub>0</sub> =  $2.2 \times 10^6$  CFU/mL, temperature = 37 °C, pH = 7.2, shaking (150 rpm)

presence of glucose and yeast extract (Elisangela et al. 2009).

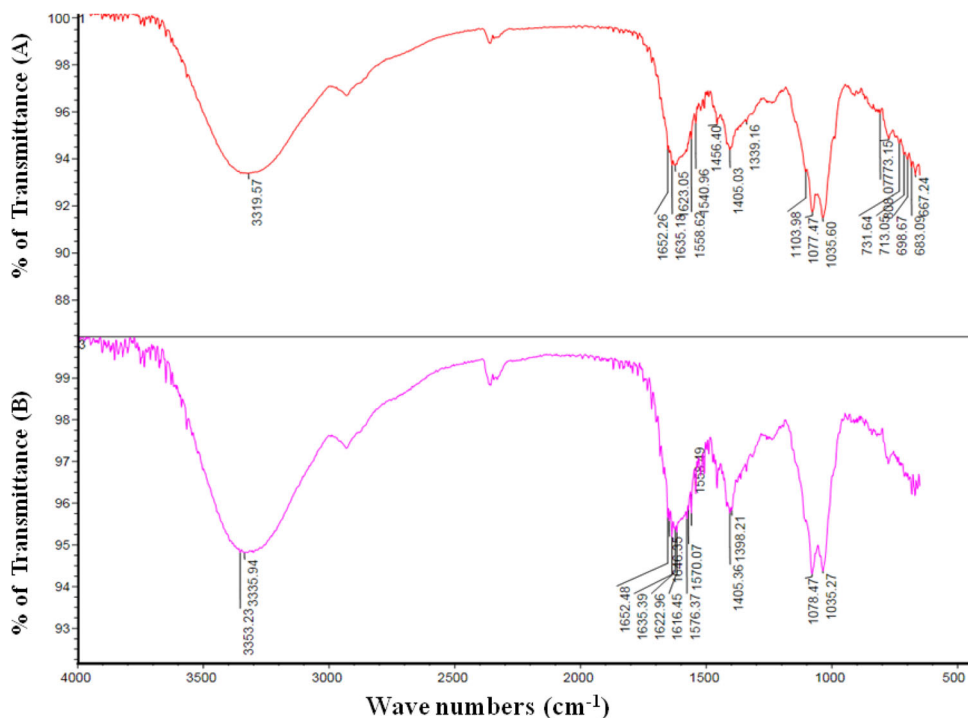
In this study, *S. lentus* was tested for its ability to decolorize three azo dyes under aerobic conditions. It has been reported that chemical structures of azo dyes are closely related to its decolorization rate and time (Pasti-Grigsby et al. 1992). The dyes with simple structures and low molecular weights are decolorized faster than those with complex structures (Pearce et al. 2003). In our study, *S. lentus* was able to decolorize dyes that contain one (Eriochrome Black T) or two (Congo red) sulfonate groups in shorter time in comparison with dyes which had four sulfonate groups (Evans blue). Similar result was obtained with *Klebsiella* sp. strain Y3 showing a difference in decolorization rates according to the number of sulfonate groups in the tested dyes (Cui et al. 2014).

We noted also that the isolated strain was more effective on Congo red and Eriochrome black T (97.63 and 96.43 %

