

## TEXTILE DYE BIOREMEDIATION POTENTIAL OF SOME RHIZOBIAL STRAINS AND THEIR HEAVY-METAL AND HIGH SALINITY TOLERANCE

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Received: 20<sup>th</sup> of March 2019 / Revised: 3<sup>rd</sup> of June 2019  
Accepted: 26<sup>th</sup> of June 2019 / Published: 20<sup>th</sup> of September 2018

**Keywords:** rhizobial strains, bioremediation, textile dyes, heavy metals, salinity, Danube –Delta Biosphere Reserve

**Abstract** The discharge of untreated textile dye effluents enriched with toxic pollutants including dyes, heavy metals and other hazardous materials may cause negative impacts on the entire ecosystem. The proposed work aimed to isolate, molecularly identify and characterize the native rhizobial strains with textile dye biodegradation potential in relation with their tolerance to high salinity and heavy metals (usually meet in high concentrations in the textile dye effluents). Native rhizobial strains were isolated from various terrestrial ecosystems originated in Danube – Delta Biosphere Reserve. Most of the strains tolerated  $\geq 2.0\%$  NaCl. Our data showed that 3 strains (*Agrobacterium* sp.CR-B19; *Rhizobium giardinii* CR-B22 and *Ensifer* sp.CR-B26) were able to tolerate 15 ppm concentration of cadmium ( $\text{Cd}^{2+}$ ), whereas all strains identified as *Rhizobium* sp. (except *R. leguminosarum* CR-B10), and *Agrobacterium* sp. could tolerate 70 ppm of chromium ( $\text{Cr}^{6+}$ ). Moreover, 3 indigenous strains (*Rhizobium giardinii* CR-B13; *Rhizobium* sp.CR-B15 and *Agrobacterium* sp. CR-B19) tolerated a concentration of 200 ppm of lead ( $\text{Pb}^{2+}$ ). In regard to azo-dye degrading potential, only *Rhizobium leguminosarum* CR-B10 was able to degrade the Reactive Orange 16 dye (90.18% decolorization) in stationary conditions, at 30°C. Comparatively, *Agrobacterium* sp. CR - B19 strain removed Reactive Orange 16 (sulphonic azo-dye) (78.92 % decolorization) and Reactive Blue 4 (antraquinonic dye) (12 % decolorization) by adsorption. Based on their bioremediation potential, the newly isolated rhizobial strains could be further used (in pure culture or in consortia) to develop a new environmental friendly and cost-effective biotechnology in order to reduce the toxicity of textile dyes effluents.

### INTRODUCTION

In the last decades, the severe environmental pollution has been associated with fast industrialization and discharge of large amounts of waste waters without pretreatment into water bodies. In some cases, the difficulty in treating industrial waste waters by conventional treatment methods has been reported (Van der Zee, 2001). Synthetic dyes and heavy metals are ones of the major xenobiotic pollutants of textile, pharmaceutical, cosmetics and food industries. Many reports confirmed the recalcitrant nature of synthetic dyes and their carcinogenic or mutagenic properties (Grover, 1999; Phugare et al, 2011). Also, soil and water contamination with heavy metals containing effluents received an increasing concern due to their direct toxicity to animals, plants and microorganisms (Hamilton and Wetterhahn, 1988; De Flora et al, 1990) and irreversible immobilization in soil components (McGrath and Lane, 1989). Textile dye effluents containing various synthetic dyes and heavy metals could induce toxic effects on agricultural plants and soil microorganisms by reducing soil fertility and agricultural output ((Puvaneswari et al, 2006). Based on environmentally friendly characteristics of bioremediation process (low cost, less sludge volume, environmentally safe) many bacteria, molds and yeasts (live or dead microbial cells, in pure culture or in consortia, free or immobilized cells) have been used for industrial waste water bioremediation (Ali, 2010; Ali et al, 2009; Allam, 2017).

Some studies showed that species of *Agrobacterium* spp., *Phyllobacterium* spp., *Rhizobium* spp., *Mesorhizobium* spp., *Ensifer* spp. and *Bradyrhizobium* spp. could detoxify the industrial waste waters or soil due to their resistance to heavy metals and their abilities to degrade organic pollutants (Ahmad, 1997; Carasco et al, 2005; Stan, 2011; Teng et al, 2015). Species of *Rhizobium* spp. și *Bradyrhizobium* spp are ones of the most frequent strains isolated from contaminated area (Teng, 2015). Also, strains of *Agrobacterium radiobacter* were isolated from activated sludge (in sewage treatment plants) (Drysdale et al, 1999; Singh et al, 2004).

