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ORIGINAL RESEARCH PAPER

Screening of environmental bacteria for multiple dye decolorization capabilities in textile wastewater

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Abstract

Textile industry effluents are rich in microorganisms capable of dye decolorization, including bacteria, actinomycetes, and yeast. Conventional microbiological techniques were used to isolate these bacteria, with 10 strains selected for screening. These cultures belonged to *Enterococcus, Bacillus, Pseudomonas, Xanthomonas, Sporolactobacillus,* and *Enterobacter* genera. The decolorization of these dyes was examined using UV-visible spectrophotometers, revealing varying resistance and sensitivity to antibiotics.

Keywords: Decolorization, Bacterial isolates, Screening, Textile dyes, Laboratory dyes, Antibiotic sensitivity.

Introduction

Health and agricultural problems are caused by wastewater discharge from textile and dye production. Decolorization techniques have been studied, but few are accepted due to high costs, low efficiency, and diverse dyes. Microbial methods for decolorizing and degrading dyes are considered cost-effective for eliminating environmental pollutants.

Biodegradation by microorganisms is a potential technique for treating dye-containing effluents, as several studies have shown.[2,14] The adaptability and activity of the chosen microorganisms[3] influence the success of microbial decolorization. Various microorganisms have been identified as capable of degrading dyes, including bacteria[10,16], filamentous fungi[1,6,11], and yeasts.[7-9] Actinomycetes[15,16], and algae.[4] It is recognized that several bacterial strains can reductively cleave certain dyes to produce aryl amines under anaerobic conditions; however, these dyes pose challenges for aerobic degradation (Balan, D.S.L. & R.T.R. Monteiro. 2001).

In this study, we screened a number of bacteria that were isolated from textile effluents and gathered at various treatment stages.

Materials and Methods

Dyes

Textile dyes, including Direct Brown 02, Direct Violet 09, Direct Red 31, Direct Green 26, Direct Black 22, and Direct Red 37 (marked T1 to T6), were obtained from a number of dye-producing companies situated in Ankelshwar, Ahmedabad, Vapi, and Jetpur. The laboratory dyes used in this investigation, designated L1 through L8, are Crystal Violet, Malachite Green, Methylene Blue, Congo Red, Phenol Red, Safranine, Brilliant Green, and Fast Green. They were purchased from Hi-Media, India. (Brown, D. & P. Laboureur. 19830

Sample collection

In order to separate dye-decolorizing bacteria, samples were taken from the Jetpur Textile Dyeing and Printing Association's common effluent treatment facility at six different stages using autoclaved bottles.

Enrichment, isolation and Identification of dye-decolorizing bacteria

The samples were gathered from the Central Effluent Treatment Plant (CETP) and the nearby agricultural areas that regularly get irrigation from the treated wastewater. The samples included textile wastewater, soil, and sludge.

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Six fabrics and eight synthetic dyes were used for this study, which employed a pH-7.0 mineral salt medium (MSM). The ultimate dye concentration was 1 $mg.mL^{-1}$, which was achieved by combining 06 different dyes. (Chen, K.C., Jane, Y.W., Liou, D.J., & J.H. Sz-Chwun 2003) The bacteria relied only on the dyes as a carbon source.

The experiment involved streaking the medium onto sterile nutrient agar plates, incubating for 24 to 72 hours, and then serially transferring the enriched culture to a fresh medium for 30 days.

Screening of potential dye-decolorizing bacterial isolates

From CETP, a total of 25 bacterial strains were initially identified. A selection of six textile colors and eight laboratory dyes were then added to 100 mL of CMB, which had been inoculated with 1-mL samples from the strains' 24-hour-old cultures. After that, the inoculation flasks were incubated for ninety-six hours under static conditions.

Primary screening of potential dye-decolorizing bacterial isolates

One milliliter of the 25 strains' 24-hour-old cultures was used to inoculate 100 mL of culture media that had been enhanced with six different textile colors. (Dilek, F.B., Taplamacioglu, H.M. & E. Tarlan. 1999) The infected flasks were shaken and left to incubate for 96 hours. To find the decolorizing activity, samples were taken out of the flasks every twelve hours and analyzed.

