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Mineralization of Congo red azo dye by *Paenibacillus populi* sp. strain JK11 isolated from high altitude region of Uttarakhand

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Azo dyes are aromatic organic compounds with toxic potential to the living organisms. Congo red azo dye is frequently using in different textile industries at commercial level. For the removal of these types of different dyes from soil and aqueous ecosystems, viable, cost effective and eco-friendly tool is required. Microorganisms are considered useful for this purpose and this was considered in the present study. For isolation, total ten soil samples were collected, seven from root nodules of plants and three soil samples fromdye contaminated soil from ten different places of different altitudes of Uttarakhand region in India.Fifty bacterial strains were screened out from100 isolates after enrichment of 21 days in ideal conditions of temperature 37^{0}C, pH 7.0 and agitation 100rpm. Strain JK11 found as most efficient bacterial strain out of 50 with 65.05% degradation of Congo red azo dye in 24 hours at 37^{0}C temperature. The cell yield, specific degradation rate and pH for “JK11” isolate was recorded as 135.37, 0.00511 and 7.87 respectively.Thin layer chromatography, LCMS, GCMS and UV-visible spectrometric techniques were confirmed the degradation of Congo red azo dye. This technology could be very effective and useful for the removal of azo dyes from the textiles effluents in minimum time at low cost with less labor intensive powers. Based on microscopic examination, biochemical estimation & 16S rDNA sequencing method, JK11 strain found as closest neighbor of *Paenibacillus populi* strain (accession no. OK602682.1).

Keywords : Cell yield, enrichment, GCMS, *Paenibacillus populi*, specific degradation rate.

INTRODUCTION

Azo dyes are vast range of synthetic organic compounds (-N = N- group) are the largest group used in textile, paper, plastic and leather manufacturing units (Guru Lakshmi *et al*. 2008, Saharan and Ranga, 2011). There are over 10, 0000 commercially available dyes with a production of over 7×10^5 tons per year. Colored industrial effluent is the most obvious indicator of water pollution and the discharge of highly colored synthetic dye effluents is aesthetically displeasing and cause considerable damage to the light (Tripathi *et al*., 2011). Waste water from fabric printing and dyeing poses serious environmental problems because of their color and high chemical oxygen demand (Ayed*et al*. 2012).Dyes are usually aromatic and heterocyclic compounds and are often recalcitrant, some of them being toxic and even carcinogenic. They include a broad spectrum of different chemical structures, primarily based on substituted aromatic and heterocyclic groups such as the aromatic amine (C_6H_5-NH_2), which is a suspected carcinogen, phenyl (C_6H_5-CH_2) and naphthyl (NO_2-OH).

Common to them is their ability to absorb light in the visible region (Rajamohan*et al*. 2006). Presence of the dyes in aqueous ecosystem diminishes the photosynthesis by impeding the light penetration into deeper layers thereby deteriorating water quality and lowering the gas solubility. If the wasted dye baths are channeled to the rest of the plant’s wastewater streams for treatment by conventional biochemical operations, the azo dyes may go through the system without degradation and ultimately reach the receiving water with the potential to produce carcinogenic amines under anaerobic conditions (Eren and Ince, 2010).

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There are several physic-chemical methods such as adsorption, flocculation- coagulation and precipitation, photolysis, chemical oxidation and reduction, electrochemical treatment for the removal of dyes from effluents(Viral *et al*. 2012). But these methods can be cost effective and some of these can generate secondary pollutions.Many organisms such as *Bacillus*, *E. coli*, *Klebsiella*, *Enterobacter*, *Pseudomonas* and a group of fungi, yeast have been studied for their decolonization of Congo red dye by many researchers (Chen *et al*. 2003;Buan *et al* 2010; Kishor *et al*. 2018; Ceretta *et al*. 2020) but there is a need to be developed an environmental viable bioprocess for the degradation of such type of xenobiotic compounds.

However, with increasing industrialization, the magnitude of the problem is constantly on rise and needs a plausible microbial solution to be used as bioremediation tools for removal of azo dyes. This current research results showed this phenomenon very successful for the treatment of the some azo dyes which are very harmful to the fauna and flora of the environment.

MATERIALS AND METHODS

Soil Samples collection

Subsurface (1-10 cm below the surface) dyes contaminated & plant roots associated soil samples were collected in sterile bottles from different sites at different geographical locations in Uttarakhand, India. These samples were coded as JKS-1 to JKS-10 and stored at low temperature (4^{0}C) for the isolation of the azo dye decolorizing bacterial strain/s and for further analysis.

Soil analysis for various parameters

pH

10% (w/v) suspension of air dried soil of each sample was prepared in de-ionized milli-Q water. This suspension was allowed to settle for one hour and filtered through Whatman filter paper no.42. pH for all the soil filtrates was checked using a calibrated pH meter (Singapore made).