In this study, the textile dye bioremediation of some soil rhizobial strains was investigated. Since the presence of elevated level of salts and heavy metals in textile dye effluents can significantly reduce the efficacy of dye biodegradation process due to their toxicity to microbial cells, a preliminary screening of bacterial resistance to salinity (NaCl) and heavy metals, as chromium ( $\text{Cr}^{6+}$ ), cadmium ( $\text{Cd}^{2+}$ ) and lead ( $\text{Pb}^{2+}$ ) was also performed. A new biotechnology – based bioremediation technique could be next developed and applied to detoxify the toxic waste waters used for agronomic practices.

## MATERIALS AND METHODS

**Sampling sites and sample collection.** Soil samples from various sites and soil type of Danube - Delta Biosphere Reserve (DDBR) were collected from the soil depth (10 -15 cm), packed in sterile bags, transported to the laboratory, homogenized and stored at 4°C for later use. The slurry was obtained by adding 1 g soil to 50 ml of sterile distilled water and mixed on an orbital shaker at 200 rpm for 1 h. Then, aliquots (0.1 ml) were plated on yeast – mannitol-agar (YMA) supplemented with Congo red dye (25µg/ml). Rhizobial strains were then isolated and purified according to standard protocols (Vincent, 1970) and further characterized. The purified isolates were maintained at -80°C in yeast – mannitol broth (YMB) containing 20% glycerol.

**Phylogenetic analysis of 16S rDNA sequences.** Bacterial strains were grown in liquid YMB medium and incubated at 28°C on a rotary shaker. Equal aliquots of bacterial cultures were collected by centrifugation and total genomic DNA was isolated using Bacteria DNA Preparation kit (Jena Bioscience, Germany) according to manufacturer instruction. The purity and concentration of genomic DNA was checked by Nano Drop measurements and electrophoresis on 0.8% agarose gel. The fD1 and rD1 universal primers (Weisburg et al, 1991) were used to amplify conserved region of 16S rDNA as previously described (Efrose et al, 2018). PCR amplification products were purified and directly sequenced on both strands using the same primers as for PCR (CEMIA, Greece) and deposited in GenBank/NCBI database for the selected bacterial strains. For phylogenetic analysis, sequences were corrected and assembled using DNA Baser v. 3.5.4 program. The sequences obtained from the newly isolated rhizobia together with the sequences retrieved from the NCBI/GenBank database were aligned using the CLUSTAL W software as implemented by implemented by MEGA7 v.7.0.26 software package (Kumar, Stecher and Tamura, 2016), trimmed to the same length and used in the phylogenetic analysis. Phylogenetic tree was built with the Neighbor-Joining method based on Kimura's two-parameter model. Bootstrap confidence levels were calculated for 1000 replicates.

**Nucleotide sequence accession numbers.** The GenBank accession numbers for the 16S rDNA sequences obtained from the two bacterial strains which exhibited multiple biotechnological potential (*Rhizobium leguminosarum* CR-B10 and *Agrobacterium sp.* CR-B19) are MH456791 and MH456793, respectively. Accession numbers of the related reference strains are individually specified in the corresponding phylogenetic trees.

**Stress tolerance.** The bacterial cultures (10µl) were point - inoculated into YMA plates supplemented with NaCl (w/v) (0.1; 0.5; 2.0; 4.0, and 8.0 %). Heavy metal resistance was determined on YMA plates supplemented with the different heavy metals: Cr<sup>6+</sup> (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) and Cd<sup>2+</sup> (CdCl<sub>2</sub>) at concentrations in a range from 0.1 to 70 ppm and, also Pb<sup>2+</sup> (Pb(NO<sub>3</sub>)<sub>2</sub>) at concentrations in a range from 15 to 600 ppm. The readings were made after 3 days of incubation at 30°C. The highest concentration of NaCl and heavy metal salt supporting strains growth on YMA plates were defined as the maximum tolerance level.

