Annals of Microbiology (2018) 68:943–952
https://doi.org/10.1007/s13213-018-1403-6

ORIGINAL ARTICLE

Isolation and characterization of a lead (Pb) tolerant *Pseudomonas aeruginosa* strain HF5 for decolorization of reactive red-120 and other azo dyes

Farhan Hafeez[1] · Huma Farheen[2] · Faisal Mahmood[2] · Tanvir Shahzad[2] · Muhammad Shahid[3] · Muhammad Iqbal[1] · Sumaira Rasul[4] · Hamid Manzoor[4] · Sabir Hussain[2]

Received: 2 June 2018 / Accepted: 1 November 2018 / Published online: 14 November 2018

Abstract

Presence of heavy metals including lead (Pb) in the textile effluents is a crucial factor affecting the growth and potential of the dye decolorizing bacterial strains. This work was planned to isolate and characterize a bacterial strain exhibiting the potential to decolorize a range of azo dyes as well as the resistance to Pb. In this study, several Pb tolerant bacteria were isolated from effluents of textile industry. These bacterial isolates were screened for their potential of decolorizing the reactive red-120 (RR120) azo dye with presence of Pb (50 mg L^{-1}). The most efficient isolate was further characterized for its potential to resist Pb and decolorize different azo dyes under varying cultural and incubation conditions. Out of the total 82 tested bacterial isolates, 30 bacteria were found to have varying potentials to resist the presence of lead (Pb) and carry out decolorization of an azo dye reactive red-120 (RR120) in the medium amended with Pb (50 mg L^{-1}). The most efficient selected bacterium, *Pseudomonas aeruginosa* strain HF5, was found to show a good potential not only to grow in the presence of considerable concentration of Pb but also to decolorize RR120 and other azo dyes in the media amended with Pb. The strain HF5 completely (> 90%) decolorized RR120 in mineral salt medium amended with 100 mg L^{-1} of Pb and 20 g L^{-1} NaCl. This strain also considerably (> 50%) decolorized RR120 up to the presence of 2000 mg L^{-1} of Pb and 50 g L^{-1} of NaCl but with reduced rate. The optimal decolorization of RR120 by HF5 was achieved when the pH of the Pb amended (100 mg L^{-1}) mineral salt media was adjusted at 7.5 and 8.5. Interestingly, this strain also showed the tolerance to a range of metal ions with varying MIC values. The *Pseudomonas aeruginosa* strain HF5 harboring the unique potentials to grow and decolorize the azo dyes in the presence of Pb is envisaged as a potential bioresource for devising the remediation strategies for treatment of colored textile wastewaters loaded with Pb and other heavy metal ions.

Keywords *Pseudomonas aeruginosa* · Lead (Pb) tolerance · Azo dyes decolorization · Heavy metal tolerance · NaCl resistance

Introduction

Textile effluents are a major source of freshwater pollution which have been reported to have impacts on different ecological components of the environment as well as the human beings (Chequer et al. 2009; Khan and Malik 2014; Gupta et al. 2015; Imran et al. 2015a). These impacts are primarily attributed towards the occurrence of enormous amounts of synthetic dyes and heavy metals in the textile effluents (Khan and Malik 2014; Imran et al. 2015a). Reports have suggested that an average concentration of dye may exist up to 300 mg L^{-1} in the effluent generated from the textile industries (Tony et al. 2009) which is less frequent than the 10–250 mg L^{-1} value as reported by O'Neill et al. (1999). Literature has reported as high as 1500 mg L^{-1} of dyes in some textile effluents (Pierce 1994). Meanwhile, these textile effluents also contain a range of metal

Farhan Hafeez and Huma Farheen contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13213-018-1403-6) contains supplementary material, which is available to authorized users.

✉ Sabir Hussain
sabirghani@gmail.com

[1] Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus, Tobe Camp University Road, Abbottabad 22060, Pakistan

[2] Department of Environmental Sciences & Engineering, Government College University, Allama Iqbal Road, Faisalabad 38000, Pakistan

[3] Department of Bioinformatics and Biotechnology, Government College University, Allama Iqbal Road, Faisalabad 38000, Pakistan

[4] Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

ions such as cobalt, chromium, manganese, copper, lead, iron, and nickle (Imran et al. 2015a). The ecotoxicological data shows that some of the synthetic dyes, aromatic in nature, have been found to be carcinogenic and mutagenic (Ma et al. 2014). Azo dyes constitute a key group of synthetic dyes which are extensively used in textile industries with about 50% share in total dyes used worldwide (Stolz 2001; Pandey et al. 2007). These are relatively less degradable and, when released along with textile effluents, they persist in different components of the environment including soil and water as a pollutant (Hussain et al. 2013; Loganathan et al. 2015). In soil resources, they do not only disturb the microbial abundance and microbial community structure but also have a negative impact on germination and growth of plants (Ghodake et al. 2009; Imran et al. 2015a, c). Impact of azo dyes to water ecosystem is also largely reported including esthetic problems, increased chemical oxygen demand (COD), and biological oxygen demand (BOD) and also hindering the light penetration thus ultimately decreasing the activity of aquatic life (Chacko and Subramaniam 2011; Imran et al. 2015a). Few azo dyes and their degradation metabolites have also been found to harbor carcinogenic and mutagenic properties having different negative impacts on health of living organisms including human beings (Chacko and Subramaniam 2011; Imran et al. 2015a). In order to cope with the harmful effects attributed towards azo dyes, devising the strategies for their remediation from different components of the environment is a topic of interest for the global scientific communities.

Recently, the use of potential microbial bioresources for remediation of azo dyes from the synthetic and real textile wastewaters has been reported as an efficient environmental friendly approach worldwide (Imran et al. 2015a; Maqbool et al. 2016; Hussain et al. 2017). For this purpose, different bacteria from a range of genera comprising *Comamonas*, *Shewanella*, *Pseudomonas*, *Acinetobacter*, *Psychrobacter*, *Serratia*, *Enterococcus*, *Bacillus*, *Staphylococcus*, *Proteus*, and *Providencia* isolated from different sources have been described for biodecolorization of various dyes in synthetic and real textile wastewaters (Kalme et al. 2007; Bafana et al. 2009; Bayoumi et al. 2010; Phugare et al. 2011; Khalid et al. 2012; Hussain et al. 2013; Anwar et al. 2014; Imran et al. 2015b; Najme et al. 2015; Abbas et al. 2016; Mahmood et al. 2017). However, it has been observed that growth as well as the decolorizing activity of such strains is affected when the media are amended with various heavy metal ions individually or in mixtures (Hussain et al. 2013; Imran et al. 2015b; Abbas et al. 2016). Henceforth, the bacterial strains with potential of simultaneously decolorizing the azo dyes and resisting various metal ions are needed to be isolated and characterized for their potentials.