Moisture content

Weighed 10 g soil of each sample and dried at 100ºC in a hot air oven for 24 hours. The final weight of each sample was recorded using an electronic weighing balance (Sansui, Japan). The moisture content of each of the sample was calculated as follows

Moisture content (mg per gram soil) = W_1-W_2,
where,W_1 is initial weight of the soil
and W_2 is final weight of the soil after drying

Total organic content (TOC)

250 mg of air dried soil of each sample was taken in 250 ml conical flasks. 5 ml of 1N potassium dichromate solution was added to it. After that, 10 ml concentrated sulfuric acid was added gradually and the contents were incubated for 30 minutes at room temperature. Then, 100 ml de-ionized water, 5 ml of concentrated Phosphoric acid, 0.1 g of dry sodium fluoride and 0.5 ml of diphenylamine indicator were added sequentially. The contents of the flask were titrated against 0.5N ferrous ammonium sulfate. The end point was noticed a change of dull green through turbid blue to brilliant green color. Distilled water blank was run simultaneously and the TOC was calculated as described by Hooda and Kaur (1999).

$$\text{TOC (mg/g soil)} = \frac{6.791}{w\left(1-\frac{T_1}{T_2}\right)} \times 10$$

Where, T_1= Volume of titrant used against samples (ml)
T_2= Volume of titrant used against distilled water blank (ml)

Inorganic phosphate content (PO_4^{-3})

Stock solution was prepared by dissolving 136 mg of KH_2PO_4 in 100 ml distilled water, and diluted ten times. Different volumes viz. 0.2, 0.4, 0.6, 0.8 and 1.0 ml of this solution was taken and the final volume was made to 1.0 ml with distilled water. Simultaneously, the test sample was also prepared by adding 0.1 ml soil suspension (10% w/v) to 1.0 ml. distilled water. Then, 1ml 5 N

H_2SO_4, 1 ml of $(NH_4)_6Mo_7O_{24}.4H_2O$ and 0.1 ml ANSA was added to each test tube containing standard phosphate and test sample. The test mixture was allowed to stand for 10 minutes. The final volume in all the test tubes was made up to 10 ml. Finally, the absorbance (O.D) was measured at 690 nm. (Hooda and Kaur, 1999)

Nitrate content (NO_3^-)

10% (w/v) soil suspension was prepared in de-ionized water and was filtered through Whatman filter paper no 42. The filtrate was treated with 0.4 ml concentrated aluminium hydroxide suspension to remove color and avoid the organic interference from the filtrate. The mixture was vortexed. The suspension was allowed to settle for 5 minutes, and filtered through Whatman filter paper no 42. Thereafter, 0.1 ml of 1 N HCL was added to 5 ml clear filtrate. A nitrate calibration curve was plotted using a standard curve in range of 0-350 µg of nitrate. The absorbance was recorded at 220 nm and 275 nm (Hooda and Kaur, 1999).

Chloride content (Cl^-)

100 ml (10%) soil suspension was taken and the pH was adjusted to 7.0. Then, added 9.0 ml of 5% K_2CrO_4. Stirred it well and titrated with 0.0282 N $AgNO_3$ to a permanent reddish tinge. The chloridecontent was calculated as outlined by Hooda and Kaur, (1999).

Chloride (mg/g) = [Volume of $AgNO_3$ used against samples (ml) - Volume of $AgNO_3$ used against blank (ml)] X [Normality of $AgNO_3$] X 35.46 X 1000/ mg of soil sample taken.

Total colony forming unit (CFU)

10% (w/v) soil suspension in sterile normal saline was serially diluted under aseptic conditions. 100 µl serially diluted soil suspension from different dilutions were spread on nutrient agar media using spread plate method. The inoculated plates were incubated at 37⁰C for 24 hours. The number of colonies were counted using colony counter and the results were recorded as total colony forming units (CFU) per gram of soil sample (Aneja, 2002).

Enrichment and Isolation of azo dye degrading pure bacterial strain/s

Collected samples were enriched in a 100ml conical flask containing containing peptone 1g/ lit. The medium, supplemented with 500ppm Congo red azo dye as sole carbon source and then it was inoculated with 10% (v/v) soil/water sample filtrate. The flasks were incubated at 37^0C at 160 rpm in an environmental shaker (REMI CIS-24BL) for 12 days. After 12 days, sub culturing was done in fresh BSM/azo dye medium and incubated for 10 days under similar conditions (second enrichment). There were five sub culturing were done for the isolation of effective bacterial population degrading azo dyes. On the completion of incubation after 5th sub culturing, enriched microorganisms were isolated and purified on nutrient agar plates. The cultures were stored on nutrient agar slants at 4^0C in refrigerator for further use.