Cell morphology & Biochemical tests

All isolates' 24-hour-old cultures were subjected to Gram staining in order to examine cell morphology and the Gram response (Felsenstein J. 1985). The media needed for each biochemical test were made, 10 µL of culture that was 24 hours old was added, and then the plates were incubated at 37℃ for 24 hours.

Identification of bacterial isolates:

Bergey's Manual of Systematic Bacteriology was used to identify bacterial isolates that can decolorize dyes. Moreover, molecular analysis, especially 16S rRNA sequencing, was used to describe the putative bacterial isolates.

16S rDNA sequencing of bacterial isolates

DNA was extracted from bacterial cultures for 16S rDNA sequencing. The quality was assessed using agarose gel. 16S rRNA genes were sequenced using universal primers. Sequences were compared against the NCBI database, and the top ten hits were selected for phylogenetic analysis. Sequence alignment was performed using ClustalW (Gold, M.H. & M. Alic, 1993).

Antibiotic sensitivity

The study used Octa discs containing various antibiotics to test antibiotic sensitivity. The discs were prepared using molten antibiotic assay medium (AAM) and inoculated with 0.1 mL 24-hour-old culture. They were then placed on the medium, incubated at 37°C for 24 hours, and tested for antibiotic resistance. (Itoh, K., Yatome, C. & T. Ogawa. 1993)

Table 1: Summary of samples collected from various sites of CETP, Jetpur

Treatment stage	*Sample*	*Color*	*pH*
Stage 1	Raw Sewage	Reddish Brown	8.1
Stage 2	Raw Filtered sewage	Reddish Brown	8.0
Stage 3	Activated sludge-1	Brown	8.0
Stage 4	Activated sludge-2	Light brown	7.5
Stage 5	Sludge Solids	Blackish Green	7.3
Stage 6	Treated Effluent	Light green	7.3

Results and Discussion

Samples were gathered from six phases of wastewater treatment facilities for textiles.

Industrial dyes give raw sewage its characteristic reddish-brown color. As the treatment progresses, the color shifts from reddish-brown to brown, light brown, blackish-green, and finally pale green. The light green color may be due to *Pseudomonas* species synthesis. The pH of untreated effluent (pH 8.1) decreased to neutral during treatment (pH 7.3).

Enrichment, isolation and screening of potential dye-decolorizing bacteria

Effluents contain microorganisms that can either actively decolorize or degrade dyes. To cultivate microbial populations capable of both processes, a mixture of selected textile dyes is introduced into a mineral salt medium supplemented with trace elements. (Martins, M.A., Cardoso, M.H., Queiroz, M.J., Ramalho, M.T., & A. M. Campos. 1999) Over time, these bacterial populations will surpass those without dyes as their exclusive carbon source. Based on visual decolorization, ten isolates were chosen, and their capacity to decolorize particular textile and lab colors was examined.

Growth characteristics

The bacterial strains were categorized as gram-positive (21 isolates) and gram-negative (4 isolates). The spore-forming, gram-positive isolates had unique coloration, borders, and sizes. (Meehan, C., Banat, I.M., McMullan, G., Nigam, P., Smyth, F., & R. Marchant. 2000) The colonies ranged in size, texture, and elevations, with opaque colonies indicating Gram-positive bacteria and transparent colonies indicating Gram-negative organism.

Table 4 displays the filiform, echinulate, and arborescent growth pattern and moderate to high abundance of the isolates on nutritional agar slant.