Lead (Pb) is one of the heavy metal ions often found in textile effluents originating from different textile industries due to their use in producing pigments for textile dyeing (Halimoon and Yin 2010; Das et al. 2011). In addition to its release from textile industries, Pb is also contributed in the soils and water resources through different natural and anthropogenic processes including mining and smelting activities, combustion of gasoline, sewage sludge, and batteries disposal (Sparks 2005; Singh and Gad 2012). The presence of Pb in the natural media affects plant growth and activity (Sharma and Dubey 2005) and the microbial communities (Blagodatskaya et al. 2006) including the dye decolorizing bacteria (Hussain et al. 2013; Abbas et al. 2016). Hence, the existence of Pb in textile wastewaters serves as a hurdle in devising the biological wastewater treatment technologies involving the use of microbial populations. In order to cope with this problem, the present study reports the isolation of a Pb tolerant *Pseudomonas aeruginosa* strain HF5 which can decolorize different dyes as well as the capability to grow and perform its decolorizing activity in the presence of considerable concentrations of Pb.

Material and methods

Dyes, chemicals, and media

The textile dyes for this study were purchased from Santa Cruz Biotechnology (Shanghai, China). General characteristics including chemical formula, molecular weight, color index number, and wavelength of maximum absorption (λ_{max}) of the dyes used in this study have been presented in Table 1. All other chemicals and reagents were also of analytical grade and purchased from Sigma-Aldrich. A mineral salt (MS) medium [composition (g L^{-1}): 1.0 NaCl, 0.1 $CaCl_2$. $2H_2O$, 0.5 $MgSO_4.7H_2O$, 1.0 KH_2PO_4, 1.0 K_2HPO_4, 3.0 yeast extract] having 7.2 pH and containing 200 mg L^{-1} of reactive red-120 (RR120) dye and 50 mg L^{-1} of Pb as Pb $(NO_3)_2$ was used to isolate lead resistance dye decolorizing bacteria. Whenever required, standard HCl or NaOH were used to adjust the pH of the MS medium. Nutrient agar (NA) medium [composition (g L^{-1}): 5.0 NaCl, 5.0 peptone, 2.0 yeast extract, 15.0 agar] was used to estimate the minimum inhibitory concentration (MIC) of Pb and other metals for HF5 and other isolates as well as for estimation of population density of the strain HF5 at various pH levels.

Isolation of Pb tolerant strain HF5

For isolation of Pb tolerant RR120 decolorizing strain, the textile wastewater samples were collected from effluent discharge sites of various textile industries in Faisalabad (Table S1). The wastewater samples were analyzed for EC and pH by using Microprocessor Conductivity Model DDS-120 W and pH meter (Model 1770 D) respectively (Table S1). Isolation of Pb tolerant RR120 decolorizing bacterial strain was done through enrichment culture technique using the MS broth containing 50 mg L^{-1} of Pb as Pb $(NO_3)_2$ and 200 mg L^{-1} of RR120 dye followed by dilution plating. Enrichment was carried out by inoculating each wastewater

Table 1 Decolorization of different azo dyes by *Pseudomonas aeruginosa* strain HF5 in the liquid media containing 100 mg L^{-1} of lead (Pb)

Dyes	Physicochemical characteristics				Color removal %		
	Molecular formula	Molecular weight	Color index no.	λ_{max}	24 h	48 h	96 h
Reactive Black-5	$C_{26}H_{21}N_5Na_4O_{19}S_6$	991.80	20,505	597	23.7 ± 3.8	67.4 ± 2.4	71.5 ± 4.8
Reactive Orange-16	$C_{20}H_{17}N_3Na_2O_{11}S_3$	617.54	17,757	494	81.9 ± 2.5	90.7 ± 3.4	92.4 ± 3.3
Reactive Red-120	$C_{44}C_{12}H_{24}N_{14}Na_6O_{20}S_6$	1469.98	292,775	535	92.1 ± 4.2	95.7 ± 3.3	96.6 ± 1.9
Reactive Yellow-2	$C_{25}H_{15}C_{l3}N_9Na_3O_{10}S_3$	872.96	18,972	404	45.9 ± 5.2	75.8 ± 2.1	83.6 ± 3.5
Direct red-28	$C_{32}H_{22}N_6Na_2O_6S_2$	969.66	22,120	497	19.7 ± 2.9	62.8 ± 7.0	94.5 ± 5.7

individually into MS broth media (1:10 ratio) added with Pb and RR120, The enrichment cultures along with their respective un-inoculated control were incubated under static condition at 30 °C in dark. After 72 h incubation, decolorization (%) was examined by comparing the absorbance of aliquots of decolorized media and controls after centrifugation (6000 rpm for 5 min) by UV-visible spectrophotometer (Shimadzu) at 540 nm (λ_{max}) using the following formula:

$$\text{Decolorization efficiency } (\%) = \frac{(I-F)}{I} \times 100$$

where I and F represent the absorbance of the MS broth media before (initial) and after (final) incubation, respectively. Once more than half of the initially added color was removed, the cultures from the first enrichment were added to next batch of fresh MS amended with RR120 and Pb in 1:10 ration and treated up to decolorization in the same way as mentioned before. After 4–5 of such cycles, 0.1 mL from each culture was inoculated on MS + Pb + RR120 agar plates and incubated in dark for 48 h at 30 °C. After the incubation, 82 fast-growing bacterial colonies with morphological differences were picked and repeatedly streaked on MS agar media plates for purification. The purified bacterial colonies were screened for their potential to decolorize RR120 in MS broth medium containing Pb. For screening, the purified 82 bacterial isolates were allowed to grow in nutrient broth added with 50 mg L^{-I} of Pb as Pb $(NO_3)_2$. The growth of each isolate was monitored by estimating their optical density (OD_{600}) and homogenized to 0.5 OD by adding the fresh medium. Two milliliters of each culture was inoculated separately in triplicates of 18 mL of freshly prepared MS broth media containing Pb (50 mg L^{-I}) and RR120 (50 mg L^{-I}) and incubated statically along with un-inoculated controls at 30 °C. After 48 h, aliquots from all cultures were centrifuged (6000 rpm for 5 min) and the cell free extracts were examined for RR120 decolorization. The isolates having the potential to decolorize RR120 (> 5.0%) were allowed to grow on NA plates added with varying concentrations (10 to 500 mg L^{-I}) of Pb and the minimum inhibitory concentration (MIC) values of Pb were estimated for the isolates. On the basis of these analyses, the isolate HF5 showing the maximum RR120 decolorization and a considerable Pb MIC value was chosen for the upcoming experiments. The isolate HF5 was again tested for decolorization of RR120 under the same conditions and the decolorization was confirmed by taking the UV-visible spectra (350–625 nm) of the culture media before and after the decolorization.

Amplification, sequencing, and analyses of 16S rRNA of HF5

In order to identify HF5, its 16S rRNA was amplified, sequenced, and analyzed through bioinformatics tools. 16S rRNA sequence was amplified, verified, and purified following the method as already described by Maqbool et al. (2016). Sequencing of 16SrRNA of HF5 was carried out by Macrogen (Seol, South Korea) and the sequence was deposited in Genbank database under accession number KF730788. Comparison of this sequence with other known sequences was carried out using the program BlastN (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This sequence was also analyzed through multiple alignments followed by the construction of a neighbor joining phylogenetic tree as already described by Maqbool et al. (2016).

