The Rhizospheric Soil of *Sparganium erectum* L. Plant: A new Source of Efficient Bacteria for Azo Dye Decolorization

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Article Info	ABSTRACT
	The purpose of our study was to identify the native bacteria with the ability to degrade azo dyes from the rhizosphere of *Sparganium erectum* L., and *Typha latifolia* L. plants that were grown on a drain of a textile mill. Eight and one strain with decolorization ability of Cibacron Brilliant Red EB and Terasil Red 3BL-01 were isolated from the saline rhizosphere of *Sparganium erectum* L. and *latifolia* L. plant respectively. Results showed that the bacteria isolated from the rhizosphere of *Sparganium erectum* L. are more capable of decolorizing azo dyes. Based on the 16S rRNA sequencing, selected strains were identified as follows: *Enterobacter ludwigii* strain SNP3 (OL719291), *Rhodococcus fascians* strain SNP5 (OL759129), *Pseudomonas aeruginosa* strain SNP10 (OL759126), and *Bacillus safensis* strain SNP13 (OL759127). The results of azo dyes biodegradation tests revealed that strains SNP10, SNP3, and SNP5 were more capable of decolorizing 94-97%, 72.53-73.8, 72.53%, and 71.13-73.5% of Cibacron Brilliant Red EB at concentration 10-20 mg/L within 72 h, respectively. Besides, strain SNP13 was the fastest strain in decolorization of Cibacron Brilliant Red EB with 68% and 59% decolorization activity at 10 and 20 mg/L respectively (24 h). Only strains SNP3 and SNP13 could decolorize 83% and 77% of Terasil Red 3BL-01 (30 mg/L), respectively. For the first time, our research findings illustrated that indigenous rhizospheric bacterial strains isolated from *Sparganium erectum* L. plants have the potential to apply as an azo dye breakdown tool in textile effluent treatment or other ecosystems.

INTRODUCTION

Textile industry wastewater is considered one of the serious pollution threats to global resources and human food security (Holkar et al., 2016; Lelis et al., 2019; Afrin et al., 2021). The worldwide production of synthetic colorants in the textile industry consumption is around 7×10^7 tons annually, of which about 60-70% are Azo dyes which are considered the most prominent group in applied dyes (Ong et al., 2010; Gomaa, 2016, Tohamy et al., 2020). Azo dyes are aromatic compounds with one or more -N=N- groups, which are classified as xenobiotic and hardly degradable contaminants (Pourbabaee et al., 2006; Singh et al., 2015; Orts et al., 2018; Mani et al., 2019). Nearly, 10,000 textile dyes are used globally, and their annual production is tantamount to 7×10^5 metric tons (Baena-Baldiris et al., 2020), in which approximately, 10-15% of azo dyes release directly into the wastewater during the manufacture and dyeing process (Gomaa,

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2016; Afrin et al., 2021), that leads to many environmental problems such as reduction dissolved oxygen levels, and the rate of photosynthesis (Imran et al., 2015; Hassan and Carr, 2018, Sarkar et al., 2021). Azo dyes can enter the food chain (Newman, 2015) which causes carcinogenic, mutagenic, and toxic effects on the human body and other organisms (Ito et al., 2016; Khan and Malik, 2018; Khatri et al., 2018). Bioremediation is the key and idealistic way to eliminate these pollutants from the ecosphere and is more economical, safe, and eco-friendly compared to existing physical and chemical methods due to its higher efficiency, less sludge production, and fewer secondary pollution creation (Hayat et al., 2015; Holkar et al., 2016; Gomaa, 2016). Biodegradation of azo dyes in aerobic and anaerobic conditions by a microbial consortium or a bacterium has been confirmed by researchers (Vijayalakshmidevi & Muthukumar, 2015; Chandra, 2016; Miran et al., 2018). *Pseudomonas aeruginosa* (Nachiyar and Rajkumar, 2003), *Shewanella putrefaciens*, *Bacillus cereus* (Pourbabaee et al., 2005), *Aeromonas punctate* (Khalid et al., 2008), *Acinetobacter* sp. SW30 (Wadhwani et al., 2018) and *Bacillus subtilis* (Barathi et al., 2020) have been reported as decolorizer bacteria. Bacteria biodegrade azo bands (-N≡N-) by producing the enzymes azoreductase (Pourbabaee et al., 2005; Saratale et al., 2011), peroxidase, laccase, tyrosinase, NADH-DCIP reductase, and MG reductase (Telke et al., 2015), and use azo dyes as a source of nitrogen and carbon (Roy et al., 2018).

Asad et al. (2007) isolated halophilic and halotolerant bacteria that belonged to the genus *Halomonas* with the capability to decolorize azo dyes in a broad range of temperature (25–40 °C), pH (5–11), and NaCl concentration (up to 20% w/v), after 4 days of incubation in static conditions. Pourbabaee et al. (2013) reported *that Klebsiella Terrigenaptcc* 1650 could entirely degrade 25 mg/L of Malachite Green in optimum conditions (30 ^{0}C, pH 6, Lactose and Ammonium nitrate as carbon and nitrogen sources respectively). Gomaa (2016) isolated *Bacillus subtilis*, *Bacillus cereus*, *Bacillus licheniformis*, and *Pseudomonas* sp, from the soil with the ability to decolorization of black B and Congo Red Shafqat et al (2017) isolated 138 bacterial strains from the soil rhizosphere with 62 to 100% dye decolorization activity which 57% of them were able to decolorize the reactive black-5 dye within 48 h. *Aeromonas hydrophila* could degrade Crystal Violet dye in the presence of sucrose and yeast extract (Bharagava et al., 2018). In another study, a *Franconibacter* sp. strain 1MS with the capability to degrade Ponceau S Red and Methyl Orange in optimum conditions (37 °C, and pH 7, static) at 100-1000 mg/L, was isolated from sediment samples in the gold mining district of San Martin de Loba, Colombia (Baena-Baldiris et al., 2020). Afrin et al. (2021) isolated a bacterial consortium (*Enterococcus faecium*, *Bacillus pumilus/ safensis*, B. *thuringiensis*, *Pseudomonas aeruginosa* (Lab1), and *Pseudomonas aeruginosa* (Lab2)) from the effluents of textile industries with the ability to degrade Novacron dyes. Novacron dyes biodegradation tests showed that the consortium began decolorization at 18 h and displayed the maximum degradation after 72 h, whereas the strains started decolorization at 24 h under optimum (pH 7.0, temperature 37 ^{0}C, 10 % inoculums, and 100 mg/L dye) and static conditions. The results revealed that all bacterial strains could separately degrade all five dyes: Novacron Black Novacron Navy, Novacron Yellow, Novacron Ruby, and Novacron Blue dk. Between all five strains, *E. faecium* and two strains of *P. aeruginosa* exhibited a superior efficacy in the breakdown of mixed plus single dye. Sarkar et al. (2021) reported that *Chryseobacterium geocarposphaerae* DD3 which was isolated from textile industry dye effluent in West Bengal, India, could decolorate 0.2 g/l Congo Red within 12 h in the presence of g/L glucose as the co-substrate (37 ^{0}C, 120 rpm).