Table 2: Decolorization (%) of textile dyes by the cultures of bacterial isolates growing on a Complete medium containing textile dyes under static condition

Isolates	*T1*	*T2*	*T3*	*T4*	*T5*	*T6*
CV1	52	69		67	58	94
CV2	2	35	78	49	55	39
CV3	44	53	32	55	24	65
CV4	49	26	89	56	15	64
CV5	31	66	47	21	66	95
CV6	42	65	58	57	35	76
CV7	37	55	26	46	95	85
CV8	46	46	27	95	48	85
CV9	47	42	83	32	36	21
CV10	79	88	91	100	88	100
Decolorization (%)		0-25	26-50		51-75	76-100

Table 3: Decolorization (%) of laboratory dyes by the culture of bacterial isolates growing on a complete medium under static condition

Isolates	*Laboratory dyes*							
	Crystal violet	*Malachite green*	*Methylene blue*	*Congo red*	*Phenol red*	*Safranine*	*Brilliant green*	*Fast green*
CV-01	24	59	57	38	07	56	84	79
CV-02	36	54	78	65	44	57	68	14
CV-03	44	75	36	29	68	48	12	34
CV-04	25	45	75	50	68	43	72	49
CV-05	57	36	88	71	53	61	30	25
CV-06	19	40	53	77	39	52	43	67
CV-07	28	35	47	20	57	68	81	35
CV-08	84	75	68	25	34	59	57	21
CV-09	55	28	36	84	58	57	60	50
CV-10	50	67	63	54	78	89	72	67
Decolorization (%)		0-25	26-50	51-75	76-100			

As seen in Table 5, the isolates developed primarily near the bottom of the nutrient broth. However, several also displayed flaky aggregates, finely distributed growth throughout the medium, or thick, pod-like growth on the surface.

Biochemical tests

Most of the isolates, except for 25, could ferment sugars. All sugars, except sorbitol, underwent fermentation, producing acid without releasing gas (refer to Table 6). None of the isolates showed the capacity to ferment sorbitol, while isolates 1, 2, 9, 10, 12, 14, 17, 22, and 25 were unable to ferment lactose. All isolates except for 25 fermented glucose, fructose, and sucrose, and all isolates except for 2, 17, and 25 fermented maltose. Furthermore, isolates 4 and 25 did not ferment the pentose sugar xylose.

During their development on triple sugar iron (TSI) agar, none of the isolates produced gas or hydrogen sulfide. While the other isolates fermented the sugars in TSI, as seen by the shift in slant color from red to yellow, isolates 3, 12, 13, and 25 did not create acid, producing red slants (Table 6).

All isolates hydrolyzed gelatin and tributyrin, according to research on the hydrolysis of different complicated polymeric macromolecules. However, all isolates except isolates 3, 10, and 13 hydrolyzed casein. Notably, isolates 2, 6, 12, 24, and 25 showed no signs of hydrolyzing starch. (Sani, R. & U. Banerjee**.** 1999) Moreover, all isolates (Table 7) were unable to use citrate as their exclusive carbon source, with the exception of 9, 22, 24, and 25. Furthermore, every isolate except for five produced catalases, and every isolate except for 1, 8, and 15 had positive oxidase activity tests (see Table 7).

The study also assessed the impact of using different nitrogen molecules. All isolates generated negative findings in the deamination test, and the results showed that none

Table 4: Colony characteristics of dye decolorizing bacterial isolates on nutrient agar

Isolates	*Colony characteristics*							
	Size	*Pigmentation*	*Form*	*Margin*	*Opacity*	*Elevation*	*Texture*	*Gram's reaction*
CV-01	M	G	Circular	Entire	Tp	Um	S	+ve
CV-02	L	G	Irregular	Filamentous	Tp	F	S	+ve
CV-03	S	LY	Circular	Entire	Tp	R	S	-ve
CV-04	M	G	Rhizoid	Filamentous	Tp	Con	S	+ve
CV-05	M	W	Circular	Entire	Op	F	S	+ve
CV-06	L	G	Irregular	Undulate	Tp	F	S	+ve
CV-07	L	LY	Irregular	Uneven	Tp	F	S	+ve
CV-08	M	W	Uneven	Uneven	Op	F	S	+ve
CV-09	M	LY	Circular	Even	Tp	SR	R	-ve
CV-10	M	G	Irregular	Filamentous	Op	SR	S	+ve
CV-11	L	W	Rhizoid	Wavy	Op	F	S	+ve
CV-12	M	G	Circular	Entire	Tp	R	S	-ve
CV-13	M	LY	Circular	Even	Tp	SR	S	-ve
CV-14	L	G	Irregular	Uneven	Op	F	S	+ve
CV-15	M	LY	Circular	Even	Tp	SR	S	-ve
CV-16	M	G	Circular	Even	Tp	R	S	+ve
CV-17	L	LY	Circular	Uneven	Tp	F	S	+ve
CV-18	L	W	Irregular	Uneven	Op	R	S	+ve
CV-19	M	G	Circular	Uneven	Op	SR	S	+ve
CV-20	S	W	Circular	Wavy	Op	F	R	+ve
CV-21	M	W	Circular	Even	Op	R	S	+ve
CV-22	L	W	Circular	Even	Op	Um	R	+ve
CV-23	L	W	Irregular	Even	Op	F	S	+ve
CV-24	L	LY	Irregular	Uneven	Op	F	S	+ve
CV-25	M	LY	Circular	Even	Tp	SR	S	-ve