Metal tolerance of HF5

The metal tolerance in terms of minimum inhibitory concentration (MIC) of the selected heavy metals (Cr, Co, Pb, Zn, Cd, Cu) for *Pseudomonas aeruginosa* strain HF5 was assessed in general purpose medium (GPM) plates by allowing it to grow in the presence of various concentrations of the individual heavy metal ions separately at 30 °C (Table S2). The growth of HF5 was examined and the concentration of the metal ion at which growth of HF5 was halted was considered as MIC of that metal.

Estimation of decolorization potential of HF5 under varying cultural and incubation conditions

Impact of Pb concentration on FH5 growth and RR120 decolorization by HF5

In order to evaluate the impact of varying levels of Pb on growth of HF5, this strain was allowed to grow separately

under shaking (150 rpm) in different nutrient broth media flasks amended with varying concentrations (0, 100, 200, 500 mg L^{-1}) of Pb. After 72 h incubation, the HF5 growth was assessed in terms of optical density (OD_{600}). Moreover, decolorization of RR120 (200 mg L^{-1}) by *Pseudomonas aeruginosa* strain HF5 was also evaluated in the presence of various concentrations (0–2000 mg L^{-1}) of Pb in MS broth media. HF5 cells grown in nutrient broth medium were centrifuged at 6000 rpm for 5 min, washed thrice with distilled water, and inoculated (to produce an initial OD_{600} of 0.5) in MS broth media amended with RR120 (200 mg L^{-1}) and varying levels of Pb. Triplicates of each culture along with triplicates of un-inoculated controls were incubated statically at 30 °C. After 48 h incubation, aliquots from all cultures were centrifuged at 6000 rpm for 5 min and cell free extracts were then used for estimating the RR120 decolorization as already described above. On the basis of this study, the media used in the rest of experiments were amended with 100 mg L^{-1} of Pb.

Effect of pH on RR120 decolorization by HF5

Potential of *Pseudomonas aeruginosa* strain HF5 to decolorize RR120 (200 mg L^{-1}) in MS broth media amended with Pb (100 mg L^{-1}) was also examined at five different levels of pH. The MS broth media containing RR120 and Pb were adjusted at various pH values (5.5, 6.5, 7.5, 8.5, and 9.5) using 0.05 M HCl and 0.05 M NaOH solutions. Triplicate sets of the media at each pH were inoculated (to develop an initial OD_{600} of 0.1) with pre-grown culture of HF5 and incubated statically at 30 °C. A triplicate set of un-inoculated media was also incubated as control along with these samples. At the end of this experiment, 0.1 mL of the diluted (up to 10^{-6}) cultures were plated separately on NA plates and incubated at 30 °C. The colony forming units (cfu) were counted for each plate.

Effect of NaCl concentrations on RR120 decolorization by HF5

Decolorization of RR120 (200 mg L^{-1}) by HF5 was also evaluated in the presence of different concentrations (0 g L^{-1}, 10 g L^{-1}, 20 g L^{-1}, 50 g L^{-1}, and 100 g L^{-1}) of NaCl in MS broth media added with 100 mg L^{-1} of Pb. Triplicates of tubes containing MS broth media with varying levels of NaCl were inoculated with HF5 (to develop an initial OD_{600} of 0.5) and incubated statically at 30 °C. Over the incubation period, aliquots from each culture were drawn and processed for estimating the decolorization of RR120 as described earlier.

Potential of HF5 for decolorization of various azo dyes

Pseudomonas aeruginosa strain HF5 was also tested for its potential of decolorizing various azo dyes including RY2, RB5, RR120, RO16, and DR28 in MS broth media containing 100 mg L^{-1} of Pb and amended with 200 mg L^{-1} of each selected azo dye separately. Triplicates of the MS broth media with various azo dyes were inoculated with strain HF5 (to develop an initial OD_{600} of 0.5) and incubated statically at 30 °C. Respective controls without inoculation were also incubated with the samples. Aliquots from triplicate samples of each dye were centrifuged (6000 rpm for 5 min) and evaluated for decolorization by taking the absorbance for each dye at their respective λ_{max} using a UV-visible spectrophotometer.

Statistical analyses

The acquired data was analyzed statically by using JMP8® (SAS Institute Inc., SAS Campus Drive, NC, USA). Wherever needed, analyses of variance (ANOVA) were carried out to statistically compare the obtained data.

Results

Isolation, screening, and identification of HF5

While isolating the strain HF5, total 82 purified bacterial colonies were screened for RR120 decolorization. Out of the 82 isolates, only 30 isolates showed the potential to decolorize (> 5%) RR120 in the media containing 50 mg L^{-1} of Pb (Fig. 1a). A huge variation in the decolorization of RR120 by different isolates was observed in this study. Over 48 h incubation, average decolorization of RR120 by all the isolates was 40.1% with the isolate HF18 showing the lowest decolorization (5.1 ± 1.3%) and the isolate HF5 showing the highest decolorization (93.5 ± 1.9%). Like RR120 decolorization, the MIC values of Pb for the same 30 bacterial isolates were also found to be variable (Fig. 1b). Most of the isolates were observed to tolerate Pb up to 200 mg L^{-1} concentration. The highest value of Pb MIC was observed for the isolates HF5 and HF29. On the basis of RR120 decolorization and Pb tolerance, the isolate HF5 was selected for further studies. 16S rRNA gene sequence of HF5 was deposited in the NCBI GeneBank (GeneBank Ac. No. KF730788). On the basis of its identity (> 99%) through BlastN analyses, the strain HF5 was found belonging to genus *Pseudomonas aeruginosa*. Moreover, affiliation of this strain with *Pseudomonas aeruginosa* was further strengthened on the basis of its grouping in phylogenetic tree with the bacteria of genus *Pseudomonas* having closest grouping with *Pseudomonas aeruginosa* strain BCH (Genbank Ac. No. FJ496659) (Fig. 2). On the basis of these analyses, the isolate HF5 was named as *Pseudomonas aeruginosa* strain HF5.