**Screening of dye degrading bacteria.** Different liquid culture media have been used in order to assess the dye biodegradation potential of newly isolated rhizobial strains, as follow: V1 culture medium (mineral salt medium – 1.0 g L<sup>-1</sup> NH<sub>4</sub>Cl, 0.28 g L<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.067 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 0.04 g L<sup>-1</sup>, MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.022 g L<sup>-1</sup> CaCl<sub>2</sub>.2H<sub>2</sub>O, 0.005 g L<sup>-1</sup> FeCl<sub>3</sub> and trace elements solution (10 ml L<sup>-1</sup>); the trace element solution contained: 10.0 mg L<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O, 3.0 mg L<sup>-1</sup> MnCl<sub>2</sub>.2H<sub>2</sub>O, 1.0 mg L<sup>-1</sup> CoCl<sub>2</sub>.6H<sub>2</sub>O, 2.0 mg L<sup>-1</sup> NiCl<sub>2</sub>.6H<sub>2</sub>O, 3.0 mg L<sup>-1</sup> NaMoO<sub>4</sub>.2H<sub>2</sub>O, 30.0 mg L<sup>-1</sup> H<sub>3</sub>BO<sub>3</sub> and 1.0 mg L<sup>-1</sup> CuCl<sub>2</sub>.2H<sub>2</sub>O - supplemented with glucose 10.0 g L<sup>-1</sup> and yeast extract 10.0 g L<sup>-1</sup>); V1.1.culture medium (mineral salt medium supplemented with 10.0 g L<sup>-1</sup> glucose and yeast extract reduced to 3.0 g L<sup>-1</sup>); V2 culture medium (TY medium – 10.0 g L<sup>-1</sup> tryptone, 5.0 g L<sup>-1</sup> NaCl and 3.0 g L<sup>-1</sup> yeast extract); and V2.1. (TY medium supplemented with yeast extract reduced to 1.5 g L<sup>-1</sup>). Synthetic dyes used in the experiments were azo-dye Reactive Orange16 (commercial name: Bezactiv Orange V-3R – λ<sub>max</sub>= 495 nm) and anthraquinonic dye Reactive Blue 4 (commercial name: Procion Blue MX-R - λ<sub>max</sub>= 595 nm).The stock solution of dyes (1000 mg L<sup>-1</sup>) were prepared, filter sterilized (Millipore filter, 0.22µm, Millipore Corp., Bedford, USA) and diluted properly before use.

The decolorization assay has been performed as follow: 250-ml flasks contained 100 ml of different culture media, supplemented with 20 ppm textile dyes (Reactive Orange 16 and, respectively Reactive Blue 4) were inoculated with 2% (v/v) suspensions from newly isolated bacterial strains and incubated at 30°C in stationary conditions. The control flasks containing the same mediums without inoculums were also kept as control. At the maximum visible dye decolorization time (72 -120 h), 5 ml of liquid media was centrifuged at 12,000 rpm for 10 min and the supernatant was analyzed for remaining dye content. The experiments were carried out in triplicates.

The decolorization efficiency of dyes was determined by measuring the absorbance of culture supernatant at their λ<sub>max</sub> for each textile dye (λ<sub>max</sub>= 495 nm (RO16) and λ<sub>max</sub>= 595 nm (RB4), respectively). The decolorization percentage (%) was calculated as follow:

$$\text{Decolorization (\%)} = \frac{\text{initial absorbance} - \text{final absorbance}}{\text{initial absorbance}} \times 100$$

**UV–Vis analysis.** The culture supernatants were analyzed by spectral scanning between 200 and 800 nm using a UV–Vis spectrophotometer (BekmanCoulter-DU730) and changes in the absorption spectra were recorded in order to analyze treated dyes degradation compared with non – treated dyes.