(S, small; M, moderate; L, Large; G, gray; W, White; LY, Light Yellow; Tp, transparent; Op, opaque; Um, Umbonate; F, flat; R, raised; SR, Slight raised; Con, convex; S, smooth; R, rough)

of the isolates formed indole (Tamura K., Stecher G., and Kumar S. 2021). Nonetheless, it was verified that all isolates produced ammonia.

It was determined that isolates 8, 9, and 11 produced H2S when growing in the medium. It was also shown that whereas the other isolates tested positive for this activity, isolates 4, 5, 6, 8, 9, 13, 19, 23, and 25 did not decrease NO3. (Swamy, J. & J.A. Ramsay. 1999)

Table 5: Growth pattern of dye decolorizing bacterial isolates in nutrient broth

Isolates	*Growth characteristics*			
	Turbidity	*Flocculants*	*Pellicle*	*Deposit*
CV-01	---	---	---	Concentration of growth at bottom of broth culture
CV-02	---	---	---	Concentration of growth at the bottom of broth culture
CV-03	---	---	Thick, Pod like growth on the surface	---
CV-04	---	---	---	Concentration of growth at the bottom of broth culture
CV-05	---	---	---	Concentration of growth at the bottom of broth culture
CV-06	---	---	---	Concentration of growth at the bottom of broth culture
CV-07	---	---	---	Concentration of growth at the bottom of broth culture
CV-08	---	---	Thick, Pod like growth on the surface	---
CV-09	Finely dispersed	---	---	---
CV-10	---	Flaky aggregate	---	---
CV-11	---	---	Thick, Pod like growth on the surface	---
CV-12	---	---	---	Concentration of growth at the bottom of broth culture
CV-13	Finely dispersed	---	---	---
CV-14	---	---	Thick, Pod like growth on the surface	---
CV-15	Finely dispersed	---	---	---
CV-16	---	---	---	Concentration of growth at the bottom of broth culture
CV-17	---	---	---	Concentration of growth at the bottom of broth culture
CV-18	---	Flaky aggregate	---	---
CV-19	---	---	---	Concentration of growth at the bottom of broth culture
CV-20	---	---	Thick, Pod like growth on the surface	---
CV-21	---	---	Thick, Pod like growth on the surface	---
CV-22	Finely dispersed	---	---	---
CV-23	---	Flaky aggregate	---	---
CV-24	---	---	---	Concentration of growth at the bottom of broth culture
CV-25	Finely dispersed	---	---	---

Table 6: Fermentation of sugars and biochemical changes on TSI by the cultures of dye-decolorizing bacterial isolates