Screening for efficient azo dye degrader bacterial strain

5 ppm aqueous solution of Congo red dye was differentially scanned for λ_{max} of this dye using UV–VIS spectrophotometer (Perkin Elmer, Lambda 365) with double distilled water as control. Nutrient broth (50 ml) was prepared (appendix-I) and supplemented with 100 ppm model azo dye (Congo red). Different pure bacterial strains were inoculated in different conical flasks. Then all flasks were incubated at 37^0C for 144 hours. After 144 hours incubation, absorbance was recorded at 495.6nm in UV-VIS spectrophotometer to calculate percentage decolorization of azo dye as follows.

$$\% \text{ decolorization} = \frac{C-T}{C} \times 100$$

(Where, C = absorbance of control sample, T = absorbance of test sample).

Bacterial strain identification

Various microscopic and biochemical tests were performed for the identification of most efficient bacterial strain.

Molecular analysis based on 16S rDNA sequence

DNA Extraction

The sample was picked up and placed in a mortar and homogenized with 1 ml of extraction buffer and the homogenate was transferred to a 2 ml-

microfuge tube.An equal volume of Phenol: Chloroform: Isoamly alcohol (25:24:1) was added to the tubes and mixed well by gently shaking the tubes.The tubes were centrifuged at room temperature for 15 min at 14,000 rpm.The upper aqueous phase was collected in a new tube and an equal volume of Chloroform: Isoamly alcohol (24:1) was added and mixed. The upper aqueous phase obtained after centrifuging at room temperature for 10 min at 14,000rpm was transferred to a new tube. The DNA was precipitated from the solution by adding 0.1 volumes of 3 M Sodium acetate pH 7.0 and 0.7 volume of Isopropanol. After 15 min of incubation at room temperature the tubes were centrifuged at 4ºC for 15 min at 14,000 rpm.The DNA pellet was washed twice with 70% ethanol and then very briefly with 100% ethanol and air dried.The DNA was dissolved in TE (Tris-Cl 10 mM pH 8.0, EDTA 1 mM). To remove RNA 5 ìl of DNAse free RNAse A (10 mg/ml) was added to the DNA.

PCR Amplification of 16S Gene

130 ng of Extracted DNA is used for amplification along with 10pM of each primer

Composition Master MIX:

1) High-Fidelity DNA polymerase
2) 0.5mM dNTPs
3) 3.2mM MgCl2
4) PCR Enzyme Buffer

Initial Denaturation	3 minutes at 94°C
Denaturation	1 minutes at 94°C
Annealing	1 minutes 50°C 30 Cycles
Extension	2 minutes at 72°C
Final Extension	7 minutes at 72°C

Identification of the isolated strain was performed by 16S rDNA sequence analysis. Genomic DNA was obtained from the culture by using Chemistry Cyle sequencing kit (Big Dye Terminator version 3.1") and Polymer and Capillary array (POP_7 pol Capillary Array). The 16S rRNA was amplified by PCR using the16SrRNA specific primers,(16S Forward primer5'-GGATGAGCCCGCGGCCTA-3' and 16SReverse primer 5'-CGGTGTGTA CAAGGCCCGG3').100 µl reaction mixtures were prepared containing 1 µl of total DNA (135 ng/ ul),3U/µl of Taq DNA polymerase, 2.5 mM each of deoxynucleoside triphosphates (4 µl d NTP's - 2.5 mM each) with10pM of each primer (2 µl each).The PCR amplifications (total 30 cycles) were done using an initial denaturation step of 3 min at 94^{0}C, followed by the denaturation step of 1 min at 94^{0}C,annealing step of 1 min at 50^{0}C, extension step of 2 min at 72^{0}C followed by final extension for 7min at 72^{0}C, in a sequencing machine (Applied Biosystems (ABI3130) Genetic Analyzer).The sequencing was carried out according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Total ten (10) different (seven from root nodules of plants and three from dye contaminated) soil sampling sites from different altitudes of Uttarakhand were randomly selected..The description of these sites with their geographical location is depicted in Table 1.Their growth on Petri plates have been shown in Fig. 1

Six Physico-Chemical parameters were analyzed and compared with total colony forming units (CFU) per gram of soil. Maximum CFU (16.6X10^9)was found at pH 7.37 with moisture content 188mg, total organic matter 216.56mg, inorganic content 15.50 mg, Nitrate 0.902mg and

DNA	1 ul
16s Forward Primer (GGATGAGCCCGCGGCCTA)	2 ul
16s Reverse Primer (CGGTGTGTACAAGGCCCGG)	2 ul
dNTPs (2.5mM each)	4 ul
10X Taq DNA polymerase Assay Buffer	10 ul
Taq DNA Polymerase Enzyme (3U/ ml)	1 ul
Water	30 ul
Total reaction volume	50 ul

Chloride content 537.44mg per gram of soil sample. However at slightly alkaline pH value (8.06) in sample JKS-3, CFU was found minimum ($8.7X10^6$). It was also reported that CFU was maximum where moisture content, total organic content & chloride content is maximum. Acidic soil was found in one sample (JKS-9)(Table 2).

The establishment and performance of these microorganisms in the field can be affected by numerous environmental variables, such as soil pH, salinity, moisture content, and temperature (Sharma, 2013). Despite these limitations, continuing research may enable more widespread use of these Biofertilizers. One benefit of inoculating fields with endospore-forming bacteria such as *Paenibacillus* is their capacity to survive for long periods in the soil under adverse environmental conditions (Duca *et al*. 2014).