## RESULTS AND DISCUSSIONS

### Isolation and identification of bacterial strains

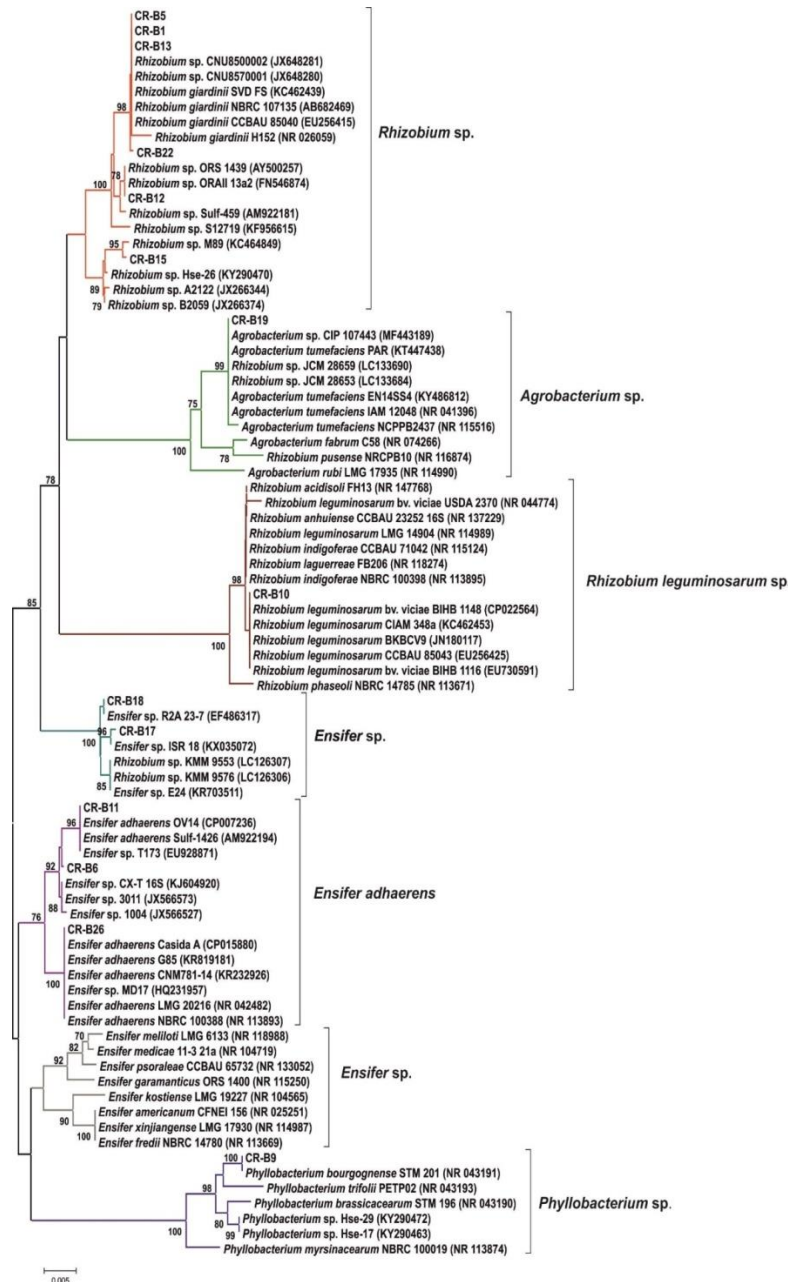
For bacteria isolation, soil samples were collected from various terrestrial ecosystems located in the Danube - Delta Biosphere Reserve (DDBR). Most of the strains (CR-B1; CR-B5; CR-B6; CR-B10-13; CR-B15; CR-B17-18) were isolated from agricultural soil originated in Chilia Veche - Pardina.

**Table 1** - Molecular identification and geographical origin of bacterial strains with biotechnological potential, isolated from various ecosystems in DDBR -Romania

No.	Bacterial isolates	Geographical origin	Identity* (%)	Identified species**
1.	CR-B1	Chilia Veche - Pardina	99.8	<i>Rhizobium giardinii</i>
2.	CR-B5	Ostrovu Tataru	100	<i>Rhizobium giardinii</i>
3.	CR-B6	Ostrovu Tataru	99.7	<i>Ensifer</i> sp.
4.	CR-B9	Chilia Veche - Pardina	100	<i>Phyllobacterium bourgognense</i>
5.	CR-B10	Chilia Veche - Pardina	100	<i>Rhizobium leguminosarum</i>
6.	CR-B11	Chilia Veche - Pardina	100	<i>Ensifer adhaerens</i>
7.	CR-B12	Chilia Veche - Pardina	100	<i>Rhizobium</i> sp.
8.	CR-B13	Chilia Veche - Pardina	99.9	<i>Rhizobium giardinii</i>
9.	CR-B15	Chilia Veche - Pardina	99.8	<i>Rhizobium</i> sp.
10.	CR-B17	Chilia Veche - Pardina	99.6	<i>Ensifer</i> sp.
11.	CR-B18	Chilia Veche - Pardina	99.8	<i>Ensifer</i> sp.
12.	CR-B19	Dunavatu de Jos	100	<i>Agrobacterium</i> sp.
13.	CR-B22	Dunavatu de Jos	99.9	<i>Rhizobium giardinii</i>
14.	CR-B26	Murighiol - Sf. Gheorghe	100	<i>Ensifer</i> sp.