Isolates	*Fermentation*								*Triple Sugar Iron Slant*			
	Suc	*Mal*	*Lac*	*Glu*	*Xyl*	*Man*	*Fru*	*Sor*	*Slant*	*Butt*	*Gas*	*H_2S*
CV-01	+/-	+/-	-/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-02	+/-	-/-	-/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-03	+/-	+/-	-/-	+/-	+/-	+/-	+/-	-/-	R	Y	-	-
CV-04	+/-	+/-	+/-	+/-	-/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-05	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-/-	Y	Y	-	-
CV-06	+/-	+/-	+/-	+/-	+/-	-/-	+/-	-/-	NP	NP	NP	NP
CV-07	+/-	+/-	+/-	+/-	+/-	-/-	+/-	-/-	Y	Y	-	-
CV-08	+/-	+/-	-/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-09	+/-	+/-	-/-	+/-	+/-	+/-	+/-	-/-	Y	Y	-	-
CV-10	+/-	+/-	-/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-11	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-12	+/-	+/-	-/-	+/-	+/-	+/-	+/-	-/-	R	Y	-	-
CV-13	+/-	+/-	-/-	+/-	+/-	+/-	+/-	-/-	R	Y	-	-
CV-14	+/-	+/-	-/-	+/-	+/-	-/-	+/-	-/-	NP	NP	NP	NP
CV-15	+/-	+/-	+/-	+/-	+/-	-/-	+/-	-/-	Y	Y	-	-
CV-16	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-17	+/-	-/-	-/-	+/-	+/-	-/-	+/-	-/-	NP	NP	NP	NP
CV-18	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-19	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-20	+/-	+/-	+/-	+/-	+/-	-/-	+/-	-/-	NP	NP	NP	NP
CV-21	+/-	+/-	+/-	+/-	+/-	-/-	+/-	-/-	NP	NP	NP	NP
CV-22	+/-	+/-	-/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-23	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-24	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-/-	NP	NP	NP	NP
CV-25	-/-	-/-	-/-	-/-	-/-	-/-	+/-	-/-	R	Y	-	-

[Glc: Glucose; Mal: Maltose; Sur: Sucrose; Lac: Lactose; Man: Manitol; Xyl: Xylose; Fru: Fructose; Sor: Sorbitol], [+/+ indicate acid production/ gas production; -/- indicate no acid/no gas production, NP- Not performed]

Every isolate grew at 25, 37, 40, and 45℃, as indicated in Table 8. While the other isolates grew at 50°C, isolates 4, 8, 9, 10, 14, 18, and 20 did not.

Identification of bacterial isolates

The isolates were provisionally identified and assigned the designations CV-1 to CV-25 based on all of the growth parameters and biochemical tests previously reported (Table 8).

Isolates CV-2, CV-4, CV-6, CV-8, CV-11, CV-14, CV-16, CV-17, CV-18, CV-19, CV-20, CV-21, CV-22, CV-23 and CV-24 belong to genus *Bacillus*. Among them, isolates CV-2 were identified as *Bacillus circulans,* respectively. Isolates CV-7 and CV-12 belong to the genus *Enterobacter.* (Wesenberg, D., Buchon, F., & S.N. Agathos**.** 2002) Isolates 1 and 5 and isolate 10 belong to the genera *Brucella, Exiguobacterium and Enterococcus*; Isolates CV-9, CV-13, CV-15 and CV-25 are identified as *Pseudomonas*, CV-3 isolates belong to genus *Xanthomonas*.

Taxonomic Identification of bacterial isolates

The study identified CV-1, CV-5, and CV-10 bacterial isolates through rRNA sequencing gene sequence analysis. They were identified as *Brucella intermedia*, *Exiguobacterium arabatum,* and *Enterococcus innesii.* The Maximum Likelihood technique and bootstrap consensus tree[13] were used to infer the evolutionary history. The neighbor-Join and BioNJ algorithms were used to automatically obtain the initial tree(s).[5]

The strain CV-1, based on 16S rRNA gene sequencing, is

Table 7: Biochemical tests for the Identification of dye-decolorizing bacterial isolate