Tolerance of HF5 against Pb and other metals

Estimation of MIC of different heavy metal ions against the strain HF5 indicated that this strain had the potential to tolerate

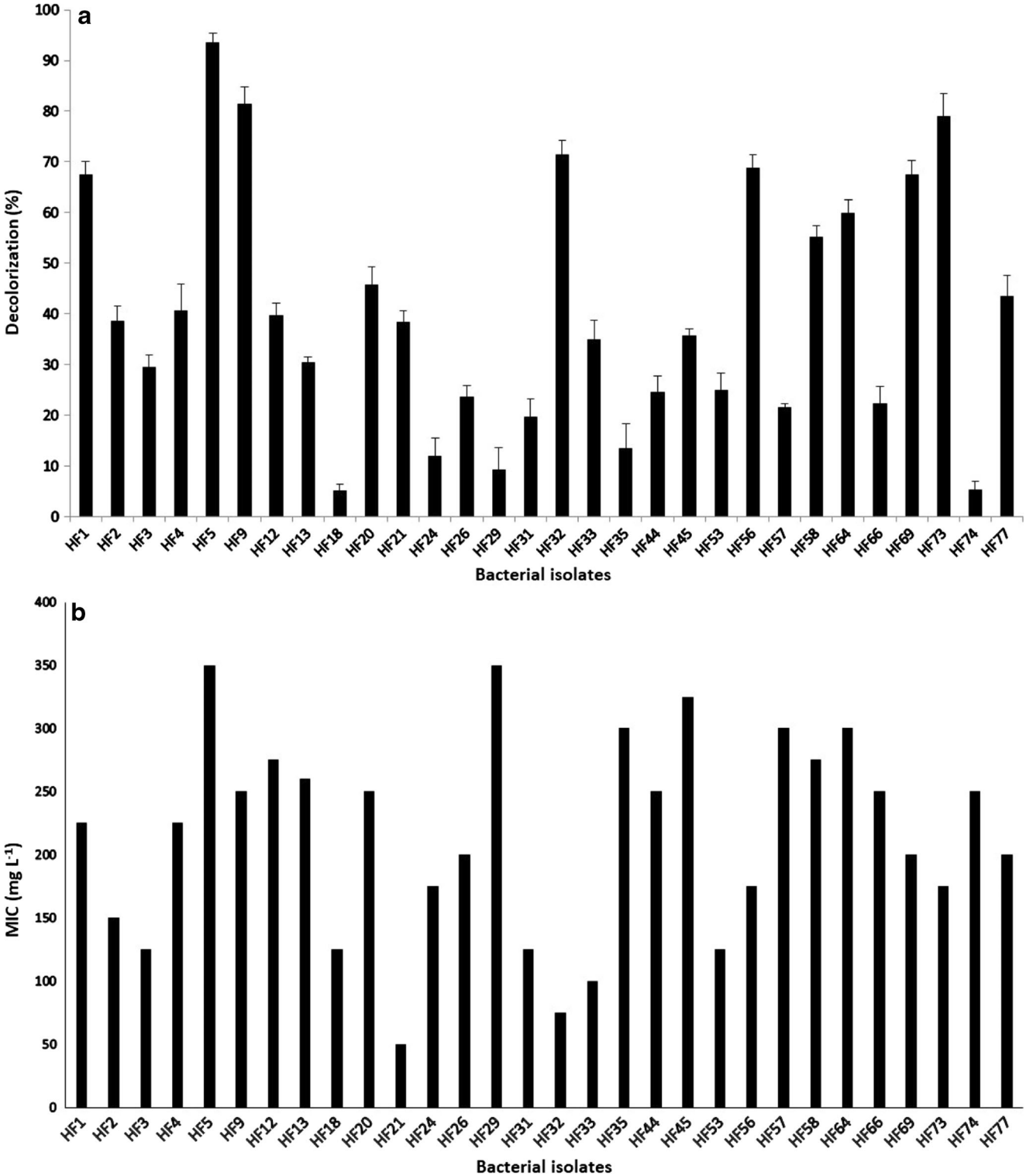

Fig. 1 **a** Decolorization of reactive red 120 by the selected bacterial isolates in the presence of 50 mg L^{-1} of lead (Pb) after 48 h, **b** minimum inhibitory concentration (MIC) of Pb for the selected bacterial isolates

variable levels of different metal ions (Table S2). The MIC values of strain HF5 for the different metals including cobalt, chromium, zinc, lead, copper, and cadmium were recorded as 10 mM (~ 590 mg L^{-1}), 0.5 mM (~ 26 mg L^{-1}), 20.0 mM (~ 1310 mg L^{-1}), 2.75 mM (~ 570 mg L^{-1}), 3.5 mM (~ 222 mg L^{-1}), and 7.5 mM (~ 843 mg L^{-1}), respectively. The resistance of HF5 against the presence of Pb was also estimated by allowing this strain to grow in the presence of different concentrations of Pb. The strain HF5 exhibited a good growth in nutrient broth medium containing 100 mg L^{-1} of Pb (Fig. 3).

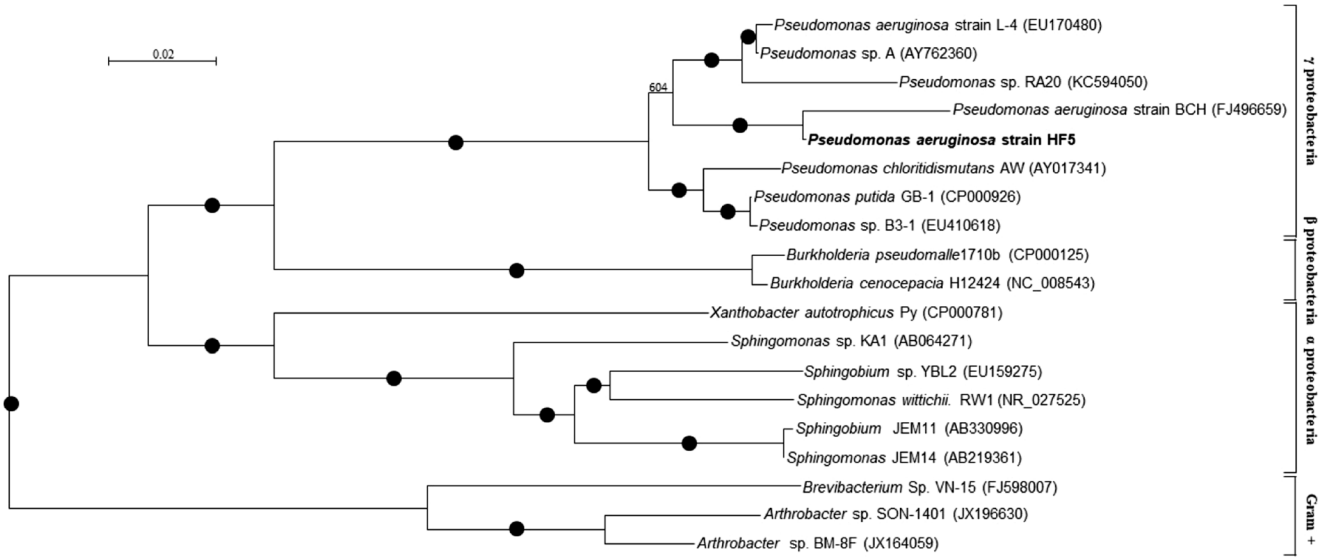

Fig. 2 Neighbor-joining phylogenetic analysis resulting from the multiple alignment of 16S rRNA gene sequence of *Pseudomonas aeruginosa* strain HF5 with those of other bacterial strains found in the GenBank database. The accession numbers of the strains from the GenBank database, used for phylogenetic analysis, are given in brackets. Bootstrap values greater than 800‰ are marked as black circles and the phylogenetic distance is shown on a scale bar

Upon 500 mg L^{-1} of Pb treatment to nutrient broth medium, the growth was found to be significantly reduced which was later reached to the minimum with the 500 mg L^{-1} of Pb.