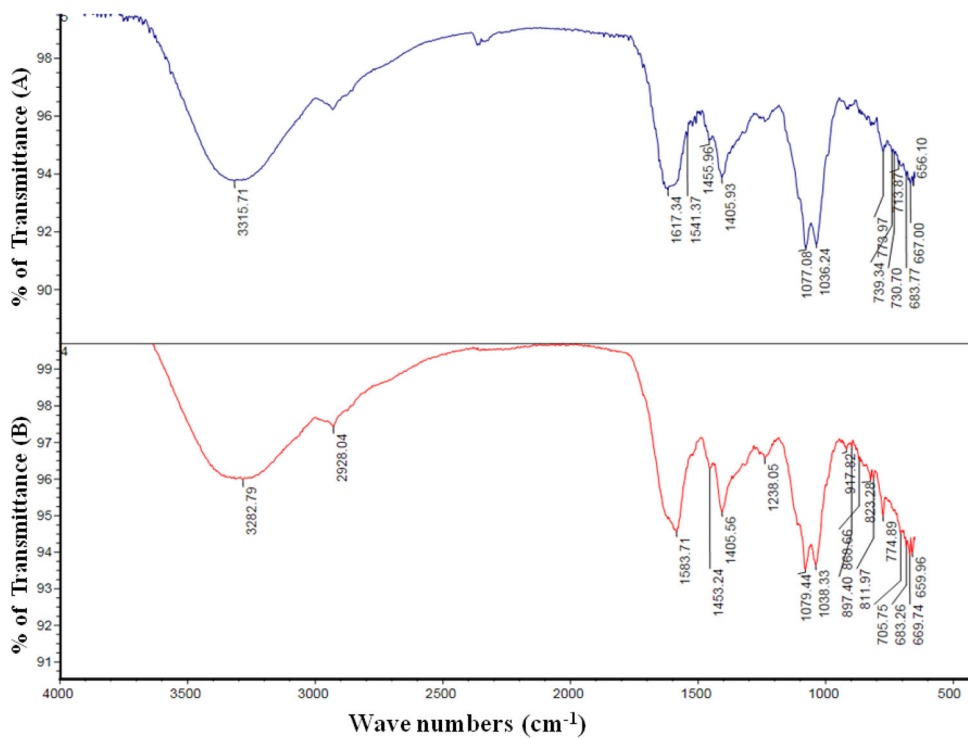
decolorization rates, respectively) than on Evans blue (19.7 %). Similar result was obtained with *S. epidermidis*, isolated from textile wastewater plant, which has been reported to be able to cause 100 % Congo Red (750 ppm) color removal (Ayed et al. 2010). Cui et al. (2012) tested the effectiveness of azo dye removal using a consortium composed of different *Klebsiella* strains; Eriochrome black T and methyl orange were less than 80 % removed after 16 h.

According to UV-Vis spectroscopic analysis of the three azo dyes (before and after treatment), we note that the absorbance peaks in the visible region decreased without any shift in  $\lambda$ -max, indicating complete decolorization of the solution (Congo red and Eriochrome black T). According to Asad et al. (2007), decolorization of dyes by bacteria may be attributed to adsorption by microbial cells or to biodegradation. It has been reported that *S. arlettae* VN-11, isolated from an activated sludge process in a