Isolates	*Hydrolysis*				*Citrate utilization*	*MR*	*VP*	*Catalase*	*Oxidase*	*Indole production*	*H_2S production*	*Ammonia production*	*NO_3 reduction*	*Deamination test*	*Motility*
	Casein	*Gelatin*	*Starch*	*Tributyrin*											
CV-01	+	+	+	+	-	NP	NP	+	-	NP	-	+	+	-	+
CV-02	+	+	-	+	-	NP	NP	+	+	NP	-	+	+	-	-
CV-03	-	+	+	+	-	+	-	+	+	-	-	+	+	-	+
CV-04	+	+	+	+	-	NP	NP	+	+	NP	-	+	-	-	+
CV-05	+	+	+	+	-	+	-	-	+	-	-	+	-	-	+
CV-06	+	+	-	+	-	NP	NP	+	+	NP	-	+	-	-	+
CV-07	+	+	+	+	-	+	-	+	+	-	-	+	+	-	+
CV-08	+	+	+	+	-	NP	NP	+	-	NP	+	+	-	-	+
CV-09	+	+	+	+	+	-	-	+	+	-	+	+	-	-	-
CV-10	-	+	+	+	-	NP	NP	+	+	NP	-	+	+	-	-
CV-11	+	+	+	+	-	NP	NP	+	+	NP	+	+	+	-	+
CV-12	+	+	-	+	-	+	-	+	+	-	-	+	+	-	-
CV-13	-	+	+	+	-	+	-	+	+	-	-	+	-	-	+
CV-14	+	+	+	+	-	NP	NP	+	+	NP	-	+	+	-	-
CV-15	+	+	+	+	-	-	-	+	-	-	-	+	+	-	-
CV-16	+	+	+	+	-	NP	NP	+	+	NP	-	+	+	-	+
CV-17	+	+	+	+	-	NP	NP	+	+	NP	-	+	+	-	+
CV-18	+	+	+	+	-	NP	NP	+	+	NP	-	+	+	-	+
CV-19	+	+	+	+	-	NP	NP	+	+	NP	-	+	-	-	+
CV-20	+	+	+	+	-	NP	NP	+	+	NP	-	+	+	-	+
CV-21	+	+	+	+	-	NP	NP	+	+	NP	-	+	+	-	+
CV-22	+	+	+	+	+	NP	NP	+	+	NP	-	+	+	-	+
CV-23	+	+	+	+	-	NP	NP	+	+	NP	-	+	-	-	+
CV-24	+	+	-	+	+	NP	NP	+	+	NP	-	+	+	-	+
CV-25	+	+	-	+	+	-	-	+	+	-	-	+	-	-	+

Table 8. Growth of dye-decolorizing bacterial isolates in nutrient broth at different incubation temperatures and tentative Identification based on morphological and biochemical characteristics

Isolates	*Incubation temperature (°C)*					*Identification*
	25	*37*	*40*	*45*	*50*	
CV-01	+	+	+	+	+	Brucella intermedia
CV-02	+	+	+	+	+	Bacillus circulans
CV-03	+	+	+	+	+	*Xanthomonas* sp.
CV-04	+	+	+	+	-	Bacillus sp.
CV-05	+	+	+	+	+	Exiguobacterium arabatum
CV-06	+	+	+	+	+	Bacillus sp.
CV-07	+	+	+	+	+	*Enterobacter sp*.
CV-08	+	+	+	+	-	Bacillus sp.
CV-09	+	+	+	+	-	Pseudomonas sp.
CV-10	+	+	+	+	-	Enterococcus sp.
CV-11	+	+	+	+	+	Bacillus sp.
CV-12	+	+	+	+	+	*Enterobacter sp*.
CV-13	+	+	+	+	+	Pseudomonas sp.
CV-14	+	+	+	+	-	Bacillus subtilis
CV-15	+	+	+	+	+	Pseudomonas sp.
CV-16	+	+	+	+	+	Bacillus sp.
CV-17	+	+	+	+	+	Bacillus sp.
CV-18	+	+	+	+	-	Bacillus sp.
CV-19	+	+	+	+	+	Bacillus sp.
CV-20	+	+	+	+	-	Bacillus sp.
CV-21	+	+	+	+	+	Bacillus sp.
CV-22	+	+	+	+	+	Bacillus sp.
CV-23	+	+	+	+	+	Bacillus sp.
CV-24	+	+	+	+	+	Bacillus sp.
CV-25	+	+	+	+	+	Pseudomonas aeruginosa

