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Characterization of Bacteria Isolated from Dye Contaminated Soil and Textile Industry Effluents

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Abstract: Synthetic dyes and their effluents are harmful, posing serious threat to the environment and hence becomes a challenge to degrade it in an environment-friendly way. Traditional methods are extremely costly, energy-consuming, non-eco-friendly as it generates a lot of toxic sludge impacting the environment. Henceforth, microorganisms especially bacteria can be imposed in treating these effluents. Bacteria were isolated from the samples collected from common effluent treatment plant, Spectrum process factory, Tirupur and dye contaminated soil, Kanchipuram. 55 morphologically different bacterial colonies were isolated and characterized. The present study illustrates the biodiversity of bacterial strains from textile industry effluents which explains their adaptive nature within the toxic dyes and also effluents as sole source of nutrients for their growth.

Keywords: Textile industry, Effluent treatment plant, Synthetic dyes, Dye degrading bacteria, Bioremediation

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Introduction

Textile industry being the ancient industries in India and are growing rapidly every day. Considering the quantity and effluent composition of the textile wastewater, it is rated as the most polluting among the growing industrial sectors. The presence of high values of biological oxygen demand (BOD) and chemical oxygen demand (COD) in the untreated textile wastewater can cause rapid depletion of dissolved oxygen if it is directly discharged into the water sources and are

highly toxic to biological life (Palamthodi *et al.*, 2011). The high-level nitrogen content in the effluents may cause eutrophication of closed water bodies and in addition, coloured water (contaminated) can interrupt with the aesthetic beauty of water bodies (Verma *et al.*, 2010). Accumulation of wide range of recalcitrant compounds from these industries can intrude with the balance of the ecosystem, causing climatic changes, water level reduction in the water bodies

(especially the ground water), melting ice caps, global warming, depletion of ozone layer due to photochemical oxidation, etc. (Varsha *et al.*, 2011). Discharging untreated textile effluents into the environment will lead to bioaccumulation affecting the food chain and consequently on human health (Rahman *et al.*, 2019). Therefore, water pollution is one of the major global concern and gaining a major attention by environmentalists (Basha and Rajaganesh, 2014).

Conventional methods are physical, chemical and physico-chemical methods and are extremely energy-consuming, not fully effective and generate a toxic sludge impacting the environment. Whereas biological methods are cheaper and environment friendly alternative for dye decolourization. Several microorganisms can be utilized to treat these effluents effectively. Microorganisms are the most abundantly and metabolically available diverse group on earth, which play the major role in neutralizing the harmful effects of most of the chemicals. The primary goal of bioremediation of textile effluent is to increase the native microorganism's capacity for natural degradation, which enables the degradation and mineralization of dyes under mild pH and temperature conditions with minimal environmental impact (Rahman *et al.*, 2019). In recent years, number of studies have focused on microorganisms that are able to degrade and absorb dyes from wastewater. An array of microorganisms are capable of decolourizing wide variety of dyes, that includes bacteria: *Escherichia coli*, *Salmonella typhi*, *Bacillus licheniformis* and *Pseudomonas fluorescens* (Basha and Rajaganesh, 2014), actinobacteria: *Streptomyces chrestomyceticus* (Vignesh *et al.*, 2020), fungi: *Arthrographis kalrae* (Yadav *et al.*, 2021), *Corioloropsis sp* (Chen and Ting, 2015), yeasts: *Sterigmatomyces halophilus*, (Al-Tohamy *et al.*, 2020), and microalgae (Premaratne *et al.*, 2021). But there are few disadvantages in fungal treatment like maintenance of low pH for optimum activity, lengthy retention time for complete decolourization etc. (Telke *et al.*, 2010).

To focus on the pollution due to dye contaminated issues, the objectives of the present research work were designed, as a first step to recover potential bacterial isolates from textile industry effluents which could withstand the extreme conditions and could decolourize wide variety of textile dyes.

Materials and Methods

Chemicals and reagents:

Chemicals and reagents were procured from Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu.

Characterization of bacteria:

Bacteria isolated by standard spread plate method by serial dilution of the five different samples (Kavitha and Sendhilvaidu, 2023) were recovered and characterized by the staining methods, hanging drop technique.

The total number of colonies forming units (CFU) of each sample was enumerated and the colonies were then studied for their morphological characteristics based on their physical appearance (colour, shape and texture) and gram staining for its grouping purpose. Colonies showing remarkable differences in their morphology were selected and re-streaked on nutrient agar plates to obtain pure cultures (Lathwal *et al.*, 2015), for further studies.