**Fig. 3** FTIR analysis of Congo red (A) and degraded products (B) (after 24-h incubation period)



**Fig. 4** FTIR analysis of Evans blue (A) and degraded products (B) (after 24-h incubation period)

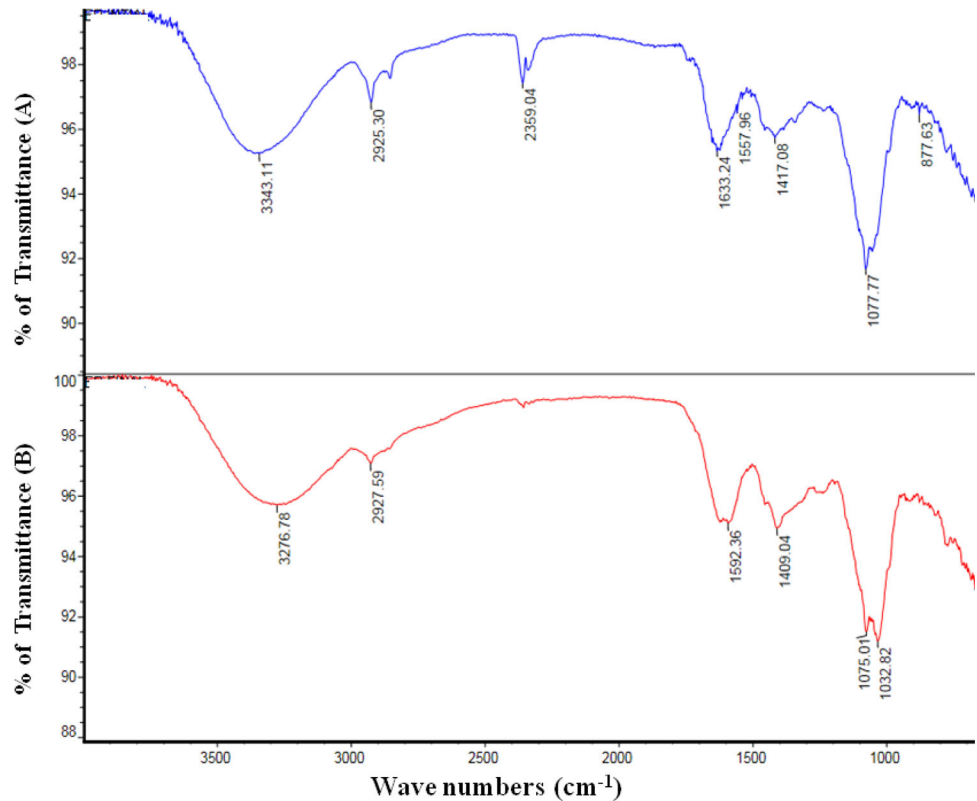


textile industry, was able to decolorize four different azo dyes (Reactive Yellow 107, Reactive Black 5, Reactive Red 198, and Direct Blue 71) at  $100 \text{ mg l}^{-1}$  under micro-aerophilic conditions with decolorization percentage being more than 97 % (Elisangela et al. 2009). Meanwhile, a salt-tolerant *S. cohnii* strain, isolated from textile wastewater,

has been found to exhibit effective decolorization activity of several kinds of azo dyes with different structures at  $\text{pH} = 7.0$  and  $T = 30 \text{ }^\circ\text{C}$  (Yan et al. 2012).

Figures 3, 4, and 5 show the FTIR spectra of the three studied azo dyes before and after decolorization. The comparison between the FTIR spectra of control dye

**Fig. 5** FTIR analysis of Eriochrome black T (A) and degraded products (B) (after 24-h incubation period)



**Table 2** Phytotoxicity study of azo dyes and its degradation product

Azo dyes	Parameters studied	<i>Sorghum bicolor</i>		<i>Triticum aestivum</i>	
		Before treatment	After biodegradation	Before treatment	After biodegradation
MSM	Germination (%)	100		100	
	Shoot (cm) ± SD	13.5 ± 1.39		12.11 ± 1.83	
	Root (cm) ± SD	17.06 ± 0.98		10.75 ± 0.72	
Congo red	Germination (%)	90	100	90	100
	Shoot (cm) ± SD	6.06 ± 0.93	10.85 ± 1	4 ± 0.53	9.85 ± 1.23
	Root (cm) ± SD	2.72 ± 0.63	6.4 ± 0.81	2.72 ± 0.42	5.8 ± 0.67
Evans blue	Germination (%)	80	100	80	100
	Shoot (cm) ± SD	5.13 ± 0.74	8.5 ± 0.78	4 ± 0.87	9.2 ± 0.86
	Root (cm) ± SD	2.81 ± 0.53	5.2 ± 0.54	2.5 ± 0.71	4.5 ± 0.62
Eriochrome black T	Germination (%)	90	100	90	100
	Shoot (cm) ± SD	4 ± 1.67	8.2 ± 0.86	4.78 ± 1.20	6.55 ± 0.76
	Root (cm) ± SD	2.3 ± 0.67	3.95 ± 0.72	2.78 ± 0.48	4.7 ± 0.64

(before degradation) with FTIR spectra of extracted metabolites after decolorization clearly indicates the biodegradation potential of parent dye compound by *S. lentus* (Fig. 3). The FTIR spectrum of treated Evans blue (Fig. 4B) and Eriochrome black T (Fig. 5B) shows a significant change in the position of peaks and disappearance of some others peaks (Sanmuga Priya et al. 2015).