In Iran, the textile industry's contribution among total industries to water pollution was about 12.5% in 2003 and rising continuously (Valizadeh and Parvin, 2014). The entry of these

pollutants into water sources without any treatment is a serious crisis for the environment. Iran Poplin textile mill located in the Guilan province, north of Iran is one of the industries that has added its wastewater without purification to the rivers. Then, this wastewater is transported through rivers to the Anzali International Wetland and to the Caspian Sea, causing many environmental problems. This indicates that textile dye removal is of great importance in that ecosystem (Saeedi and Jamshidi-Zanjani, 2015; Pirsaheb et al., 2015). Anzali Lagoon was registered as an international wetland under Ramsar Convention (Ramsar site #40, wetlands international Site Reference No.: 2IR005) in 1975 (Mousazadeh et al., 2015; Shariati et al., 2021). This international wetland is of great importance in the world ecosystem due to its diverse species of animals, plants, and fish, and as a natural habitat for breeding, staging, and wintering waterbirds according to the International BirdLife Organization (Kazemi et al., 2012). Furthermore, the birds and fish, and other organisms of this wetland are used as human food. Many reports indicate the crucial situation of the Anzli wetland from the point of pollution (Akbarzadeh et al., 2008; Mirzajani et al., 2010, Pirsaheb et al., 2015). Isolation of bacteria from the rhizosphere of the plants that grow in contaminated sites takes into consideration in research due to the high number of metabolically active microorganisms in that zone. Releasing some compounds including simple sugars, amino acids, enzymes, aliphatic, and aromatics by plant roots encourages the growth of specific microbial communities (Lelis et al., 2019; Yue et al., 2020). Therefore, in our study, regarding the extensive growth of two aquatic plants *Sparganium erectum* L. and *Typha latifolia* L. in the drain of the Iran Poplin textile mill, the purpose of this study was to assess the potential of these plants' rhizospheric bacteria to decolorize the Cibacron Brilliant Red EB and Terasil Red 3BL-01 which are widely used in Iran Poplin textile mill.

MATERIALS AND METHODS

Cibacron Brilliant Red EB and Terasil Red 3BL-01 were produced from the Iran Poplin textile mill with commercial grade. All chemicals used in the culture medium and biodegradation experiments were bought from Merck Company (Darmstadt, Germany).

The Iran Poplin textile industry is located in Rasht, Guilan Province, northern Iran (Longitude=37º12'30", Latitude=49º38'31"). Soil drain of industry and centers adjacent to this textile factory have a variety of plants grown in aquatic environments. Vegetation on the inner surface of the soil drain of the Iran Poplin factory is mainly inclusive of *Sparganium erectum* L. and *Typha latifolia* L. while the outer surface of this drainage, which is not mainly exposed to colored effluent has a cover of different plants. The colored effluent of the Iran Poplin industry is discharged into the drain (independently of the surrounding factories), then enters the river outlet after passing through it. In other words, the crop area contains only the effluent of the Iran Poplin textile factory. The soil samples from the rhizosphere of *Sparganium erectum* L. and *Typha latifolia* L. grown at the Iran Poplin mill drain were collected in sterile screw-capped vials and transported to the laboratory by using an icebox from the sites. Samples were stored at 4 °C and some chemical characteristics of the rhizospheric soil including pH, organic carbon (OC), electrical conductivity (EC), available phosphorus and potassium, and soluble calcium and magnesium were further measured (Carter and Gregorich, 2008).

Isolation of bacteria was carried out through the serial dilution method (Somasegaran and Hoben, 1994). 5 g of soil samples was added in 9 mL saline solution (0.85% NaCl, w/v) and serially diluted. 1 mL of each dilution was spread on nutrient agar plates by using the pour plate technique and incubated at 28 ± 2 °C for 48 h. To obtain pure colonies in a

plate, colonies and their differences were observed regularly and the prominent growing single colonies were picked up and re-cultured. Then, the morphological and biochemical properties of each bacterial strain were determined. Besides, the ability of the isolates to grow at different salt concentrations (2, 5, 7, 10, and 12%) and temperatures (5, 10, 30, 40, and 55 ^{0}C) in the nutrient broth medium was separately investigated (Parry et al., 1988; Holt et al., 1994; Shahid et al., 2018).