Characterization of the decolorizing capabilities of HF5

While studying the decolorization of RR120 by HF5 in the media amended with different concentrations of Pb, it was observed that HF5 showed almost complete (> 95%) and statistically at par decolorization of RR120 in the media added with 0, 50, and 100 mg L^{-1} of Pb (Fig. 4). However, when the concentration of Pb was increased up to 500, 1000, and 2000 mg L^{-1}, it resulted into a significant reduction in RR120 decolorization at each level with 88.2, 74.3, and 57.5% decolorization of RR120, respectively.

Under the conditions set in this study, the strain HF5 carried out the maximum decolorization in the media whose pH was adjusted at 7.5 and 8.5, respectively (Fig. 5a). Over 24 h incubation, this strain decolorized 90.9% and 82.5% of the initially added RR120 at pH values of 7.5 and 8.5, respectively. However, over the same incubation period, only 34.8, 43.3, and 43.5% decolorization was observed at pH 5.5, 6.5, and 9.5, respectively. Similarly, over 48 h incubation, this strain decolorized 92.8% and 90.6% of the initially added RR120 at pH values of 7.5 and 8.5, respectively. However, over this incubation period, 70.3, 74.2, and 76.2% of the initially added RR120 decolorized at pH 5.5, 6.5, and 9.5, respectively.

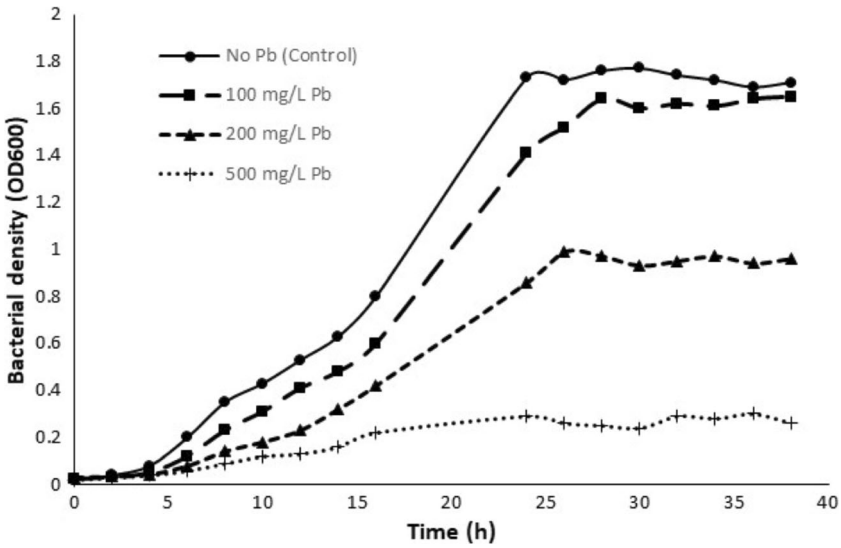

Fig. 3 Growth of *Pseudomonas aeruginosa* strain HF5 in nutrient broth medium in the presence of different concentrations of lead (Pb). *Filled circles*: no Pb (control), *filled squares*: 100 mg L^{-1} Pb, *filled triangles*: 200 mg L^{-1} Pb, *times*: 500 mg L^{-1} Pb

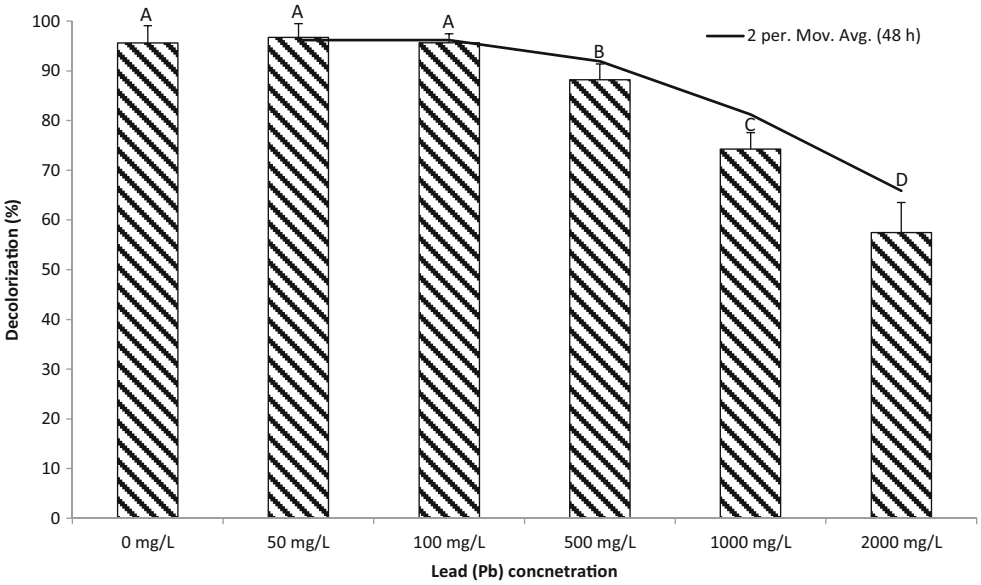

Fig. 4 Decolorization of reactive red 120 by *Pseudomonas aeruginosa* strain HF5 in the presence of different lead (Pb) concentrations after 48 h. The different letters on the bars (A, B, C, and D) represent the significant difference between the treatment means