\*Estimates of evolutionary identity between 16S rDNA sequences of the test strains and recognized rhizobia species, based on pair-wise analysis of the obtained sequences.

\*\* The GenBank accession numbers for the sequences obtained from CR-B10 and CR-B19 rhizobial strains are MH456791 and MH456793, respectively.



**Figure 1. Phylogenetic trees of the 16S rDNA sequences** showing the relationships of the representative bacterial strains isolated from various habitats from DDBR with selected reference strains for defined bacterial species. The Neighbor-Joining dendrogram was constructed using the Kimura 2-parameter model. Bootstrap values (based on 1000 replicates) below 70% are not shown. The scale bar represent 0,005 nucleotide substitutions.

The fields were cultivated with corn, wheat, barley and rape (Chilia –Veche – Pardina) or alfalfa (Ostrovu Tataru). Three strains (CR-B19; CR-B22 and CR-B26) were isolated from natural habitats (e.g. alluvial soils) located in Murighiol – Dunavatu de Jos area and Sf. Gheorghe branch (Saraturi Lake) (Table 1). The newly rhizobial strains were selected based on typical colonies appearance on Congo Red supplemented culture medium (Kuykendall, 2015). The taxonomic identification of the isolates and their relatedness to known bacterial strains was assessed by the sequence analysis of 16S rRNA gene (Figure 1; Table 1). Phylogenetic analysis revealed that the newly isolated strains clustered in seven well defined phyletic groups highly similar with previously described bacteria strains belonging to the Order of *Rhizobiales*.

### Salinity and heavy metal tolerance of soil rhizobia

Most of the bacterial isolates could tolerate concentrations up to 2.0 % NaCl. However, the isolates identified as *Rhizobium giardinii* (CR-B5) and the *Ensifer* sp. (CR-B6), originated in agro ecosystems (sodium saline alluvial soil type) as well as *Rhizobium leguminosarum* (CR-B10) showed increased tolerance to salinity (4% NaCl) (Table 2).

**Table 2 - Salinity tolerance of selected rhizobial strains**

Rhizobial strains	NaCl (%)			
	0.5	2.0	4.0	8.0
CR-B1 ( <i>Rhizobium giardinii</i> )	+	-	-	-
CR-B5 ( <i>Rhizobium giardinii</i> )	+	±	±	-
CR-B6 ( <i>Ensifer</i> sp.)	+	+	±	-
CR-B9 ( <i>Phyllobacterium bourgognense</i> )	+	-	-	-
CR-B10 ( <i>Rhizobium leguminosarum</i> )	+	+	+	-
CR-B11 ( <i>Ensifer adhaerens</i> )	+	+	-	-
CR-B12 ( <i>Rhizobium</i> sp.)	+	+	-	-
CR-B13 ( <i>Rhizobium giardinii</i> )	+	+	-	-
CR-B15 ( <i>Rhizobium</i> sp.)	+	+	-	-
CR-B17 ( <i>Ensifer</i> sp.)	+	+	-	-
CR-B18 ( <i>Ensifer</i> sp.)	+	+	-	-
CR-B19 ( <i>Agrobacterium</i> sp.)	+	+	-	-
CR-B22 ( <i>Rhizobium giardinii</i> )	+	+	-	-
CR-B26 ( <i>Ensifer</i> sp.)	+	+	-	-

+ good growth; ± weak growth ; - no growth

Since undiluted wastewaters from dyestuff industries usually contain salt concentrations up to 15 - 20 % (EPA, 1997) a high salinity tolerance of newly isolated bacteria should be considered as a prerequisite for those strains that are intended to be used as bioremediation agents for textile dye waste waters. For example, *Stentrophomonas maltophilia* RSV – 1 was able to decolorized (82.72 %) a mixture of dyes (Blue RR, Black B, Red RR and Yellow RR) within 6 days of incubation in the presence of 3% NaCl (Rajeswari et al, 2013).

The newly free – living bacterial isolates presented different responses to heavy metal stress (Table 3). For example, for those originated in soils collected from flooded areas (natural ecosystems), the highest tolerance to Cd<sup>2+</sup> was 15 ppm (*Agrobacterium sp.* CR-B19; *Rhizobium giardinii* CR-B22 and *Ensifer sp.* CR-B26) (Table 3). In terms of tolerance to Cr<sup>6+</sup>, bacterial strains belonging to the species *Rhizobium sp.* and *Agrobacterium sp.* were tolerant at maximum concentration of 70 ppm. Most strains tolerated 100 ppm Pb<sup>2+</sup> concentration. Much adapted to heavy metal stress, the isolates *Rhizobium giardinii* CR-B13, *Rhizobium sp.* CR-B15 and *Agrobacterium* CR-B19 were able to tolerate 200 ppm Pb<sup>2+</sup>.