The bacterial isolates were assessed to estimate their potential to decolorize two azo dyes, Cibacron Brilliant Red EB and Terasil Red 3BL-01 as typical dyes with complex and resistant structures, under static and shacking conditions. For this purpose, the mineral salt medium (3.5 g /L K_2HPO_4, 1.5 KH_2PO_4, 0.5 NaCl, 0.14 Na_2SO_4, 0.15 $MgCL_2$, 0.1 Sucrose, 0.05 Yeast extract) plus azo dye concentrations (0.01 g/ L) was used and incubated at 30 °C (Moosvi e al., 2005). The Terasil Red 3BL-01 was solved in ethanol and then added to MSM. In the static condition, decolorization experiments were performed in a test tube filled with 15 ml of MSM containing dyes. To investigate decolorization in shaking conditions, the 100 ml Erlenmeyer flasks containing 30 ml of MSM- dyes mixture were used. Decolorization assay of Cibacron Brilliant Red EB was carried out at concentrations of 10 and 20 mg/L and for Terasil Red 3BL-01 at 30 mg/L. The sterilized mediums containing dye were inoculated with one loop of each bacterial colony individually. All the experiments were conducted in triplicates and control treatments containing MSM+ dye without microorganisms were run in parallel. The test tubes were placed at 30 °C under the static condition and the Erlenmeyer flasks were incubated at 120 rpm and 30 °C under shaking conditions. The concentration of azo dyes in the supernatant was analyzed after centrifugation at 6,000g for 10 min. Aliquots for Cibacron Brilliant Red EB decolorization assay were collected after 20, 40, 60, and 80 h and for Terasil Red 3BL-01, the aliquots at final decolorization time were analyzed. The concentration of Cibacron Brilliant Red EB and Terasil Red 3BL-01 in the cultures were determined using a spectrophotometer UV-2100 (Unico, Wisconsin, United States) at 508 nm and 512 nm respectively. The decolorization ratio was calculated using the following equation: Decolorization (%) = $(A_0 - A_1)/A_0 \times 100$, where A_0 and A_1 represented the absorbance of the control and samples inoculated with isolates (Saratale et al., 2006).

To prove the decolorization of azo dyes by selected strains, Thin Layer Chromatography (TLC) and Gas Chromatography were used. MSM+ dyes (Terasil Red 3BL-01 30 mg/L and 20 mg/L of Cibacron Brilliant Red EB) were prepared. At the final time point, the decolorized medium was centrifuged at 7000 rpm for 10 min, and the supernatant was filtered by Whatman filter paper (to remove the bacterial culture). Organic phase extraction of the decolorized solution was done in a separatory funnel with equal volumes of ethyl acetate and the extracted phase was evaporated in a rotary evaporator over anhydrous Na_2SO_4 and dried out. The concentrated extract was dissolved in HPLC-grade methanol and used for TLC. Extraction was also performed for the abiotic control sample. Metabolite formation was examined by TLC through developing prepared plates in the chromatographic tank previously saturated with the developing mobile phase, ethyl acetate: methanol (1:1), for Cibacron Brilliant Red EB and acetonitrile for Terasil Red 3BL-01. TLC analysis was done for the best decolorizer among the selected strains (Watharkar et al., 2013). Besides, azo dye degradation was confirmed by using Gas Chromatography 7890A (Agilent, USA). It was equipped with HP 5 column (30 m long, 0.25 mm i.d., nonpolar) and Helium gas (>99.99% purity) was used as the carrier gas at a flow rate of 1.1 mL per min. The injector temperature was maintained constant at 300 °C; oven conditions were kept stable at 100 °C for 2 min, raised to 250 °C with 10 °C per min, and again increased up to 280 °C with 30 °C per min rate (Mane et al., 2008).

After screening of dye- decolorizing bacteria by morphological and biochemical tests, the genomic DNA isolation and PCR amplification of the 16S rRNA gene were done (Chaturvedi et al., 2006). The genomic DNA of six bacteria with higher decolorization potential was isolated by the commonly used QIAGENE® DNA Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instruction. Universal pair of primers using primers, forward 27F (5′ AGA GTT TGA TCC TGG CTC AG 3′) and reverse 1520R (5′ AAG GAG GTG ATC CAGCCGCA 3′), was used for the amplification of 16SrRNA gene of isolates. Then the 16S rRNA gene was sequenced by Macrogen Company (South Korea). Then, edited sequences were blasted in the EzBioCloud gene bank. Subsequently, edited sequences were submitted to the NCBI gene bank, and accession numbers were received.

To investigate the interaction effect of initial Cibacron Brilliant Red EB concentration and bacterial strains on biodegradation of this dye, experiments were carried out in a completely randomized block design and the factorial based. Mean comparisons between different treatments were performed using Duncan's Multiple range test at a significance level of 99% ($P<0.01$). Average mean values from each experiment are shown with standard deviation (SD). Data were statistically analyzed through ANOVA by SAS 9.2.

RESULTS AND DISCUSSION

As can be seen in Table 1, the study areas have saline soils with high organic matter. Also, the concentration of the available form of nutrients P, K, Ca, and Mg is much more than in agricultural soil which may be due to the entry of Iran Poplin wastewater.

21 bacterial strains were isolated from the rhizosphere of *Sparganium erectum* L. and *Typha latifolia* L. plants. Azo dye decoloration ability of isolated strains was measured through the spectrophotometric method. Out of 21 strains, four rhizospheric strains of *Typha latifolia L.* (57.14%) were capable of decolorizing Cibacron Brilliant Red EB in the first generation (decolorization as a temporary trait) and one strain (TNP6) (14.28%) could do that permanently, however, eight rhizospheric strains (SNP3, SNP5, SNP6, SNP7, SNP8, SNP10, SNP11, SNP13) of *Sparganium erectum L.* (57.14%) were able to decolorize the dye as a permanent trait. Soil rhizosphere colony-forming unit (CFU) of *Sparganium erectum L.* and *Typha latifolia* L. were 75×10^8 and 11.3×10^5 CFU/g respectively. Depending on the sampling location of each plant from different drain points, a large difference was observed between the CFU of rhizospheres. *Typha latifolia* L. and *Sparganium erectum L.* were sampled from the place of color and salt deposition (EC= 11.12 dS/m) and drain inlet of the textile industry (EC= 5.8 dS/m), respectively. Morphological and biochemical characteristics of efficient bacterial strains are presented in Table 2. Results showed that all strains were able to grow at 5% salinity and SNP6 and SNP13 had the most tolerance to the salinity (10%). Also, four strains SNP5, SNP6, SNP7, and SNP11 could grow until 55⁰C. 100% of gram-positive bacteria and 33% of gram-negative bacteria isolated from the rhizosphere of *Sparganium*

Table 1. Chemical characteristics of *Sparganium erectum* L. and *Typha latifolia* L. rhizospheric soils