The mechanisms of how microorganisms decolorize (i.e., reduce azo bonds to aromatic amines) and degrade (i.e., break down azo dyes into small molecules leading to H_2O, CO_2, and mineral by-products) azo dyes have been a subject of interest in different studies (Khan *et al*. 2013;Yesilada *et al*. 2018;Akansha *et al*. 2019;Barathi *et al*. 2020;Yang, 2022; Dhir *et al*. 2022). In present study, JK11 isolated from dye contaminated soil sample and found as most efficient bacterial strain out of 50 with 65.05% degradation of Congo red azo dye in 24 hours at 37^0C temperature (Table 3).The cell yield, specific degradation and pH for "JK11" isolate was recorded as 135.37, 0.00511 and 7.87 respectively on the final (6th) day of incubation.
Methyl Orange (MO) showed 98% decolorization in 96 hours but could only achieve 10% decolorization under agitated condition for the same time period. Similar trend was observed for Congo red it showed 95% decolorization in static and 18% decolorization in shaking condition in 96 hours. Eriochrome Black T showed 90% decolorization in static and 35% decolorization in shaking condition for the same time period. Dye decolorization was reduced significantly in shaking condition as compared to static condition (Fig.2). This agrees with the results previously demonstrated studies (Telke *et al*. 2008;Ghodake *et al*. 2011).

The data was analyzed using SeqScape_v5.2software. Identification for isolated strains 16S rRNA sequences was achieved by comparing the contiguous sequences obtained with the 16S rRNA sequences from the reference and type strains available in public databases Gen Bank using the BLAST. The sequences were alignedusing the Jukes-Cantor corrected Model and Seq Scape_ v 5.2. The phylogenetic reconstruction was done using the neighbour-joining (NJ) algorithm, with boots trap values (Bruno *et. al*. 2000). The phylogenetic tree was created using Wighbot with alphabet size 4 and length size 1000.Based on microscopic examination, biochemical estimation & 16S rDNA sequencing method, JK11 strain found as closest neighbor of *Paenibacillus populi* strain (accession no. OK602682.1)(Figs. 3 & 4).