**Table 3** – Highest heavy metal tolerance of selected rhizobial strains

+ good growth; ± weak growth ; - no growth

This behavior is encouraging since some heavy metals as cadmium (Cd) and lead (Pb) are usually quantified in industrial effluents in high concentrations, being widely used for production of colour pigments of textile dyes. The above mentioned heavy metals have no known biological and/or physiological, functions (Gadd, 2010), but their presence could strongly inhibit the dye decolorization process by living microorganisms (Gadd, 1992). Abd - Alla et al. (2012) reported isolation and characterization of a heavy metal resistant isolate of *Rhizobium leguminosarum* bv. *viciae*, as a potentially efficient biosorbent for Cd<sup>2+</sup> and Co<sup>2+</sup>. The new isolate was resistant to 10 ppm Cd<sup>2+</sup>. Comparatively, chromium (Cr) is an essential trace element, but higher levels are considered toxic and mutagenic in humans, animals and plants (Léonard and Lauwerys, 1980; De Flora et al, 1990) and can significantly reduce also, the efficacy of biological sewage treatment.

Rhizobial strains	Cd <sup>2+</sup> (ppm)		Cr <sup>6+</sup> (ppm)		Pb <sup>2+</sup> (ppm)		
	5.0	15	50	70	70	100	200
CR-B1 ( <i>Rhizobium giardinii</i> )	+	-	+	-	-	-	-
CR-B5 ( <i>Rhizobium giardinii</i> )	+	-	+	+	±	-	-
CR-B6 ( <i>Ensifer sp.</i> )	+	-	+	-	+	+	-
CR-B9 ( <i>Phyllobacterium bourgognense</i> )	+	-	+	+	+	+	-
CR-B10 ( <i>Rhizobium leguminosarum</i> )	+	-	±	±	+	+	-
CR-B11 ( <i>Ensifer adherens</i> )	+	-	-	-	+	-	-
CR-B12 ( <i>Rhizobium sp.</i> )	+	-	+	+	+	+	-
CR-B13 ( <i>Rhizobium giardinii</i> )	+	-	+	+	+	+	+
CR-B15 ( <i>Rhizobium sp.</i> )	+	-	+	+	+	+	+
CR-B17 ( <i>Ensifer sp.</i> )	+	-	-	-	+	+	-
CR-B18 ( <i>Ensifer sp.</i> )	+	-	+	-	+	+	-
CR-B19 ( <i>Agrobacterium sp.</i> )	+	+	+	+	+	+	+
CR-B22 ( <i>Rhizobium giardinii</i> )	+	+	+	+	+	+	-
CR-B26 ( <i>Ensifer sp.</i> )	+	+	+	-	+	+	-

Smith and Giller (1992) first isolated strains of heavy metal - resistant *R. leguminosarum* from soil contaminated sewage sludge. Raaman (2012) reported that *R. leguminosarum* could detoxify a medium containing chromium by adsorption, but also by reduction of CrVI to CrIII. As result, bacteria can grow and could be a potential agent for the bioremediation purposes (heavy metals and dyes contaminated waste waters).

## Screening of bacterial isolates with bioremediation abilities

The bioremediation capacity of the RO16 and RB4 textile dyes by the newly isolated rhizobial strains is diverse as efficiency and mechanism, mainly due to the differences in the chemical structure of the dyes. From a total of fourteen tested bacterial strains belonging to four bacterial genera (*Rhizobium* spp., *Ensifer* spp., *Agrobacterium* spp. and *Phyllobacterium* spp.) only two strains, namely *Agrobacterium* spp. CR-B19 and *Rhizobium leguminosarum* CR-B10, showed a decolorization capacity over 78 % against Reactive Orange dye 16 within 120 hr of incubation (Table 4). The increased efficiency, in terms of shortest the decolorization time, was obtained by reducing the quantity of yeast extract (source of nitrogen and vitamins) from the composition of culture media (Table 5). The best results have been obtained on V2.1 (up to 90.18 % decolorization - *R. leguminosarum* CR-B10) culture media within 72 hr of incubation at 30°C, under stationary conditions (Table 5). As referring to the RB4 bioremediation process, only *Agrobacterium* sp. CR-B19 strain exhibited an adsorption capacity of the dye (up to 12 % decolorization on V1.1 culture medium). The decolorization percent (%) was not increased with prolonged incubation time. Also, no other new tested bacterial strains have demonstrated the potential to remove the RO16 and RB4 dyes from the aqueous media.