A broad stretching vibrational band appeared at  $3319\text{ cm}^{-1}$  in Fig. 3A (untreated Congo red) corresponding to N–H functional group, while saturated C–H stretching vibration band at  $2855\text{ cm}^{-1}$ , peak at  $1652\text{ cm}^{-1}$  corresponding to N–H bending vibration, and N=N azo stretching vibration band at  $1615\text{ cm}^{-1}$ . The bands at 1600 and  $1556\text{ cm}^{-1}$  correspond to the stretching vibrations of

aromatic ring double bonds. Moreover, the bands at 1450 and 1405  $\text{cm}^{-1}$  are attributed to C–H bending. The band at 1078 and 1035  $\text{cm}^{-1}$  are assigned to R-SO<sub>3</sub> groups and –S=O bands, respectively. Figure 3B indicates FTIR spectra of decolorized sample where a significant change in the position of peaks is remarked for the degradation spectrum of Congo red.

The spectrum of Evans blue (Fig. 4A) indicates a peak at 3315  $\text{cm}^{-1}$  assigned to stretching vibrations of O–H and N–H bond, peak at 2890  $\text{cm}^{-1}$  corresponding to saturated C–H stretching vibration, peak at 2325  $\text{cm}^{-1}$  assigned to C=O, band at 1617  $\text{cm}^{-1}$  corresponding to N=N azo stretching vibration, band at 1456  $\text{cm}^{-1}$ , band at 1405  $\text{cm}^{-1}$  to C–H bending characteristics, band at 1238  $\text{cm}^{-1}$  and peaks at 1077 and 1036  $\text{cm}^{-1}$  are assigned to R-SO<sub>3</sub> groups and –S=O (Sanmuga Priya et al. 2015). Figure 4B illustrates FTIR spectrum of treated Evans blue. The broadening of the band at 3283  $\text{cm}^{-1}$ , weakening of the peak at 2928  $\text{cm}^{-1}$ , disappearance of the peak at 2325  $\text{cm}^{-1}$ , and appearance of new peaks at 1583, 1453, 1405, 1238, 1079, 1038, 917, and 775  $\text{cm}^{-1}$  are indicative of the degradation.

FTIR spectrum (Fig. 5A) confirms the microbial degradation of Eriochrome black T where there is a significant change in the position of peaks and disappearance of some peaks as compared to untreated dye spectrum. Meanwhile, the spectrum of treated dye (Fig. 5B) shows the disappearance of peaks at 3277, 2927, 2850, and 2359  $\text{cm}^{-1}$  as well as 1592, 1409, 1225, 1075, and 1033  $\text{cm}^{-1}$ .

The assessment of decolorized solution on vegetation is essential to explore the possible re-use of treated water for irrigation purpose (Pourbabaee et al. 2006). The relative sensitivity of the *T. aestivum* and *S. bicolor* seeds against Congo red, Evans blue, and Eriochrome Black T (100  $\text{mg l}^{-1}$ ) before and after biodegradation is presented in Table 2. We noticed that the exposure of seeds to the untreated dye affects the germination rate (90, 80, and 90 %, respectively), while degraded solution may enhance the growth of shoot and root.

The Wilcoxon signed-rank test revealed a statistically significant difference in shoot and root lengths between the control and the treated seeds with degraded dye solution ( $P < 0.05$ ).

Similar result was obtained with degraded Congo red (100  $\text{mg l}^{-1}$ ) solution by *Pseudomonas* sp. SU-EBT on *Sorghum bicolor*, *Vigna radiata*, *Lens culinaris*, and *Oryza sativa* plants as compared to parent dye (Telke et al. 2010). More recently, Saratale et al. (2015) noticed that the degraded metabolites showed non-toxic nature revealed by phytotoxicity on *Sorghum vulgare* and *Phaseolus mungo* plants. Meanwhile, Zablocka-Godlewska et al. (2015)

reported no change in toxicity of Evans Blue on *Lemna minor* after degradation by *Klebsiella* Bz4 strain.

Our study demonstrates that *S. lentus* may achieve approximately 100 % decolorization of Congo red and Eriochrome Black T (100  $\text{mg l}^{-1}$ ) by the end of treatment. UV–Visible and FTIR analyses confirmed the biodegradation potential of the tested strain. These results support the potential use of *S. lentus* for bioremediation of azo dye solution.