Results and Discussion

Through previous literatures available it is evident that, bacteria isolated from textile effluent (native microbial diversity) is the best choice for bioremediation process of textile dyes because of their toxicity resistance and greater adaptability capacity, and hence well suits for the decolorization study (Samuciwal *et al.*, 2021). Aiming this, totally 55 different bacterial colonies were isolated from the effluents, and sludge of the textile industry (Thirupur) and from the dye contaminated soil (Kanchipuram) (Kavitha and Sendhilvaidu, 2023). These bacterial colonies were named and characterized. Bacteria are

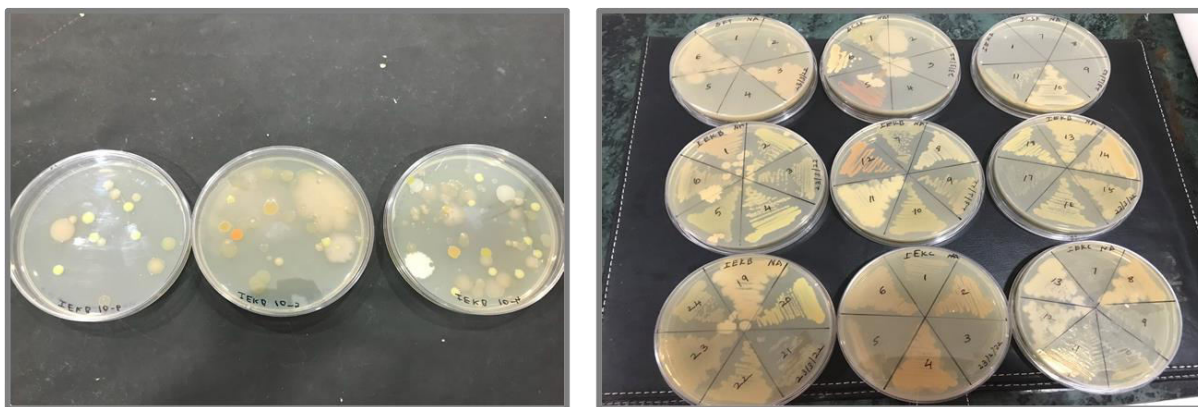


Fig. 1: Nutrient agar plates showing the growth of bacterial cultures isolated from dye contaminated samples.

considered as “unexplored reservoirs of resources”, which could be useful for innovative applications that can benefit mankind (Telke *et al.*, 2010).

Out of 55 bacteria, 6 were isolated from Spectrum Factory Tirupur and hence named as (SFT1-6), 11 were isolated from Dye contaminated soil Kanchipuram and hence named as (DCSK1-11), only 1 was isolated from an Industrial effluent treatment plant, Kallikadu and hence named as (IEKA1), 24 were isolated from Industrial effluent treatment plant, Kallikadu biological sludge and hence named as (IEKB1-24) and 13 were isolated from Industrial effluent treatment plant, Kallikadu and hence named as (IEKC1-13) (Fig. 1, Table 1).

Industrial effluent parameters are not constant always and keeps changing due to the processes practised and chemicals used (Telke *et al.*, 2010). Many researchers (Khadijah *et al.*, 2009; Neelambar *et al.*, 2013) have supported our view that native bacteria's ability of dye degradation is always on the higher side, as they have got natural tendency to survive in the presence of the toxic dyes.

This idea was clearly demonstrated by Olukanni *et al.* (2006), out of the 24 bacterial strains isolated, only 18 bacterial strains were actually more adaptive (isolated from textile effluents) in the treatment process when compared to the non-adaptive ones (isolated from land-fill sites). Potential bacterial consortium

isolated from soil samples collected from areas around dye industries (contaminated sites) in Vatva industrial estate, Ahmedabad and washing sites of textiles near textile industrial estate of Jetpur, Gujarat was also an evidence for their efficiency in decolourizing potential of textile dyes (Moosvi *et al.*, 2007).

The microorganisms were isolated from the soil samples of the effluent disposal site of a textile dyeing industry in Ichalkaranji, India, aiming to decolourize a mixture of textile dyes which includes Congo red, Reactive red HE3B, Eriochrome black T, Remazol blue 3R, Trypan blue, Reactive navy blue RX and Reactive navy blue HE2R. The potential organism were further analyzed, based on their morphological and biochemical characteristics (Telke *et al.*, 2010).