Aligned sequence Data of sample (JK 11) (1306 bp) is given below:

```
GACCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCAATT
CCGACTTCATGAGGCGAGTTGCAGCCTGCAATCCGAACTGAGACCA GCTTT
GATAGGATTGGCTCCCTCTCGCGAGTTCGCTTCCCGTTGTACTGGCCATTGT
AGTACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCC
CACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCATCCGAAAT
GCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACAT
CTCACGACACGAGCTGACGACAACCATGCACCACCTGTCTCCTCTGTCCCGA
AGGAAAGCCCTATCTCTAGGACGGTCAGAGGGATGTCAAGACCTGGTAAGG
TTCTTCGCGTTGCTTCGAATTAAACCACATACTCCACTGCTTGTGCGGGTCC
CCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAATG
CTTAATGTGTTAACTTCGGCACCAAGGGTATCGAAACCCCTAACACCTAGCA
TTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGT  TTGCTCCCCA
CGCTTTCGCGCCTCAGCGTCAGTTACAGCCCAGAAAGTCGCCTTCGCCACTG
GTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTTTC
CTCTTCTGCACTCAAGTCCACCAGTTTCCAGTGCGAACCAGGGTTGAGCCCC
AGCCTTAAACACCAGACTTAATGAACCGCCTGCGCGCGCTTTACGCCCAAT
AATTCCGGACAACGCTTGCCCCCTACGTATTACCGCGG  CTGCTGGCACGTAG
TTAGCCGGGGCTTTCTTCTCAGGTACCGTCACCTTGAGAGCAGTTACTCTCC
CAAGCGTTCTTCCCTGGCAACAGAGCTTTACGATCCGAAAACCTTCATCACT
CACGCGGCGTTGCTCCGTCAGGCTTTCGCCCATTGCGGAAGATTCCCTACTG
CTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCAC
CCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTG AGCCGTTACCTCACCAAC
TAGCTAATGCGCCGCAGGTCCATCCAACAGTGACAGATTGCTCCGTCTTTCC
CGATTTGCCCATGCGAGGAAATCGCGTATCCGGTATTAGCATTCGTTTCCGA
ATGTTATCCCGGTCTGTCGGGCAGGTTACCTACGTGTTACTCACCCGTCCGC
CGTAAGCATC
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The genus *Paenibacillus* contains many species which are known to promote the growth of plants including maize, Populus (Han *et al.* 2014), pumpkin (Fürnkranz*et al*. 2012), rice (De Souza*et al*.2014), switchgrass (Ker *et al.* 2014), and many others. Like other plant growth-promoting bacteria, they accomplish this through various facets. Plant-associated species of *Paenibacillus* can directly influence plant growth by producing indole-3-acetic acid (IAA) and other auxin phytohormones, solubilizing inaccessible phosphorous into form that can be taken up by plant roots, and some species can also fix atmospheric nitrogen. In addition, *Paenibacillus* helps to control phytopathogens by triggering

induced systemic resistance (ISR) and/or producing a variety of biocidal substances

Multiple metabolites were identified for parent compound such as Dodecane, Tetradecanoic acid, 2,4-Di-tert-butylphenol, Cetene, Hexacosane, exadecanoic acid, 1,6-methanonaphthalen-1(2H)-ol, octahydro, Octadecanoicacid, methyl ester, n-Hexadecanoic acid, Heneicosane, 2,5-Piperazinedione, 5-methylhex-2-yl butyl ester, 2,5-Piperazinedione etc after bacterial treatment (Fig. 5). These metabolites are reported to be highly toxic not only to aquatic life but also cause increased incidences of breast cancer and disrupt endocrine system in mammals (Kumar *et al*. 2019; Garg *et al*. 2020).

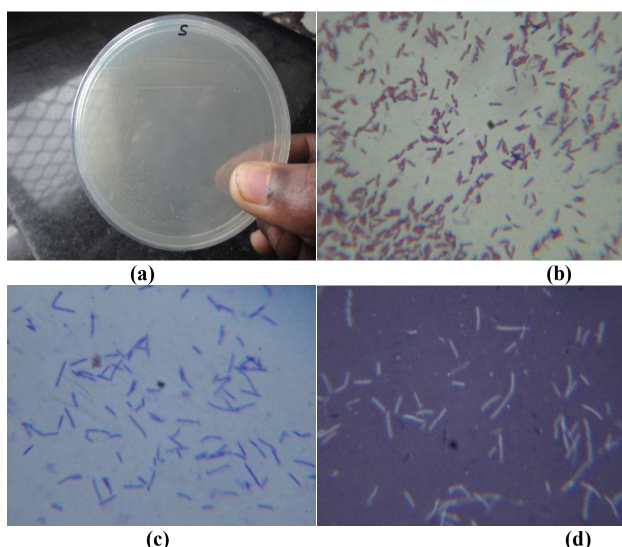


Fig. 1: (a) Growth of bacterial strain JK 11 on solid medium, (b) Gram stained cells of JK11, (c) capsule staining & (d) Negative staining of bacterial cells.