## References

- Asad S, Amoozegar M, Pourbabaee AA, Sarbolouki M, Dastgheib S (2007) Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. *Bioresour Technol* 98:2082–2088
- Ayed L, Chaieb K, Cheref A, Bakhrouf A (2009) Biodegradation of triphenylmethane dye Malachite Green by *Sphingomonas paucimobilis*. *World J Microbiol Biotechnol* 25:705–711
- Ayed L, Khelifi E, Jannet HB, Miladi H, Cheref A, Achour S, Bakhrouf A (2010) Response surface methodology for decolorization of azo dye methyl orange by bacterial consortium: produced enzymes and metabolites characterization. *Chem Eng J* 165:200–208
- Ayed L, Mahdhi A, Cheref A, Bakhrouf A (2011) Decolorization and degradation of azo dye Methyl Red by an isolated *Sphingomonas paucimobilis*: biotoxicity and metabolites characterization. *Desalination* 274:272–277
- Chen B-Y, Hsueh C-C, Chen W-M (2011) Exploring decolorization and halotolerance characteristics by indigenous acclimatized bacteria: chemical structure of azo dyes and dose–response assessment. *J Taiwan Inst Chem Eng* 42:816–825
- Christensen GD, Simpson W, Younger J, Baddour L, Barrett F, Melton D, Beachey E (1985) Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol* 22:996–1006
- Cui D, Li G, Zhao D, Gu X, Wang C, Zhao M (2012) Microbial community structures in mixed bacterial consortia for azo dye treatment under aerobic and anaerobic conditions. *J Hazard Mater* 221:185–192
- Cui D, Li G, Zhao M, Han S (2014) Decolourization of azo dyes by a newly isolated *Klebsiella* sp. strain Y3, and effects of various factors on biodegradation. *Biotechnol Biotechnol Equip* 28:478–486
- de los Cobos-Vasconcelos D, Ruiz-Ordaz N, Galíndez-Mayer J, Poggi-Varaldo H, Juárez-Ramírez C, Aarón LM (2012) Aerobic biodegradation of a mixture of sulfonated azo dyes by a bacterial consortium immobilized in a two-stage sparged packed-bed biofilm reactor. *Eng Life Sci* 12:39–48
- Ding J, Zhang Y, Quan X, Chen S (2014) Anaerobic biodecolorization of AO7 by a newly isolated Fe(III)-reducing bacterium *Sphingomonas* strain DJ. *J Chem Technol Biotechnol* 90:158–165
- Elisangela F, Andrea Z, Fabio DG, de Menezes Cristiano R, Regina DL, Artur C-P (2009) Biodegradation of textile azo dyes by a facultative *Staphylococcus arlettae* strain VN-11 using a sequential microaerophilic/aerobic process. *Int Biodeterior Biodegrad* 63:280–288
- Jadhav J, Govindwar S (2006) Biotransformation of malachite green by *Saccharomyces cerevisiae* MTCC 463. *Yeast* 23:315–323