Speices	pH (1:1)	EC (1:1) (dS.m⁻¹)	N (%)	P_a (mg.kg⁻¹)	K_a (mg.kg⁻¹)	OC%	Ca (mg.kg⁻¹)	Mg (mg.kg⁻¹)
Typha latifolia **L.**	7.6	11.12	0.4	29.56	570	6.45	17500	292
Sparganium **erectum L.**	7.7	5.8	0.13	21.88	188	2.58	10150	485

Table 2. Morphological and biochemical properties of efficient strains in decolorization isolated from the rhizosphere of *Sparganium erectum* L. and *Typha latifolia* L. grown in textile effluent contaminated sites.

| Isolate name | Morphological properties | | | | | Shape of isolates | Spore |
	Colony characteristics						
	Shape	margin	elevation	Consistency	color		
TNP6	Concentric	Irregular	Convex	Dry	Yellow	Rod	-
SNP3	Smooth	Entire	Convex	Sticky	Cream	Coccobacili	-
SNP8	Smooth	Entire	Convex	Sticky	White	Rod	-
SNP10	Smooth	Curled	Drop-like	Sticky	Milky	Rod	-
SNP5	Smooth	Entire	Raised	Sticky	Orange	Rod	-
SNP6	Concentric	Entire	Drop-like	Dry	Milky	Rod	+
SNP7	Smooth	Entire	Convex	Sticky	Yellow	Coccobacilli	-
SNP11	Concentric	Irregular	Drop-like	Sticky	Milky	Rod	-
SNP13	Concentric	Lobate	Drop-like	Dry	Milky	Rod	+

Bacterium	Biochemical properties									
Gram-Negative				TSI			SIM			
	Oxidase	Catalase	Citrate test	Area	Depth	Gas	Sulfide	indole	Motility	EMB
TNP6	-	+	+	ALK	ALK	-	-	-	-	-
SNP3	-	+	+	A	A	-	-	-	+	+
SNP8	+	+	+	ALK	ALK	-	-	-	-	-
SNP10	-	+	+	ALK	ALK	-	-	-	+	-

Gram-Positive				Temperature (^{0}C)					Salinity (%)						
	Oxidase	Catalase	Citrate test	5	10	30	40	55	2	3	5	7	10	Amylase	lecithinase
SNP5	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-
SNP6	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-
SNP7	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-
SNP11	-	+	-	+	+	+	+	+	+	+	+	-	-	-	-
SNP13	-	+	+	-	-	+	+	-	+	+	+	+	+	-	+

Table 3. Analysis of variance for Cibacron Brilliant Red EB

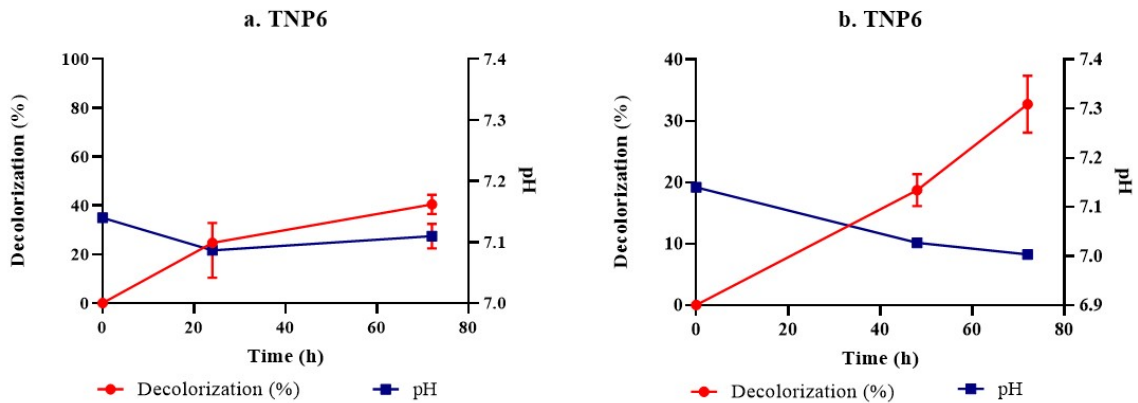| Source	DF	Anova SS	Mean Square	F Value	Pr > F
Bacteria	8	10553.29	1319.16	118.96	<.0001
Concentration	1	26.97	26.97	2.43	0.1276
bac*con	8	677.88	84.73	7.64	<.0001

Fig 1. Decolorization of reactive Cibacron Brilliant Red EB at two different concentrations (10 mg/L: (a), and 20 mg/L: (b)) by dye-degrading bacterial isolates during the time. The values presented in the graph are means of three replicates ± SD.
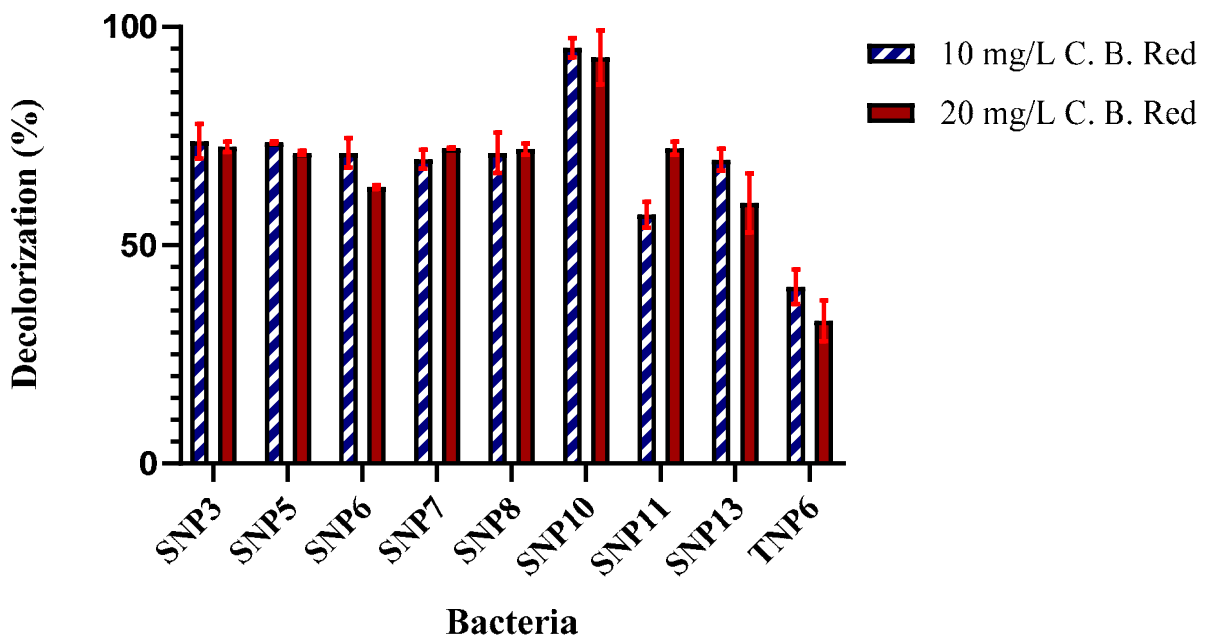
Continued Fig 1. Decolorization of reactive Cibacron Brilliant Red EB at two different concentrations (10 mg/L: (a), and 20 mg/L: (b)) by dye-degrading bacterial isolates during the time. The values presented in the graph are means of three replicates ± SD.