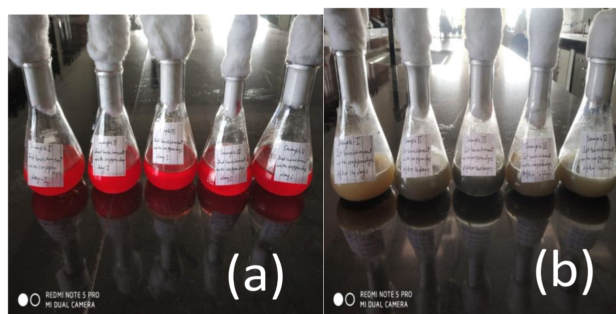


Fig.2: (a) first enrichment for 15 days (b) second enrichment for 6 days of the samples.

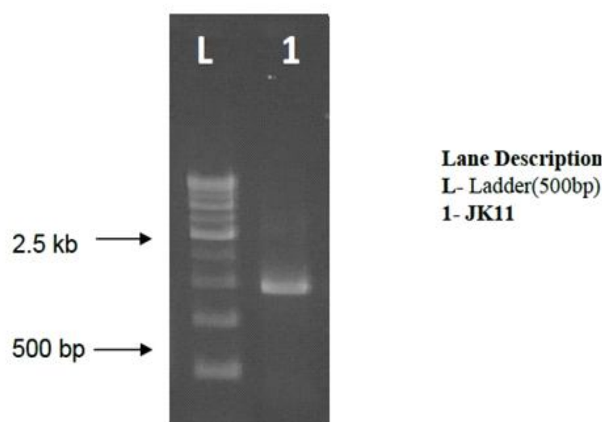


Fig. 3: Agarose gel electrophoresis of sample DNA

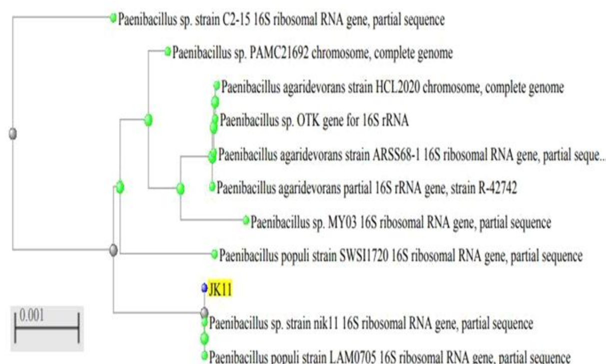


Fig. 4: The phylogenetic construction with the neighbour-joining (NJ) algorithm.

CONCLUSION

The Congo red decolorizing strains, JK11", isolated from JKS-3 sampling site from Kankhal, Haridwar (29^0.9275426N & 78^0.1443251E), Uttarakhand. JK11 strain was identified based on microscopic, biochemical & 16S rDNA sequence, as *Peanibacilius* sp. Strain. Strain JK11 found as most efficient bacterial strain out of 50 with 65.05% degradation of Congo red azo dye in 24 hours at 37^0C temperature. The cell yield, specific degradation rate and pH for "JK11" isolate was recorded as 135.37, 0.00511 and 7.87 respectively on the final (6^{th}) day of incubation. Thin layer chromatography, LCMS, GCMS and UV-visible spectrometric techniques were confirmed the degradation of Congo red azo dye.However, further studies on the isolated strains could explore new tools and techniques to evolve viable, low cost effective and eco-friendly microbial solutions for treatment of dye industry effluents(Kishor *et al*., 2018; Ceretta *et al*., 2020).

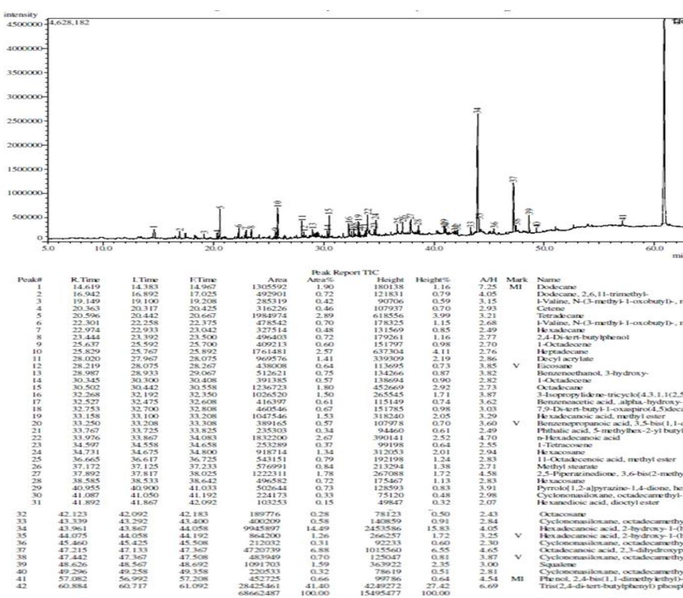
Table 1. Description of soil samples

Sample code	Geographical Location (longitude& latitude)	Collection Site	Root nodule associated/dye contaminated
JKS-1	30^0.2478528N & 78^0.0835190E	Laxman Sidh Mandir Harrawala, Dehradun, UK	Root nodule associated
JKS-2	30^0.4458532N & 78^0.0890145E	Winberg Allen School, Mussoorie, UK	Root nodule associated
JKS-3	29^0.9275426N & 78^0.1443251E	Kankhal, Haridwar, UK	Dye contaminated
JKS-4	30^0.4114188N & 78^0.2884773E	Surkanda Mata Mandir, Dhanolti, UK	Root nodule associated
JKS-5	30^0.7301236N & 78^0.4331512E	Joshiyada, Uttrakashi, UK	Root nodule associated
JKS-6	30^0.7261607N & 78^0.4373311E	Uttrakashi city, UK	Root nodule associated
JKS-7	30^0.7091573N & 78^0.3533381E	Dunda, Uttrakashi, UK	Root nodule associated
JKS-8	30^0.1221654N &78^0.3061306E	MunikeRetti, Rishikesh, UK	Dye contaminated
JKS-9	29^0.9671142N & 78^0.0596148E	SIDCUL, Haridwar, UK	Dye contaminated
JKS-10	30^0.1777107N & 77^0.9017468E	Mohand, Dehradun, UK	Root nodule associated

Table 2: Physico-chemical parameters of the soil samples and their CFUs

Sample Codes	pH of the Samples	Moisture Content (mg/gmsoil) ±S.D	Total organic matter (mg/gm soil)±S.D	Inorganic PO_4^{-3} (mg/gm soil)±S.D	NO_3^- (mg/gm soil)±S.E	Cl^-content (mg/gm soil)±S.E	CFU (No. colonies/gm soil)
JKS-1	8.05	119±0.48	123.15±0.02	10.40±0.004	3.52±0.002	112.60±0.35	5.4X10^8
JKS-2	7.33	83±0.35	159.26±0.02	16.20±0.014	0.14±0.006	396.98±0.31	8.7X10^7
JKS-3	8.06	95±0.26	72.28±0.10	02.88±0.004	2.65±0.003	47.00±0.26	8.7X10^6
JKS-4	7.20	132±0.38	217.13±0.04	09.60±0.026	2.031±0.000	487.09±0.45	9.4X10^9
JKS-5	7.37	188±0.33	216.56±0.35	15.50±0.048	0.902±0.000	537.44±0.50	16.6X10^9
JKS-6	7.54	117±0.39	83.91±0.08	01.49±0.002	2.3±0.004	17.34±0.64	12.8X10^8
JKS-7	7.87	19±0.14	129.51±0.02	15.20±0.066	2.4±0.005	56.24±0.66	6.2X10^7