- Jadhav UU, Dawkar VV, Ghodake GS, Govindwar SP (2008) Biodegradation of Direct Red 5B, a textile dye by newly isolated *Comamonas* sp. UVS. *J Hazard Mater* 158:507–516
- Kalyani DC, Telke AA, Dhanve RS, Jadhav JP (2009) Ecofriendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas* sp. SUK1. *J Hazard Mater* 163:735–742
- Knobloch JK-M, Bartscht K, Sabottke A, Rohde H, Feucht H-H, Mack D (2001) Biofilm formation by *Staphylococcus epidermidis* depends on functional RsbU, an activator of the sigB operon: differential activation mechanisms due to ethanol and salt stress. *J Bacteriol* 183:2624–2633
- Lang W, Sirisansaneeyakul S, Martins LO, Ngiswara L, Sakairi N, Pathom-aree W, Okuyama M, Mori H, Kimura A (2014) Biodecolorization of a food azo dye by the deep sea *Dermacoccus abyssi* MT1.1T strain from the Mariana Trench. *J Environ Manag* 132:155–164
- Moutaouakkil A, Zeroual Y, Blaghen M (2002) Décoloration et biodégradation du colorant azoïque toxique Rouge de Méthyle par *Enterobacter agglomerans*. *Congrès de Biochimie, Casablanca* 9:136–141
- Nachiyar CV, Rajakumar GS (2005) Purification and characterization of an oxygen insensitive azoreductase from *Pseudomonas aeruginosa*. *Enzyme Microb Technol* 36:503–509
- Pan H, Xu J, Kweon O-G, Zou W, Feng J, He G-X, Cerniglia C, Chen H (2015) Differential gene expression in *Staphylococcus aureus* exposed to Orange II and Sudan III azo dyes. *J Ind Microbiol Biotechnol* 42:745–757
- Parshetti G, Kalme S, Gomare S, Govindwar S (2007) Biodegradation of Reactive blue-25 by *Aspergillus ochraceus* NCIM-1146. *Bioresour Technol* 98:3638–3642
- Pasti-Grigsby M, Paszczynski A, Goszczynski S, Crawford D, Crawford R (1992) Influence of aromatic substitution patterns on azo dye degradability by *Streptomyces* spp. and *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 58:3605–3613
- Pearce C, Lloyd J, Guthrie J (2003) The removal of colour from textile wastewater using whole bacterial cells: a review. *Dyes Pigment* 58:179–196
- Pourbabae A, Malekzadeh F, Sarbolouki M, Najafi F (2006) Aerobic decolorization and detoxification of a disperse dye in textile effluent by a new isolate of *Bacillus* sp. *Biotechnol Bioeng* 93:631–635
- Rafii F, Franklin W, Cerniglia CE (1990) Azoreductase activity of anaerobic bacteria isolated from human intestinal microflora. *Appl Environ Microbiol* 56:2146–2151
- Robinson T, McMullan G, Marchant R, Nigam P (2001) Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresour Technol* 77:247–255
- Sanmuga Priya E, Senthamil Selvan P, Umayal A (2015) Biodegradation studies on dye effluent and selective remazol dyes by indigenous bacterial species through spectral characterisation. *Desalination Water Treat* 55:241–251
- Saratale RG, Saratale GD, Govindwar SP, Kim DS (2015) Exploiting the efficacy of *Lysinibacillus* sp. RGS for decolorization and detoxification of industrial dyes, textile effluent and bioreactor studies. *J Environ Sci Health Part A* 50:176–192
- Solís M, Solís A, Pérez HI, Manjarrez N, Flores M (2012) Microbial decolouration of azo dyes: a review. *Process Biochem* 47:1723–1748
- Telke AA, Joshi SM, Jadhav SU, Tamboli DP, Govindwar SP (2010) Decolorization and detoxification of Congo red and textile industry effluent by an isolated bacterium *Pseudomonas* sp. SU-EBT. *Biodegradation* 21:283–296
- Tony BD, Goyal D, Khanna S (2009) Decolorization of textile azo dyes by aerobic bacterial consortium. *Int Biodeterior Biodegrad* 63:462–469
- Waghmode TR, Kurade MB, Govindwar SP (2011) Time dependent degradation of mixture of structurally different azo and non azo dyes by using *Galactomyces geotrichum* MTCC 1360. *Int Biodeterior Biodegrad* 65:479–486
- Ning X-A, Yang C, Wang Y, Yang Z, Wang J, Li R (2014) Decolorization and biodegradation of the azo dye Congo red by an isolated *Acinetobacter baumannii* YNWH 226. *Biotechnol Bioprocess Eng* 19:687–695
- Yan B, Du C, Xu M, Liao W (2012) Decolorization of azo dyes by a salt-tolerant *Staphylococcus cohnii* strain isolated from textile wastewater. *Front Environ Sci Eng* 6:806–814
- Zablocka-Godlewska E, Przysaś W, Grabińska-Sota E (2015) Dye decolourisation using two *Klebsiella* strains. *Water Air Soil Pollut* 226:1–15