Continued Fig 1. Decolorization of reactive Cibacron Brilliant Red EB at two different concentrations (10 mg/L: (a), and 20 mg/L: (b)) by dye-degrading bacterial isolates during the time. The values presented in the graph are means of three replicates ± SD.


Fig 2. Final decolorization of reactive Cibacron Brilliant Red EB at two different initial concentrations (10, and 20 mg/L) by dye-degrading bacterial isolates. The values presented in the graph are means of three replicates ± SD.

erectum L. and only 25% of bacteria isolated from the rhizosphere of *Typha latifolia* L. were able to decolorize Cibacron Brilliant Red EB.

In the assessment of the aeration on dye decolorization, tests were carried out on shaking and static conditions. In this research, decolorization was only observed at the static condition which was expected to be due to the inhibitory effect of oxygen on decolorization. Statistical analysis of data indicated that the Cibacron Brilliant Red EB decolorization percentage was highly affected by bacteria strain. Although a non-significant p-value was obtained for Cibacron Brilliant Red EB decolorization at different dye concentrations, there were significant interactions between the bacteria strains and dye concentration (Table 3).

Table 4. Identification of selected bacterial strains capable of dye decolorization

Bacteria	Hit strains	Similarity (%)	Accession number
SNP3	*Enterobacter ludwigii* strain En-119	99.64	OL719291
SNP5	*Rhodococcus fascians* strain LMG 3623	95.49	OL759129
SNP6	*Paenibacillus glucanolyticus* strain DSM 5162	99.86	OL759105
SNP7	*Microbacterium oxydans* strain DSM 20578	100	OL759106
SNP10	*Pseudomonas aeruginosa* strain JCM 5962	100	OL759126
SNP13	*Bacillus safensis* strain FO-36b	99.79	OL759127
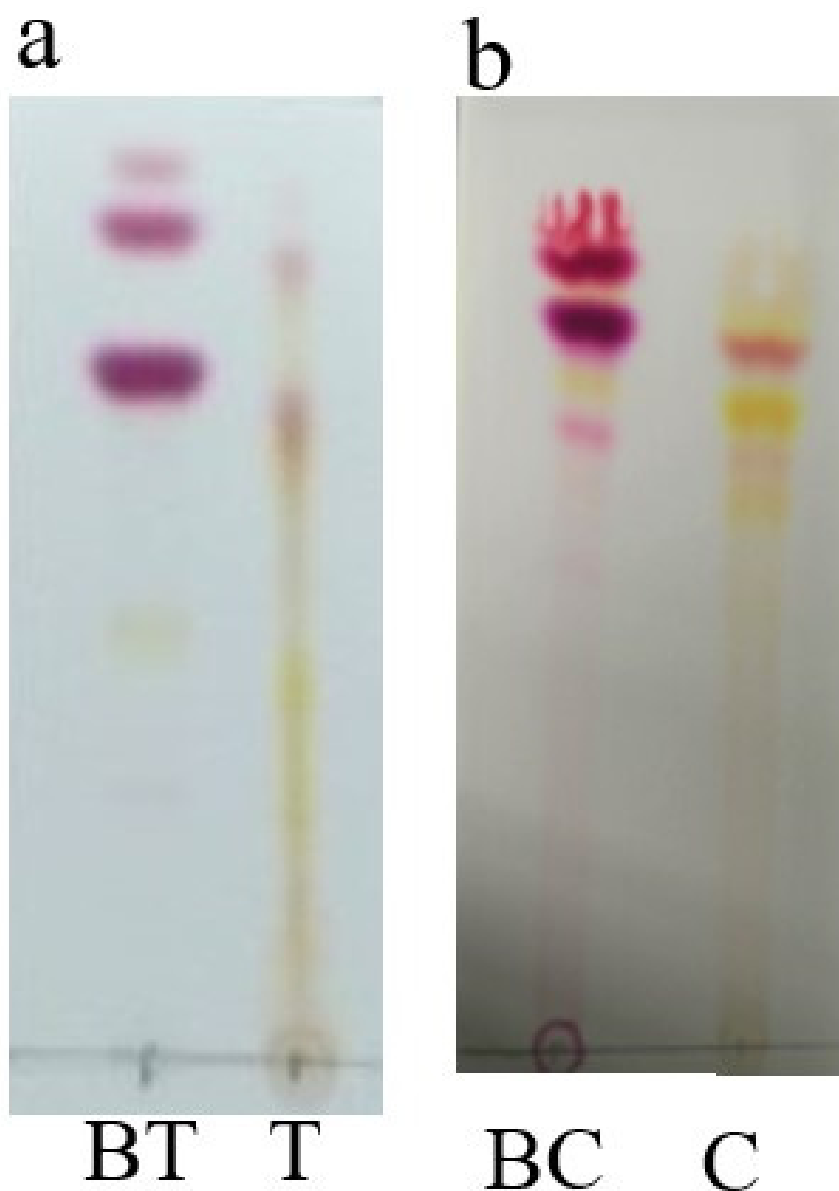
Fig 3. Comparison of TLC chromatograms for Terasil Red 3BL-01: (a) organic phase extract after decolorization by SNP3 (T) with its control (BT), and for Cibacron Brilliant Red EB: (b) organic phase extract after decolorization by SNP3 (C) with its control (BC).
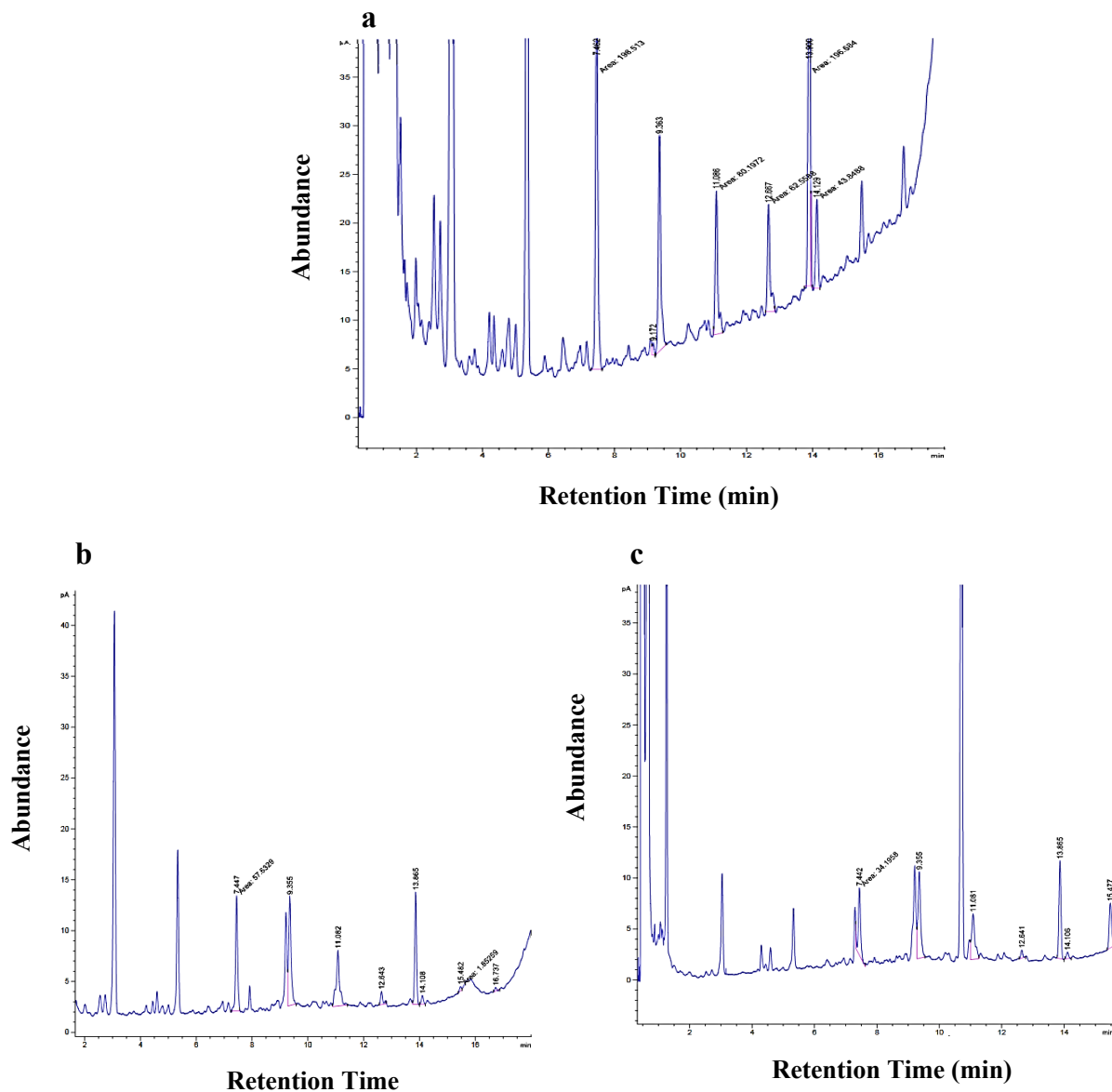