JKS-8	7.51	73±0.38	87.14±0.09	00.26±0.002	1.58±0.004	157±0.64	6.9X10^6
JKS-9	6.33	119±0.28	53.03±0.07	19.65±0.018	2.0±0.007	59.64±0.33	5.7X10^6
JKS-10	7.48	83±0.28	84.35±0.003	03.90±0.018	3.12±0.003	121.0±0.31	3.7X10^8

*Values are mean of five different determinations



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	14.619	14.383	14.967	1305592	1.90	180138	1.16	7.25	MI	Dodecane
2	16.942	16.892	17.025	492901	0.72	121831	0.79	4.05		Dodecane, 2,6,11-trimethyl-
3	19.149	19.100	19.208	285319	0.42	90706	0.59	3.15		l-Valine, N-(3-methyl-1-oxobutyl)-, m
4	20.363	20.317	20.425	316226	0.46	107937	0.70	2.93		Cetene
5	20.596	20.442	20.667	1984974	2.89	618556	3.99	3.21		Tetradecane
6	22.301	22.258	22.375	478542	0.70	178325	1.15	2.68		l-Valine, N-(3-methyl-1-oxobutyl)-, m
7	22.974	22.933	23.042	327514	0.48	131569	0.85	2.49		Hexadecane
8	23.444	23.392	23.500	496403	0.72	179261	1.16	2.77		2,4-Di-tert-butylphenol
9	25.637	25.592	25.700	409213	0.60	151797	0.98	2.70		1-Octadecene
10	25.829	25.767	25.892	1761481	2.57	637304	4.11	2.76		Heptadecane
11	28.020	27.967	28.075	969576	1.41	339309	2.19	2.86		Decyl acrylate
12	28.219	28.075	28.267	438008	0.64	113695	0.73	3.85	V	Eicosane
13	28.987	28.933	29.067	512621	0.75	134266	0.87	3.82		Benzeneethanol, 3-hydroxy-
14	30.345	30.300	30.408	391385	0.57	138694	0.90	2.82		1-Octadecene
15	30.502	30.442	30.558	1236723	1.80	452669	2.92	2.73		Octadecane
16	32.268	32.192	32.350	1026520	1.50	265545	1.71	3.87		3-Isopropylidene-tricyclo[4.3.1.1(2,5;
17	32.527	32.475	32.608	416397	0.61	115149	0.74	3.62		Benzeneacetic acid, .alpha.-hydroxy-,
18	32.753	32.700	32.808	460546	0.67	151785	0.98	3.03		7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-
19	33.158	33.100	33.208	1047546	1.53	318240	2.05	3.29		Hexadecanoic acid, methyl ester
20	33.250	33.208	33.308	389165	0.57	107978	0.70	3.60	V	Benzenepropanoic acid, 3,5-bis(1,1-d
21	33.767	33.725	33.825	235303	0.34	94460	0.61	2.49		Phthalic acid, 5-methylhex-2-yl butyl-
22	33.976	33.867	34.083	1832200	2.67	390141	2.52	4.70		n-Hexadecanoic acid
23	34.597	34.558	34.658	253289	0.37	99198	0.64	2.55		1-Tetracosene
24	34.731	34.675	34.800	918714	1.34	312053	2.01	2.94		Hexacosane
25	36.665	36.617	36.725	543151	0.79	192198	1.24	2.83		11-Octadecenoic acid, methyl ester
26	37.172	37.125	37.233	576991	0.84	213294	1.38	2.71		Methyl stearate
27	37.892	37.817	38.025	1222311	1.78	267088	1.72	4.58		2,5-Piperazinedione, 3,6-bis(2-methyl
28	38.585	38.533	38.642	496582	0.72	175467	1.13	2.83		Hexacosane
29	40.955	40.900	41.033	502644	0.73	128593	0.83	3.91		Pyrrolo[1,2-a]pyrazine-1,4-dione, hex
30	41.087	41.050	41.192	224173	0.33	75120	0.48	2.98		Cyclononasiloxane, octadecamethyl-
31	41.892	41.867	42.092	103253	0.15	49847	0.32	2.07		Hexanedioic acid, dioctyl ester
32	42.123	42.092	42.183	189776	0.28	78123	0.50	2.43		Octacosane
33	43.339	43.292	43.400	400209	0.58	140859	0.91	2.84		Cyclononasiloxane, octadecamethyl-
34	43.961	43.867	44.058	9945897	14.49	2453586	15.83	4.05		Hexadecanoic acid, 2-hydroxy-1-(hyd
35	44.075	44.058	44.192	864200	1.26	266257	1.72	3.25	V	Hexadecanoic acid, 2-hydroxy-1-(hyd
36	45.460	45.425	45.508	212032	0.31	92233	0.60	2.30		Cyclononasiloxane, octadecamethyl-