[(+) Indicate presence of growth and (-) absence of growth] [Identification is based on Bergey's Manual of Systematic Bacteriology, Vol. 2 & 3, Edi. 1986)

closely related to *Brucella intermedia* strain NBRC 15820, *B. ovis* ATCC 25840, and *B. melitensis* strain 16M. Despite high similarity, significant phenotypic variations led to its classification as *B. intermedia.*

The strain CV-5, based on 16S rRNA gene sequencing, is most closely related to *Exiguobacterium arabatum* strain RFL1109, followed by *E. profundum* strain 10C and *E. qingdaonense* strain S82. Despite high similarity, significant phenotypic variations exist, indicating a complex taxonomic classification.

The strain CV-10, based on 16S rRNA gene sequencing, is most closely related to *Enterococcus innesii* strain GAL7, followed *by E. casseliflavus* strain NCIMB 11449 and *E. casseliflavus* strain NBRC, but due to significant phenotypic variations, it was classified as *Enterococcus innesii.*

Decolorization assay and secondary screening

The dye concentration in industrial wastewater typically ranges from 10 to 50 mg.l-1. However, fluctuations in operational conditions can result in even higher dye concentrations, making it essential to determine whether the Indigenous microbial community responsible for dye decolorization can tolerate these elevated levels. (Zhou, W. & W. Zimmermann**.** 1993)

In light of this, we employed a dye concentration of 50 mg. l-1 to isolate bacteria capable of decolorizing dyes. The isolated microbial strains' average decolorization rates ranged from 50 to 100% (refer to Tables 2 and 3).

Phylogenetic Tree:

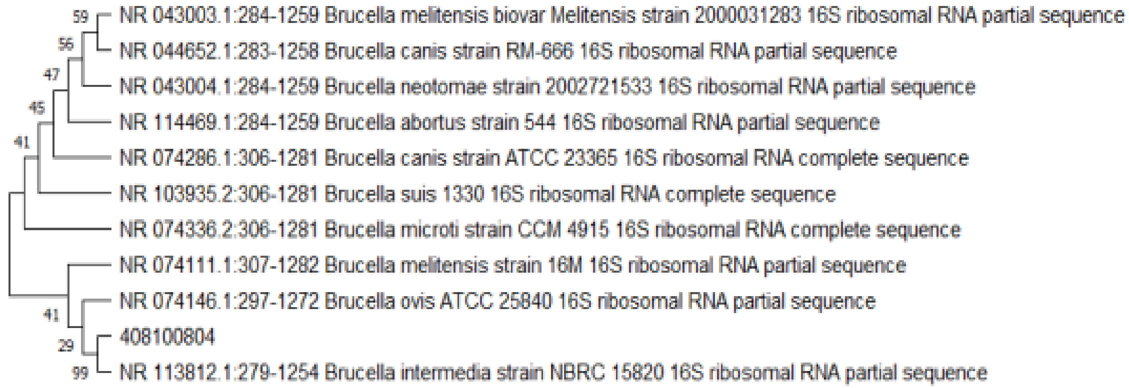

Figure 1: Phylogram of isolate CV-1 based on 16S rRNA gene analysis (Phylogram: Constructed by MEGA version 6.0.).