Fig 4. GC chromatogram of Terasil Red 3BL-01. (a), control, (b) decolorized media by SNP3, and (c), SNP13

Decolorization percentage of Cibacron Brilliant Red EB and pH variability during the time of incubation are shown in Fig 1 at two different concentrations (10 and 20 mg/L), respectively. The results of the Cibacron Brilliant Red EB decolorization assay showed that maximum decolorization activity of strains was observed at 10 mg/L of dye by SNP10 with 97% followed by strains SNP8, SNP3, and SNP5 with more than 73%. In 20 mg/L concentration of Cibacron Brilliant Red EB, strain SNP10 displayed the maximum decolorization ability with 94%. Also, the strains SNP8, SNP3, and SNP5 demonstrated remarkable decolorization activity with more than 70%. The minimum time of decolorization was achieved by SNP13 with 68% and 59% decolorization activity at 10 and 20 mg/L Cibacron Brilliant Red EB respectively in 24 hours. The results of pH measurements revealed the various trend in the pH of the medium during the

decolorization process by bacterial strains (Fig 1). This study explored the decolorization of Cibacron Brilliant Red EB by strains that were affected by changes in initial dye concentration from 10 to 20 mg/L). In response to increasing the initial concentration of Cibacron Brilliant Red EB, the final decolorization percentage by all strains decreased except SNP8 and SNP11 ($P > 0.05$) (Fig 2, Table 3). Although, initial dye concentration affected the performance of bacteria in Cibacron Brilliant Red EB decolorization, but did not significantly affect the final decolorization percentage of Cibacron Brilliant Red EB by strains after incubation time (80h) (Fig 2, Table 3). In the assessment of the ability of bacteria in decolorization of Terasil Red 3BL-01, the final decolorization activity was considered for each bacterium. Among the bacterial isolates, only two strains were able to achieve >75% decolorization of Terasil Red 3BL-01 in 3 days. Strains SNP3 and SNP13 could decolorize 83% and 77% of Terasil Red 3BL-01 at 30 mg/L, respectively.