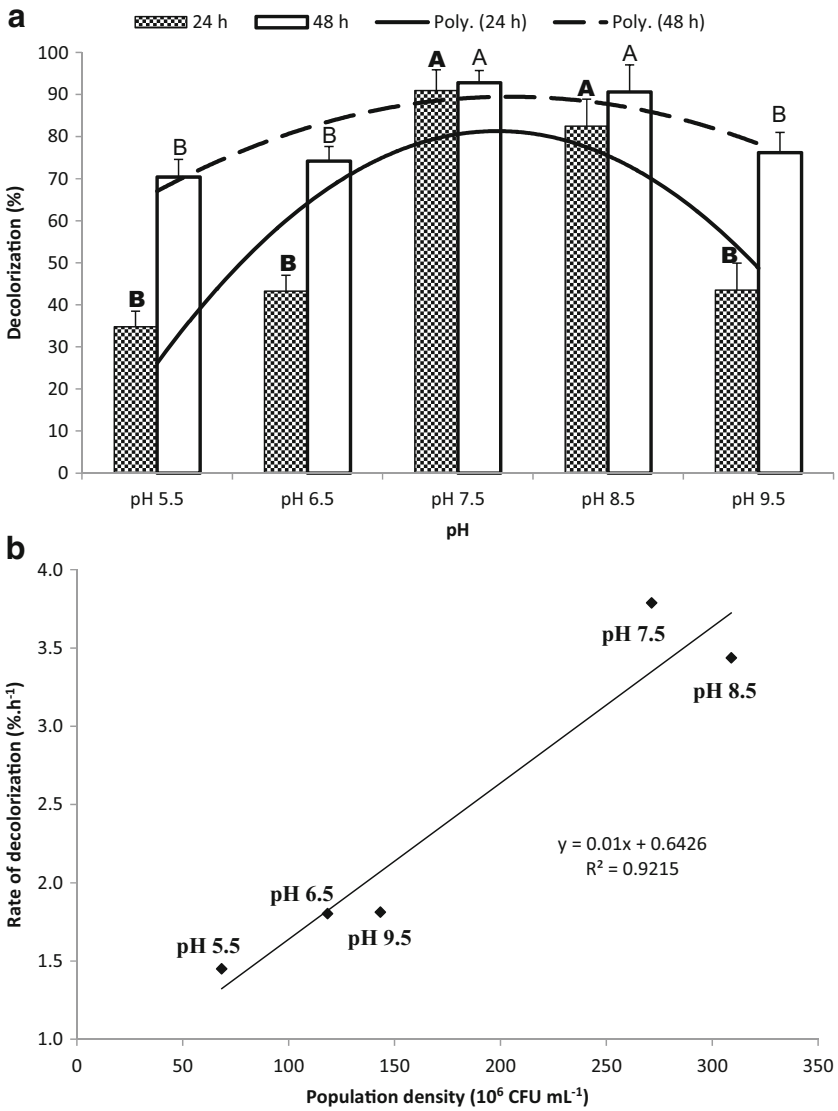

Fig. 5 **a** Effect of pH on the decolorization of reactive red 120 by *Pseudomonas aeruginosa* strain HF5 in liquid culture containing 100 mg L^{-1} of lead (Pb). Error bars indicate standard error ($n = 3$) while the letters indicate the statistical significance. **b** Correlation between the rate of decolorization (% h^{-1}) and the number of *Pseudomonas aeruginosa* strain HF5 colonies enumerated on nutrient agar medium (CFU mL^{-1}) after their incubation at different pH values. Poly. (24 h) and Poly. (48 h) represent the polynomial curves at 24 and 48 h indicating the trend of decolorization at varying pH values. The different letters on the bars (A and B) represent the significant difference between the treatment means

Similar to the decolorization (%), the cfu values for HF5 were similarly higher in the media at pH values of 7.5 and 8.5 as compared to that of in the media at pH values 5.5, 6.5, and 9.5. At varying pH values, the rate of decolorization (% h^{-1}) was found to be significantly correlated (R^2 of 0.9215) with the growth (cfu) of HF5 (Fig. 5b).

While evaluating decolorization of RR120 in the presence of different levels of NaCl (0 to 100 g L^{-1}) in MS broth media added with Pb (100 mg L^{-1}), it was interestingly found that this strain almost completely (> 95%) decolorized RR120 in the media having 0, 10, and 20 g L^{-1} of NaCl (Fig. 6). Nevertheless, 77.9% and 27.5% of the added RR120 was found to undergo decolorization when the media were added with 50 and 100 g L^{-1} of NaCl, respectively.

Besides decolorizing the RR120, the ability of *Pseudomonas aeruginosa* strain HF5 to decolorize various structurally related azo dyes was also investigated. Results suggested that *Pseudomonas aeruginosa* strain HF5 effectively decolorized all the mentioned reactive dyes and direct dye (Table 1). The decolorization of DR28, RB5, RY2, RO16, and RR120 by the strain HF5 was recorded to be 19.7 ± 2.9%, 23.7 ± 3.8%, 45.9 ± 5.2%, 81.9 ± 2.5%, and 92.1 ± 4.2%, respectively, over 24 h incubation. However, over 48- and 96-h incubation, the most of all the dyes was decolorized by HF5.

Discussion

Presence of metal ions including lead (Pb) along with the azo dyes in textile wastewaters has a negative impact on living organisms including the microbial populations involved in decolorization of dyes and create problems while devising the strategies for bioremediation of such textile wastewaters. This article reports the isolation and characterization of a Pb tolerant *Pseudomonas aeruginosa* strain HF5 for decolorization of different azo dyes.

In this study, 30 out of the total 82 purified bacterial colonies isolated from textile effluents were found to harbor highly variable (from 5.1 to 93.5%) potential to decolorize RR120 in the presence of 50 mg L^{-1} of Pb. Moreover, on the basis of MIC values of Pb, these 30 isolates also showed enormous variability in tolerance against the presence of Pb. This finding indicates that different bacterial isolates have a varying potential to resist the presence of Pb as well as a differential potential or adaptability for decolorization of RR120 dye in the media containing Pb. Such variation in decolorization activity by different microbial strains has also been reported by several researchers working on microbial decolorization of various dyes (Pandey et al. 2007; Lin et al. 2010; Hussain et al. 2013; Anwar et al. 2014; Maqbool et al. 2016). Among the various studied bacteria, HF5 strain showed a great potential to grow and decolorizing the RR120 in media having Pb. This decolorizing ability was further tested and verified on UV-visible spectrophotometer (350–650 nm) by analyzing the media before and after RR120 decolorization (Fig. S1). On the basis of its 16S rRNA sequence, it was designated as *Pseudomonas aeruginosa* strain HF5. Few *Pseudomonas aeruginosa* strains having the ability to decolorize different azo dyes are previously reported and their decolorizing potential has also been described (Kalme et al. 2007; Joe et al. 2011; Maqbool et al. 2016). In contrast, the strain HF5 has shown the ability to grow and decolorize the azo dyes even in the presence of considerable concentrations of Pb. In addition to Pb, this strain also showed a good tolerance in terms of MIC towards the presence of few other metal ions including Co, Cr, Zn, Cu, and Cd in the medium which shows a good potential of this strain to resist the metal ions which are extensively found in textile wastewaters. From this information, it can be inferred that this strain can be exploited for removal of dyes even in the wastewaters loaded with heavy metal ions. Such metal tolerance has also been previously reported in few bacteria having the capability of decolorizing different dyes (Hussain et al. 2013; Abbas et al. 2016; Maqbool et al. 2016).

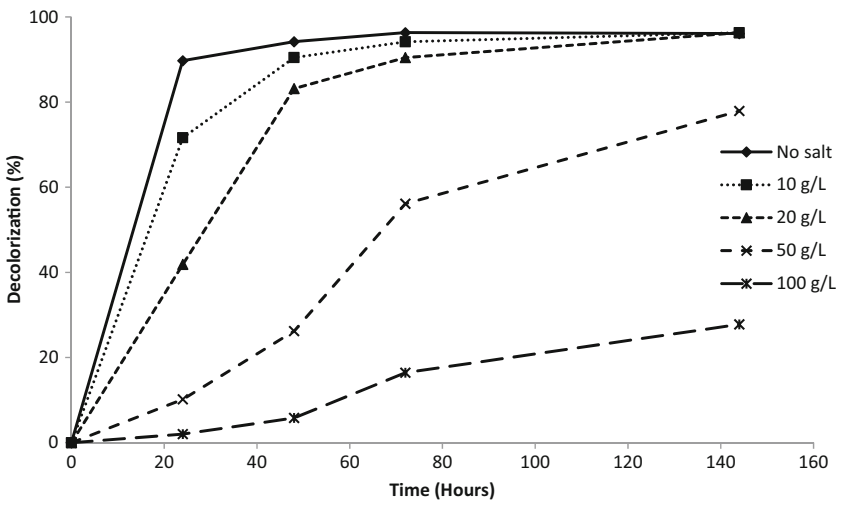

Fig. 6 Effect of different salt concentrations on decolorization of reactive red 120 by *Pseudomonas aeruginosa* strain HF5 in the liquid media containing 100 mg L^{-1} of lead (Pb)