**Table 4** - Maximum textile dyes decolorization potential (%) of some rhizobial strains isolated from soil (DDBR - Romania) after 120 hour of incubation at 30°C

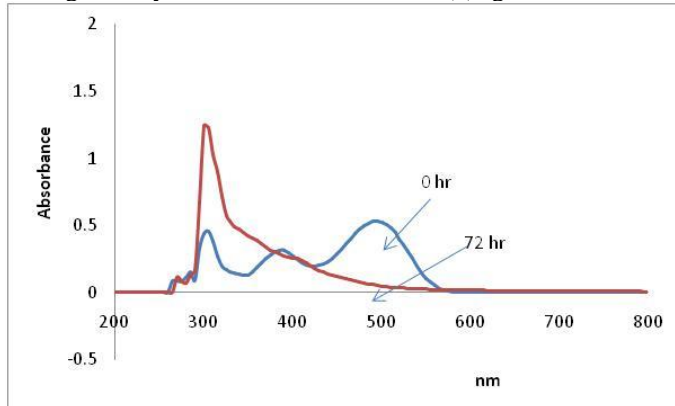
Rhizobial strains	Decolourization (%)	
	Culture medium V1	Culture medium V2
	<b>Azo – dyes (Reactive Orange 16 – 20 ppm)</b>	
CR-B9 ( <i>Phyllobacterium bourgognense</i> )	10.21	13.29
CR-B10 ( <i>Rhizobium leguminosarum</i> )	21.12	78.56
CR-B19 ( <i>Agrobacterium</i> sp.)	95.05	61.41
	<b>Antraquinonic dyes (Reactive Blue 4 – 20 ppm)</b>	
CR-B19 ( <i>Agrobacterium</i> sp.)	10.23	5.89

**Table 5** - Remediation of RO16 and RB4 dyes (20 ppm) by some rhizobial strains isolated from soil, after 72 h of incubation at 30°C

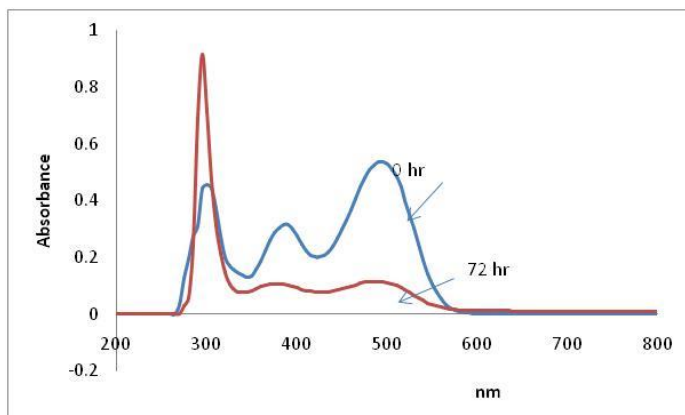
Rhizobial strains	Decolorization (%)	Cell growth (OD <sub>640nm</sub> )	pH	Color of biomass
	<b>Reactive Orange 16 (20 ppm) - medium V1.1</b>			
CR-B19 ( <i>Agrobacterium</i> sp.)	78.92	1.583	3.14	orange
	<b>Reactive Orange 16 (20 ppm) - medium V2.1</b>			
CR-B10 ( <i>Rhizobium leguminosarum</i> )	90.18	1.281	8.38	white
	<b>Reactive Blue 4 (20 ppm) – medium V1.1</b>			
CR-B19 ( <i>Agrobacterium</i> sp.)	12.00	1.023	5.85	blue

UV- Vis spectrum analysis (200-800 nm) was performed in order to elucidate the mechanisms involved in the bioremediation of RO16 and RB4 dyes by the most efficient bacterial strains. As was noticed, *R. leguminosarum* CR-B10 was able to degrade the RO16 dye: the characteristic peak at 495 nm almost disappeared within 72 hr of incubation and white

biomass was obtained at the final of the biodegradation process (Figure 2 and Table 5). Comparatively, *Agrobacterium spp.* CR-B19 removed the tested dye through adsorption: the colored biomass which was noticed at the final of the incubation time was correlated with no modifications into UV- Vis spectra of both dyes (except that the main peaks in the visible spectrum decreased gradually within 72 hr of incubation)(Figure 3 – 4 and Table 5).