The results of 16S rRNA gene sequence analysis for six selected bacteria with remarkable dye decolorization capacity were shown in Table 4. Based on the analysis of sequence similarity, strains SNP3, SNP5, SNP6, SNP7, SNP10, and SNP13 exhibited homology percentages of 99.64, 95.49, 99.86, 100, 100, and 99.79 to strains *Enterobacter ludwigii* (En-119) (T), *Rhodococcus fascians* (LMG 3623) (T), *Paenibacillus glucanolyticus* (DSM 5162) (T), *Microbacterium oxydans* (DSM 20578) (T), *Pseudomonas aeruginosa* (JCM 5962) (T), *Bacillus safensis* (FO-36b) (T), respectively. The edited 16S rRNA sequence of the strains has been deposited in NCBI GenBank (Table 4).

TLC chromatograms (TLC) of extracted organic media after decolorization by the *Enterobacter ludwigii* strain SNP3 and its comparison with the control sample indicated the appearance of new bands which might have stemmed from dye metabolites (Fig 3). The disappearance of the dye band in decolorized media also illustrated the complete decolorization of Terasil Red 3BL-01 and Cibacron Brilliant Red EB. Considering the TLC test results, it can be concluded that the SNP3 is a capable strain in decolorizing Terasil Red 3BL-01 and Cibacron Brilliant Red EB.

Gas chromatography analysis was used to investigate the T. Red 3BL-01 dye decolorizing mechanism by two supreme strains: SNP3 and SNP13. The GC chromatogram of the control sample showed the azo dye peak at the retention time of 15.5 min (Fig 4) which was decreased by 87.14 and 24.76% in GC chromatograms of decolorized media by the SNP3 and SNP13 strain. As the decolorization happened the emergence of the additional peak was observed due to degradation of dye at retention times 8 min and 10.7 min in SNP3 and SNP13 decolorized media GC chromatogram, respectively.

The widespread pollution of the textile industry in soil, water, and sediments has raised the concerns of the international community (Afrin et al., 2021). Azo dyes are the most usable group of dyes in the textile industry which are considered xenobiotic and recalcitrant contaminants (Asad et al., 2007; Orts et al., 2018; Mani et al., 2019). During the production and dyeing process, these dyes enter the wastewater and human food chain, which subsequently can cause different diseases in the human body (Khan and Malik, 2018; Khatri et al., 2018). Nowadays, many researchers have confirmed that in-situ azo dyes biodegradation with native strains is the most important and effective way with an eco-friendly attitude to eliminate this contaminant from the environment (Gomaa, 2016; Krishnamoorthy et al., 2018; Miran et al., 2018). Iran Poplin textile industry is located in Rasht, Guilan Province, northern Iran. Improper discharge of wastewater into rivers without any treatment caused many problems to the environment, especially in Anzali international wetland and the Caspian Sea (Saeedi and Jamshidi-Zanjani, 2015; Pirsaheb et al., 2015).

For the first time, in an attempt to find native bacteria in the biodegradation of azo dyes, 9 strains with the ability to degrade Cibacron Brilliant Red EB and Terasil Red 3BL-01 were isolated from the saline rhizosphere of *Sparganium erectum* L. and *Typha latifolia* L. plants. The salinity and temperature tolerance test showed that strains SNP6 and SNP13 could grow with 10% of salinity and the strains SNP5, SNP6, SNP7, and SNP11 could growth at 55^0C.

The biodegradation test of azo dyes by isolated strains was performed in static and shaking conditions. The results revealed that decolorization of azo dyes only happened at the static condition which was mainly due to the inhibitory effect of oxygen on decolorization. Tripathi and Srivastava (2011) emphasized the need for electrons for azo-reductive to carry out the reduction process and also reported that the presence of oxygen impedes the ability of azo-reductase to obtain electrons from NADH/NADPH. Saratale et al (2009) also demonstrated that azo-reductase is often less effective under aerobic conditions because of the competition of oxygen for electrons under oxidizing conditions. However, the ability of some microbial strains was also reported to produce the azo-reductase that can effectively decolorize azo dyes in the presence of oxygen (Jang et al., 2007; Imran et al., 2015). Among the 21 isolated strains, 8 strains (SNP3, SNP5, SNP6, SNP7, SNP8, SNP10, SNP11, SNP13) from the rhizosphere of *Sparganium erectum* L. (TNP6) and 1 strain from *Typha latifolia.* could decolorize azo dyes. The results of the CFU test revealed that the microbial population in the rhizosphere of *Sparganium erectum L.* (75×10^8) was much more than *Typha latifolia* L (11.3×10^5). The large difference between the CFU of rhizospheres may be due to the sampling location of each plant from different parts of the drain. Although the rhizospheric soil of *Sparganium erectum* L. was sampled from the drainage inlet of the textile industry (EC= 5.8 dS/m), *Typha latifolia* L. was taken from the place of color and salt deposition (EC= 11.12 dS/m).

The results of the Cibacron Brilliant Red EB decolorization assay showed that strains SNP10, SNP3, SNP5, and SNP8 could decolorize 97%, 73.80 %, 73.54, 71.15% of azo dyes (at a concentration of 10 mg/L) respectively. Besides, in 20 mg/L concentration of Cibacron Brilliant Red EB, 94% of decolorization was recorded by strain SNP10 and more than 70% for strains SNP8, SNP3, and SNP5. Also, the fastest strain in degradation of azo dyes was strain SNP13 with 68% and 59% decolorization performance at 10 and 20 mg/L Cibacron Brilliant Red EB respectively (24 h). Many studies have confirmed that bacteria biodegrade azo bands (-N≡N-) by producing the enzymes azoreductase (Pourbabaee et al., 2005; Saratale et al., 2011), peroxidase, laccase, tyrosinase, NADH-DCIP reductase, and MG reductase (Telke et al., 2015), and use azo dyes as a source of nitrogen and carbon (Roy et al., 2018).