In this study, higher concentrations (500, 1000, and 2000 mg L^{-1}) of Pb resulted into a significant reduction in RR120 decolorization while the lower concentrations (50 and 100 mg L^{-1}) did not have any impact on RR120 decolorization by the strain HF5. This suggests that the activity of this strain is not affected at lower concentrations of Pb but affected at higher concentrations. It is also supported by few previous findings which reported that the microbes can resist the presence of Pb only up to a certain limit and, when the concentration exceeds this limit, the Pb become toxic for the same microbes and affects their growth and activity (Aktan et al. 2013; Naik et al. 2012). It is also noteworthy that this strain carried out a significant decolorization of RR120 also in the presence of 2000 mg L^{-1} of Pb which indicates for its unique decolorizing capabilities.

Decolorizing activity of *Pseudomonas aeruginosa* strain HF5 monitored at varying pH of the media indicated that a pH from neutral to somewhat alkaline is optimal for achieving optimal decolorization of RR120 in the presence of 100 mg L^{-1} of Pb. This optimal pH for RR120 decolorization by HF5 is not only relevant with the pH value of the environmental samples from which it was isolated but also in line with several previous findings focused on studying the impacts of pH on bacterial decolorization of different dyes (Suzuki et al. 2001; Kalme et al. 2007; Yan et al. 2012; Hussain et al. 2013; Anwar et al. 2014). Correlation of cfu with decolorization at varying pH values indicates that pH may have influenced the growth of the bacterial strain HF5 which ultimately might have affected the decolorization. However, the detail of the effect of pH is still to be explored.

As the salt is also one of the main constituents found in textile wastewater, therefore, there is need to see if the dye decolorizing strain would be able to resist the presence of these salts. In this study, *Pseudomonas aeruginosa* strain HF5 was evaluated for decolorization of RR120 in MS having 100 mg L^{-1} of Pb and different levels of NaCl salt stress. The presence of salt up to 20 g L^{-1} did not have any significant impact on decolorization. Nevertheless, a considerable decolorization was observed even in the media added with higher levels of NaCl salt. This finding points out that HF5 would be able to resist the existence of salts in the wastewater while carrying out decolorization activity. This potential is better than several other bacterial strains which can decolorize the dyes in the media added with NaCl salt. For example, *Pseudomonas* sp. RA20 could decolorize only about 25% of the initially added RB5 in the presence of 50 g L^{-1} of NaCl (Hussain et al. 2013) which is much lower as compared to 77.9% of RR120 decolorization by HF5 even in the presence of 100 mg L^{-1} of Pb. Relatively low RR120 decolorization at higher NaCl concentrations might be due to the excess of Na^+ inflow that finally ruptures the microbial cell membrane as stated earlier by (Peyton et al. 2002; Tan et al. 2009; Zilly et al. 2011; Khalid et al. 2012; Mahmood et al. 2012; Anwar et al. 2014). The strain HF5 was also found to decolorize different reactive and direct azo dyes but with varying extents which shows the adaptability of the strain for variety of dyes. It also shows that this strain can be exploited for decolorization of different azo dyes.

On the basis of findings of this study, it can be inferred that the lead (Pb) resistant *Pseudomonas aeruginosa* strain HF5 might serve as a new potential bioresource which can be exploited for devising the strategies for bioremediation of different azo dyes from the textile wastewaters also contaminated with Pb and other metal ions.

Funding This research work was funded by Government College University, Faisalabad, from its own resources under a GCUF2012 grant.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals A statement for research involving human participants and/or animals is not applicable for this study.

Informed consent A statement regarding informed consent is not applicable for this study.

References

Abbas N, Hussain S, Azeem F, Shahzad T, Bhatti SH, Imran M, Ahmad Z, Maqbool Z, Abid M (2016) Characterization of a salt resistant bacterial strain *Proteus* sp. NA6 capable of decolorizing reactive dyes in presence of multi-metal stress. World J Microbiol Biotechnol 32(11):181. https://doi.org/10.1007/s11274-016-2141-1.

Aktan Y, Tan S, Icgen B (2013) Characterization of lead-resistant river isolate *Enterococcus faecalis* and assessment of its multiple metal and antibiotic resistance. Environ Monit Assess 185(6):5285–5293

Anwar F, Hussain S, Ramzan S, Hafeez F, Arshad M, Imran M, Maqbool Z, Abbas N (2014) Characterization of Reactive Red-120 decolorizing bacterial strain *Acinetobacter junii* FA10 capable of simultaneous removal of azo dyes and hexavalent chromium. Water Air Soil Pollut 225(8):1–16

Bafana A, Krishnamurthi K, Devi SS, Chakrabarti T (2009) Biological decolourization of C.I. direct black 38 by *E. gallinarum*. J Hazard Mater 157:187–193

Bayoumi RA, Musa SM, Bahobil AS, Louboudy SS, El-Sakawey TA (2010) Biodecolorization and biodegradation of azo dyes by some bacterial isolates. J Appl Environ Biol Sci 1:1–25

Blagodatskaya EV, Pampura TV, Dem'yanova EG, Myakshina TN (2006) Effect of lead on growth characteristic of microorganisms in soil and rhizosphere of *Dactylis gloerata*. Eur Soil Sci 39(6):653–660

Chacko JT, Subramaniam K (2011) Enzymatic degradation of azo dyes - a review. Int J Environ Sci 1(6):1250–1260

Chequer FM, Angeli JP, Ferraz ER, Tsuboy MS, Marcarini JC, Mantovani MS et al (2009) The azo dyes Disperse Red 1 and Disperse Orange 1 increase the micronuclei frequencies in human lymphocytes and in HepG2 cells. Mutat Res 676(1):83–86

Das M, Ahmed K, Islam S, Islam M, Akter MS (2011) Heavy metals in industrial effluents (tannery and textile) and adjacent rivers of Dhaka city, Bangladesh. Terre Aquat Environ Toxicol 5(1):8–13

Ghodake GS, Kalme SD, Jadhave JP, Govindwar SP (2009) Purification and partial characterization of lignin peroxidase from *Acinetobacter*

calcoaceticus NCIM 2890 an dits application in decolorization of textile dyes. Appl Biochem Biotechnol 152:6–14

Gupta VK, Khamparia S, Tyagi I, Jaspal D, Malviya A (2015) Decolorization of mixture of dyes: a critical review. Glob J Environ Sci Manag 1(1):71–94

Halimoon N, Yin RGS (2010) Removal of heavy metals from textile wastewater using zeolite. Environ Asia 3:124–130