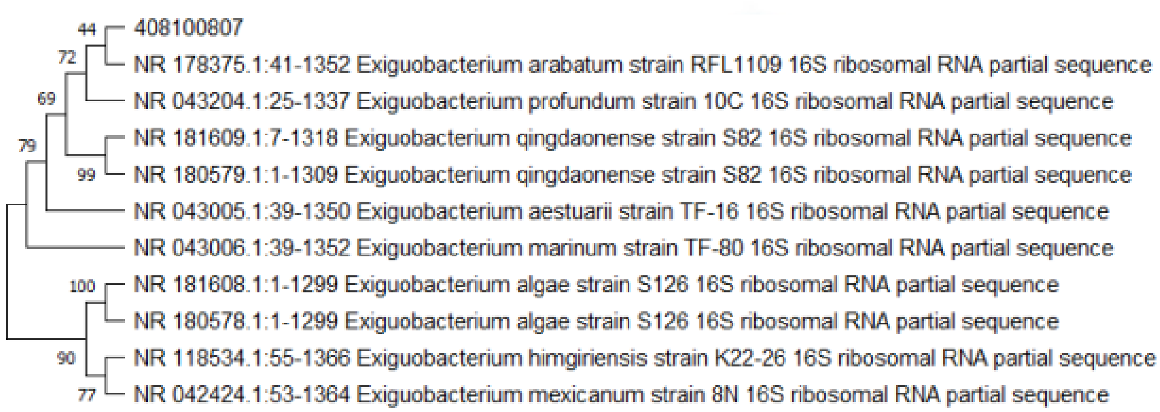

Figure 2: Phylogram of isolate CV-5 based on 16S rRNA gene analysis (Phylogram: Constructed by MEGA version 6.0).

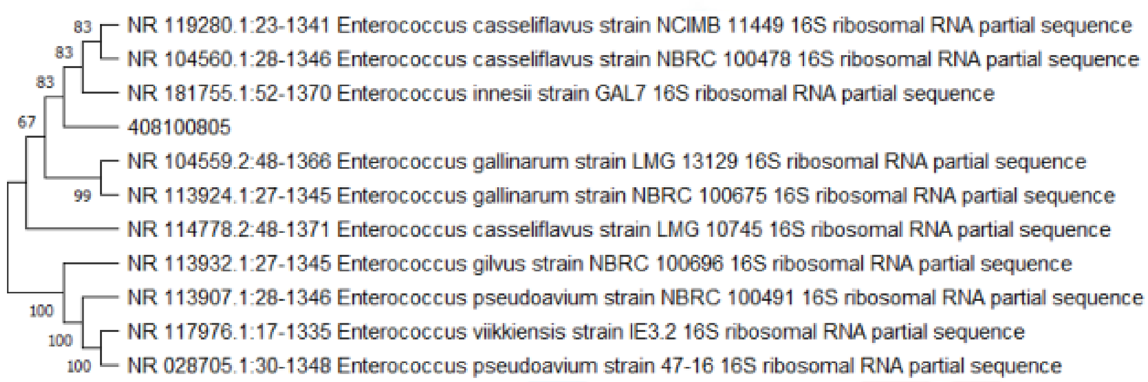

Figure 3: Phylogram of isolate CV-10 based on 16S rRNA gene analysis (Phylogram: Constructed by MEGA version 6.0.).

Antibiotic Sensitivity Testing

Inhibition zones were revealed by analyzing sixteen antibiotics against isolates numbered one through twenty-five. There are three groups of isolates: those with an inhibition zone larger than 20 mm, those with a zone between 11 and 20 mm, and those with a zone less than 10 mm. Compared to isolates exposed to tetracycline, those with an inhibition zone larger than 20 mm were less sensitive to amoxicillin.

Isolates with greater sensitivity showed greater sensitivity to co-trimoxazole, ciprofloxacin, gentamicin, erythromycin, chloramphenicol, and streptomycin (Zimmerman, T., Kulla, H.G., & T. Leisinger. 1982). Only isolate 21 was most resistant to Kanamycin and isolates 6 and 7 were resistant with a 2 mm inhibition zone. The study highlights the importance of sensitivity in molecular biology examination.

Conclusion

Textile effluents are a significant reservoir for bacterial populations capable of dye decolorization. A total of 25 bacterial isolates were initially collected, each exhibiting varying levels of dye decolorization activity. Ten isolates were chosen based on their decolorization percentage. Each isolate underwent characterization based on growth patterns, Gram staining, and biochemical assays. The isolates were tentatively identified according to these characteristics and were determined to be distinct.

Enterococcus innesii species were predominant in the effluents, followed closely by *E. arabatum* species and other genera. Among the 10- isolates, *Enterococcus innesii* species CV-10 demonstrated the highest capacity for decolorizing all the dyes tested, leading to its selection for further investigation. The study included 06 textile dyes and 8 laboratory dyes, all decolorized by every bacterial isolate. Nearly all isolates exhibited resistance to a range of tested antibiotics.

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