Furthermore, during the decolorization of azo dyes by different selected strains, the pH changes slightly. Generally, increasing pH in the dyeing process of azo dyes is related to the production of aromatic amines, which are more alkaline. This outcome is in agreement with Hu et al. (1994) and Knapp and Newby (1995) who reported that the azo dye reduction by bacterial cultures to more basic aromatic amines leads to a rise in the pH of the medium (about 0.8–1.0 values) (Junnarkar et al., 2006; Knapp and Newby, 1995; Hu et al., 1994). In addition, the decrease in pH is probably due to producing various organic acids during the metabolization of the Azo dyes which led to the reduction in pH. Therefore, the increase in pH in the treatments was probably due to incomplete biodegradation of azo dyes and the production of aromatic amines, but in the treatments with a decrease in pH, the complete decomposition and metabolism of the azo dyes probably occurred (Pourbabaee et al., 2005, 2013; Lelis et al., 2019).

Based on the results, SNP10 was the most capable strain for removal of the Cibacron Brilliant Red EB (94.14%) followed by SNP3 (73.18%). Although the increase in dye concentration did not significantly affect the decolorization performance of SNP10 and SNP3, the final decolorization percentage by SNP3, SNP5, SNP6, SNP10, SNP13, and TNP6 decreased in response to increasing initial Cibacron Brilliant Red EB concentration. Several studies reported that the initial concentration of dye affected its degradation efficiency and a higher concentration caused considerable inhibition (Fu et al. 2019; Das et al. 2015; Roy et al 2018). Regarding Terasil Red 3BL-01, the results of the decolorization test at a concentration of 30 mg/L showed that only two strains SNP3 and SNP13 could decolorize 83% and 77% of this dye, respectively.

Afterward, the identification and registration of the top bacteria in the decolorization of azo dyes was carried out as follows: *Enterobacter ludwigii* strain SNP3 (OL719291), *Rhodococcus fascians* strain SNP5 (OL759129), *Paenibacillus glucanolyticus* strain SNP6 (OL759105), *Microbacterium oxydans* strain SNP7 (OL759106), *Pseudomonas aeruginosa* strain SNP10 (OL759126), and *Bacillus safensis* strain SNP13 (OL759127). *Pseudomonas aeruginosa* (Nachiyar and Rajkumar, 2003; Afrin et al., 2021, Nho et al., 2021), *Microbacterium oxydans* (Juarez-Ramirez et al., 2012), *Bacillus pumilus/ safensis* (Afrin et al., 2021), *Enterobacter ludwigii* (Haque et al., 2021), and *Paenibacillus glucanolyticus* (Nho et al., 2021) have been reported as the azo dyes decolorizing bacteria by many researchers. Afrin et al. (2021) isolated a bacterial consortium (*Enterococcus faecium*, *Bacillus pumilus/ safensis*, B. *thuringiensis*, and *Pseudomonas aeruginosa* from the effluents of textile industries with the ability to degrade Novacron dyes. Our study demonstrates that the saline rhizosphere of *Sparganium erectum* L. plants can be considered a novel source of bacteria with the capability to decolorize azo dyes.

Results of the decolorization test showed that only *Enterobacter ludwigii* strain SNP3 (OL719291) could decolorize both Cibacron Brilliant Red EB (73%) and Terasil Red 3BL-01 (83%). Therefore, the azo dyes decolorization ability of *Enterobacter ludwigii* strain SNP3 (OL719291) was evaluated by TLC and GC. TLC chromatograms showed that *Enterobacter ludwigii* strain SNP3 was able to decolorize both Terasil Red 3BL-01 and Cibacron Brilliant Red EB which was confirmed by the appearance of a new band as the metabolite and vanishing of the band of azo dyes. Also, the decolorization of Terasil Red 3BL-01 by *Enterobacter ludwigii* strain SNP3 and *Bacillus safensis* strain SNP13 (OL759127) was confirmed by GC-FID. The peak area related to the Terasil Red 3BL-01 was decreased by 87.14% and 24.59% in culture media of *Enterobacter ludwigii* strain SNP3 and *Bacillus safensis* strain SNP13 respectively. Besides, main peaks related to the azo bond were overall or partly disappeared, shifted, or deformed, and the new peak as the intermediate of biodegradation was found in media containing strain SNP3 and SNP13 as well. Based on the results, it can be derived that the growth of fresh and vibrant plants (*Sparganium erectum* L., and *Typha latifolia* L.) at the effluent crossing may be due to the role of these decolorizer bacteria in reducing the toxicity of the dyes and their synergistic effects with plants.

CONCLUSION

In this study, for the first time, the six efficient azo dyes decolorizing bacteria including *Enterobacter ludwigii* strain SNP3 (OL719291), *Rhodococcus fascians* strain SNP5 (OL759129), *Paenibacillus glucanolyticus* strain SNP6 (OL759105), *Microbacterium oxydans*

strain SNP7 (OL759106), *Pseudomonas aeruginosa* strain SNP10 (OL759126), and *Bacillus safensis* strain SNP13 (OL759127) were isolated from the rhizosphere of *Sparganium erectum* L. plant. *Pseudomonas aeruginosa* strain SNP10 showed the maximum Cibacron Brilliant Red EB decolorization activity of about 97% and 94% at 10 and 20 mg/L concentrations, respectively. Furthermore, *Bacillus safensis* strain SNP13 required a minimum time of 24 h for Cibacron Brilliant Red EB decolorization (68%). According to our findings, among 9 strains with the ability to degrade Cibacron Brilliant Red EB, only two strains SNP3 and SNP13 could biodegrade Terasil Red 3BL-01. *Enterobacter ludwigii* strain SNP3 could decolorize 83% and 74% of Terasil Red 3BL-01 (30mg/L) and Cibacron Brilliant Red EB (20 mg/L) respectively, in 3 days. Therefore, it can be concluded that the rhizosphere of *Sparganium erectum* L. plant is a novel and suitable source for the isolation of azo dyes decolorizer bacteria. The finding of this research demonstrates that the isolated bacteria *Enterobacter ludwigii, Pseudomonas aeruginosa* strain SNP10, and *Bacillus safensis* strain SNP13 have a high potential for the waste management plan and refining and clean-up of natural ecosystems.

GRANT SUPPORT DETAILS

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript and the authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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