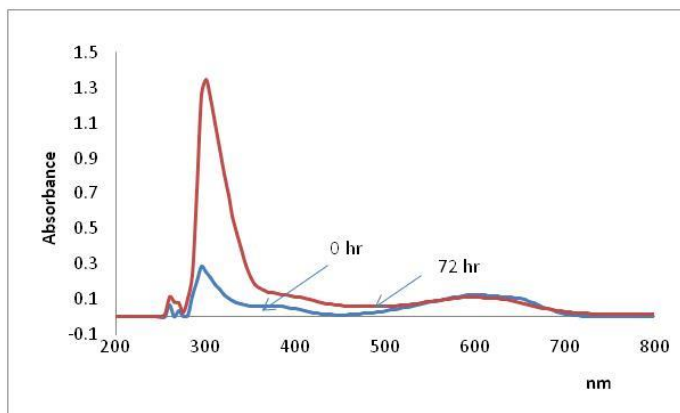


**Figure 2** - UV-Vis spectra of Reactive Orange 16 (20 ppm) before and after bioremediation by *R. leguminosarum* CR-B10 in V2.1 culture medium



**Figure 3** - UV-Vis spectra of Reactive Orange 16 (20 ppm) before and after bioremediation by *Agrobacterium spp.* CR-B19 in V1.1 culture medium





**Figure 4** - UV-Vis spectra of Reactive Blue 4 (20 ppm) before and after bioremediation by *Agrobacterium spp.* CR-B19 in V1.1 culture medium

Other studies reported that *Agrobacterium radiobacter* cells induced maximum decolorization of Crystal Violet (10 mg/L) (triphenyl methane dye type) within 8 hr under static anoxic conditions, due to lacase and aminopirin N- demetilase activities (Parshetti 2011). The enhanced efficiency on heavy metal removal and decolorization of other textile azo-dyes (Methyl Orange and Congo Red) has been obtained by treating the industrial waste water with a bacterial consortium consisted of *Rhizobium radiobacter*, *Sphingomonas paucimobilis* and *Bacillus subtilis* (Allam, 2017). The experiment confirmed the importance of synergic activity of different metabolites of bacterial cultures in bioremediation of industrial effluents.

## CONCLUSIONS

In the present study we have reported on isolation, molecular identification and characterization of the native rhizobial strains with textile dye biodegradation potential, in correlation with their tolerance to high salinity and heavy metals. Native rhizobial strains were isolated from various environments from Danube - Delta Biosphere Reserve and were grouped based on 16S rRNA gene phylogeny in seven well defined clusters.

In terms of their resistance to abiotic stress, most of the strains tolerated  $\geq 2.0\%$  NaCl and *R. leguminosarum* CR-B10 grew up until 4 % NaCl. The *Agrobacterium sp.* CR-B19, *Rhizobium giardinii* CR-B22 and *Ensifer sp.*CR-B26 strains were able to tolerate 15 ppm concentration of cadmium ( $\text{Cd}^{2+}$ ), whereas the strains identified as *Rhizobium sp.* and *Agrobacterium sp.* could tolerate 70 ppm of chromium ( $\text{Cr}^{6+}$ ). Three indigenous strains (*Rhizobium giardinii* CR-B13; *Rhizobium sp.*CR-B15 and *Agrobacterium sp.*CR-B19) tolerated a concentration of 200 ppm of lead ( $\text{Pb}^{2+}$ ). Moreover, *Rhizobium leguminosarum* CR-B10 strain was able to degrade the RO16 (sulphonic azo-dye), while both dyes (RO16 and RB4) have been removed from aqueous medium by *Agrobacterium sp.*CR-B19 cells through an adsorbtion mechanism. Based on their bioremediation potential, the two newly isolated rhizobial strains could be further used to develop a new environmental friendly and cost-effective biotechnology in order to reduce the toxicity of textile dyes effluents.

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**Acknowledgments:** This work was supported by the Romanian Ministry of Research and Innovation through the NUCLEU program (BIODIVERS) and Program 1 - Development of the National R & D System, Subprogram 1.2 - Institutional Performance - Projects for Excellence Financing in RDI (Contract no. 22PFE / 2018).

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