37	47.215	47.133	47.367	4720739	6.88	1015560	6.55	4.65		Octadecanoic acid, 2,3-dihydroxyprop
38	47.442	47.367	47.508	483949	0.70	125047	0.81	3.87	V	Cyclononasiloxane, octadecamethyl-
39	48.626	48.567	48.692	1091703	1.59	363922	2.35	3.00		Squalene
40	49.296	49.258	49.358	220533	0.32	78619	0.51	2.81		Cyclononasiloxane, octadecamethyl-
41	57.082	56.992	57.208	452725	0.66	99786	0.64	4.54	MI	Phenol, 2,4-bis(1,1-dimethylethyl)-, p
42	60.884	60.717	61.092	28425461	41.40	4249272	27.42	6.69		Tris(2,4-di-tert-butylphenyl) phosphat
				68662487	100.00	15495477	100.00			

Fig. 5: Compounds identified in untreated and bacteria treated textile industry wastewater by LC–MS analysis.

Table 3: Comparative Congo red azo dye degradation ability of various bacterial strains isolated from different soil samples after 1 days (24hr) of incubation at 37^{0}C

Soil sample	Strain code	Final day pH	% Degradation	Specific degradation*	Cell yield#
JKS-1	JK01	6.98±0.010	9.11%	0.00077	1305.3
	JK02	6.76±0.011	5.05%	0.00108	923.76
	JK03	7.32±0.009	5.78%	0.00137	724.78
	JK04	7.14±0.010	13.05%	0.00103	965.56
	JK05	7.45±0.013	7.77%	0.00476	209.67
JKS-2	JK06	7.84±0.012	4.06%	0.00359	281.07
	JK07	6.94±0.012	7.87%	0.00642	155.75
	JK08	6.66±0.015	2.05%	0.00672	148.63
	JK09	7.87±0.012	11.07%	0.00818	122.17
	JK10	7.89±0.011	3.86%	0.00601	166.31
JKS-3	JK11	7.87±0.010	65.05%	0.00511	135.37
	JK12	7.87±0.001	48.56%	0.00526	190.06
	JK13	6.95±0.012	30.07%	0.00379	275.00
	JK14	6.50±0.013	10.11%	0.00780	128.12
	JK15	6.87±0.013	25.07%	0.00387	258.06
JKS-4	JK16	8.45±0.015	9.78%	0.00376	265.14
	JK17	7.43±0.014	5.08%	0.00402	248.29
	JK18	8.44±0.013	4.78%	0.00188	530.70
	JK19	8.05±0.010	8.07%	0.00177	562.83
	JK20	8.42±0.011	5.08%	0.00157	634.69
JKS-5	JK21	8.12±0.013	6.05%	0.00269	370.78
	JK22	7.69±0.013	9.88%	0.00813	122.90
	JK23	8.23±0.011	7.89%	0.00130	765.48
	JK24	7.93±0.010	5.47%	0.00458	218.11
	JK25	7.98±0.010	10.97%	0.00245	407.35
JKS-6	JK26	6.40±0.013	8.89%	0.00385	259.42
	JK27	8.05±0.014	6.05	0.00199	500.24
	JK28	6.52±0.014	8.09%	0.00638	610.08
	JK29	8.16±0.015	8.01%	0.00183	543.97
	JK30	6.81±0.013	7.04%	0.00143	691.60
JKS-7	JK31	7.79±0.012	11.99%	0.00385	259.34
	JK32	8.08±0.010	7.11%	0.00167	296.22
	JK33	8.18±0.010	13.78%	0.00107	931.18
	JK35	7.82±0.012	16.98%	0.00454	220.10
	JK36	6.98±0.010	1.78%	0.00077	1305.3
JKS-8	JK38	6.76±0.012	29.54%	0.00108	923.76
	JK39	7.32±0.012	28.78%	0.00137	724.78
	JK41	7.14±0.013	17.77%	0.00103	965.56
	JK43	7.4540.015	31.45%	0.00476	209.67
	JK44	7.84±0.014	24.75%	0.00359	281.07
JKS-9	JK46	6.87±0.013	25.75	0.00387	258.06
	JK47	6.95±0.012	16.08%	0.00379	275.00
	JK51	7.87±0.013	29.75	0.00526	190.06
	JK54	7.89±0.014	43.87%	0.00601	166.31
	JK55	7.87±0.012	24.01%	0.00511	195.37
JKS-10	JK56	7.87±0.013	7.05%	0.00818	122.17
	JK63	6.50±0.014	3.45%	0.00780	128.12
	JK66	6.66±0.010	5.95%	0.00672	148.63
	JK79	6.94±0.010	6.89%	0.00642	155.75
	JK81	7.25±0.012	2.07%	0.00176	115.14

*Gram of Congo red azo dye utilized per gram biomass generated.
#Gram of biomass generated per gram of Congo red azo dye utilized.

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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