Hussain S, Maqbool Z, Ali S, Yasmeen T, Imran M, Mahmood F, Abbas F (2013) Biodecolorization of reactive black-5 by a metal and salt tolerant bacterial strain *Pseudomonas* sp. RA20 isolated from Paharang drain effluents in Pakistan. Ecotoxicol Environ Saf 98:331–338

Hussain S, Quinn L, Li J, Casey E, Murphy CD (2017) Simultaneous removal of malachite green and hexavalent chromium by *Cunninghamella elegans* biofilm in a semi-continuous system. Int Biodeterior Biodegrad 125:142–149

Imran M, Crowley DE, Khalid A, Hussain S, Mumtaz MW, Arshad M (2015a) Microbial biotechnology for decolorization of textile wastewater. Rev Environ Sci Biotechnol 14(1):73–92

Imran M, Hussain S, Mumtaz MW, Crowley DE (2015b) Decolorization of reactive black-5 by *Shewanella* sp. In the presence of metal ions and salts. Water Environ Res 87:579–586

Imran M, Shahroona B, Crowley DE, Khalid A, Hussain S, Arshad M (2015c) The stability of textile azodyes in soil and their impact on microbial phospholipid fatty acid profiles. Ecotoxicol Environ Saf 120:163–168

Joe J, Kothari RK, Raval CM, Kothari CR, Akbari VG, Singh SP (2011) Decolorization of textile dye Remazol Black B by *Pseudomonas aeruginosa* CR-25 isolated from the common effluent treatment plant. J Bioremed Biodegr 2(2):2155–6199

Kalme S, Ghodake G, Gowindwar S (2007) Red HE7B degradation using desulfonation by *Pseudomonas desmolyticum* NCIM 2112. Int Biodeterior Biodegrad 60(4):327–333

Khalid A, Kausar F, Arshad M, Mahmood T, Ahmed I (2012) Accelerated decolorization of reactive azo dyes under saline conditions by bacteria isolated from Arabian seawater sediment. Appl Microbiol Biotechnol 96(6):1599–1606

Khan S, Malik A (2014) Environmental and health effects of textile industry wastewater. In: Malik A, Grohmann E, Akhtar R (eds) Environmental deterioration and human health. Springer, Dordrecht

Lin J, Zhang X, Li Z, Lei L (2010) Biodegradation of reactive blue 13 in a two-stage anaerobic/aerobic fluidized beds system with a *Pseudomonas* sp. isolate. Bioresour Technol 101(1):34–40

Loganathan K, Ali KS, Purushothaman M, Silambarasan S, Jamal ANA (2015) Synthesis and characterisation of 2-(substituted Phenyl)azo-4, 6-dipropionylresorcinol derivatives. World J Pharm Res 4:1694–1701

Ma L, Zhuo R, Liu H, Yu D, Jiang M, Zhang X, Yang Y (2014) Efficient decolorization and detoxification of the sulfonated azo dye reactive Orange 16 and simulated textile wastewater containing Reactive Orange 16 by the white-rot fungus Ganoderma sp. En3 isolated from the forest of Tzu-Chin Mountain in China. Biochem Eng J 82(15):1–9

Mahmood S, Khalid A, Mahmood T, Arshad M (2012) Potential of newly isolated bacterial strains for simultaneous removal of hexavalent chromium and reactive black-5 azodye from tannery effluent. J Chem Technol Biotechnol 88(8):1506–1513

Mahmood F, Shahid M, Hussain S, Shahzad T, Tahir M, Ijaz M, Hussain A, Mahmood K, Imran M, Babar SAK (2017) Potential plant growth-promoting strain *Bacillus* sp. SR-2-1/1 decolorized azo dyes through NADH-ubiquinone:oxidoreductase activity. Bioresour Technol 235:176–184

Maqbool Z, Hussain S, Ahmed T, Nadeem H, Imran M, Khalid A, Abid M, Martin-Laurent F (2016) Use of RSM modeling for optimizing decolorization of stimulated textile wastewater by *Pseudomonas aeruginosa* strain ZM130 capable of simultaneous removal of reactive dyes and hexavalent chromium. Environ Sci Pollut Res 23: 11224–11239

Naik MM, Pandey A, Dubey SK (2012) Biological characterization of lead-enhanced exopolysaccharide produced by a lead resistant *Enterobacter cloacae* strain P2B. Biodegrad 23(5):775–783

Najme R, Hussain S, Maqbool Z, Imran M, Mahmood F, Manzoor H, Yasmeen T, Shehzad T (2015) Biodecolorization of reactive yellow-2 by *Serratia sp.* RN34 isolated from textile wastewater. Water Environ Res 87(12):2065–2075

O'Neill C, Hawkes FR, Esteves SRR, Hawkes DL, Wilcox SJ (1999) Anaerobic and aerobic treatment of a simulated textile effluent. J Chem Technol Biotechnol 74:993–999

Pandey A, Poonam S, Leela I (2007) Bacterial decolorization and degradation of azo dyes. Int Biodeterior Biodegrad 59(2):73–84

Peyton BM, Wilson T, Yonge DR (2002) Kinetics of phenol biodegradation in high salt solutions. Water Res 36(19):4811–4820

Phugare SS, Kalyani DC, Surwase SN, Jadhav JA (2011) Ecofriendly degradation and detoxification of textile effluent by a developed bacterial consortium. Ecotoxicol Environ Saf 74:1288–1296

Pierce J (1994) Colour in textile effluents—the origins of the problem. J Soc Dye Colour 110:131–133

Sharma P, Dubey RS (2005) Lead toxicity in plants. Braz J Plant Phsysiol 17(1):35–52

Singh N, Gad R (2012) Studies on biosorption of Pb (II) by the nonliving biomasses of *Pseudomonas oleovorans* and *Brevundimonas vesicularis* and its removal from wastewater samples. Eur J Sci Res 69(2):279–289

Sparks DL (2005) Toxic metals in the environment: the role of surfaces. Elements 1(4):193–197

Stolz A (2001) Basic and applied aspects in the microbial degradation of azo dyes. Appl Microbiol Biotechnol 56(1):69–80

Suzuki T, Timofei S, Kurunczi L, Dietz U, Schuurmann G (2001) Correlation of aerobic biodegradability of sulfonated azo dyes with the chemical structure. Chemosphere 45(1):1–9

Tan L, Qu Y, Zhou J, Ma F, Li A (2009) Dynamics of microbial community for X-3B wastewater decolorization coping with high-salt and metal ions conditions. Bioresour Technol 100(12):3003–3009

Tony BD, Goyal D, Khanna S (2009) Decolorization of textile azo dyes by aerobic bacterial consortium. Int Biodeterior Biodegrad 63(4): 462–469

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(6):806–814

Zilly A, da Silva CMJ, Bracht A, de Souza MCG, Carvajal AE, Koehnlein EA, Peralta RM (2011) Influence of NaCl and Na_2SO_4on the kinetics and dye decolorization ability of crude laccase from *Ganoderma lucidum*. Int Biodeterior Biodegrad 65(2):340–344