Isolated organisms from textile effluents were identified as *Bacillus* species which was observed as pale yellow colour colony on NA plates showed maximum efficiency for dye degradation and was identified as *Paenibacillus azoreducens* by biochemical tests and 16S rRNA gene sequencing. The gram staining of isolated organism showed that the organism is gram variant. (Palamthodi *et al.*, 2011). Another evidence was provided by Shah (2013), through the isolation of microbes from the effluent sample collected from the industrial site of Ankleshwar Textile Industries, Gujarat, as they were able to exist even in toxic environment with synthetic chemicals and dyes.

Table 1: Morphological characteristics, sample type and collection site of bacterial cultures

S. No.	Isolates	Sample	Location	Color	Shape	Texture	Gram Staining
1.	SFT1	Effluent	Spectrum Factory	Non-Pigmented	Irregular	Dry	Gram +ve
2.	SFT2	Effluent	Spectrum Factory	Creamy White	Irregular	Moist	Gram -ve
3.	SFT3	Effluent	Spectrum Factory	White	Spherical	Smooth	Gram +ve
4.	SFT4	Effluent	Spectrum Factory	Non-Pigmented	Irregular	Moist	Gram -ve
5.	SFT5	Effluent	Spectrum Factory	Non-Pigmented	Irregular	Dry	Gram -ve
6.	SFT6	Effluent	Spectrum Factory	Non-Pigmented	Irregular	Dry	Gram -ve
7.	DCSK1	Soil	Kanchipuram	Yellow	Spherical	Moist	Gram +ve
8.	DCSK2	Soil	Kanchipuram	Non-Pigmented	Spherical	Moist	Gram +ve
9.	DCSK3	Soil	Kanchipuram	Red	Irregular	Moist	Gram -ve
10.	DCSK4	Soil	Kanchipuram	Non-Pigmented	Irregular	Moist	Gram +ve
11.	DCSK5	Soil	Kanchipuram	Orange	Irregular	Dry	Gram +ve
12.	DCSK6	Soil	Kanchipuram	Non-Pigmented	Irregular	Dry	Gram +ve
13.	DCSK7	Soil	Kanchipuram	Non-Pigmented	Spherical	Dry	Gram -ve
14.	DCSK8	Soil	Kanchipuram	Non-Pigmented	Irregular	Moist	Gram -ve
15.	DCSK9	Soil	Kanchipuram	Non-Pigmented	Irregular	Dry	Gram -ve
16.	DCSK10	Soil	Kanchipuram	Half White	RodType	Dry	Gram +ve
17.	DCSK11	Soil	Kanchipuram	Yellow	Irregular	Moist	Gram +ve
18.	IEKA1	Effluent	Treatment plant	Non-Pigmented	Spherical	Moist	Gram -ve
19.	IEKB1	Sludge	Treatment plant	Orange	Irregular	Dry	Gram +ve
20.	IEKB2	Sludge	Treatment plant	Yellow	Irregular	Dry	Gram +ve
21.	IEKB3	Sludge	Treatment plant	Light Yellow	Spherical	Mucoid	Gram +ve
22.	IEKB4	Sludge	Treatment plant	Light Yellow	Spherical	Mucoid	Gram +ve
23.	IEKB5	Sludge	Treatment plant	Yellow	Spherical	Dry	Gram -ve
24.	IEKB6	Sludge	Treatment plant	Non-Pigmented	Irregular	Moist	Gram -ve
25.	IEKB7	Sludge	Treatment plant	Non-Pigmented	Irregular	Moist	Gram -ve
26.	IEKB8	Sludge	Treatment plant	Creamy white	Irregular	Moist	Gram -ve
27.	IEKB9	Sludge	Treatment plant	Non-Pigmented	Spherical	Moist	Gram -ve
28.	IEKB10	Sludge	Treatment plant	Yellow	Irregular	Moist	Gram -ve
29.	IEKB11	Sludge	Treatment plant	Non-Pigmented	Irregular	Dry	Gram +ve
30.	IEKB12	Sludge	Treatment plant	Orange	Spherical	Moist	Gram -ve
31.	IEKB13	Sludge	Treatment plant	Pale Orange	Irregular	Moist	Gram -ve
32.	IEKB14	Sludge	Treatment plant	Greenish	RodType	Dry	Gram -ve
33.	IEKB15	Sludge	Treatment plant	Non-Pigmented	Irregular	Dry	Gram -ve
34.	IEKB16	Sludge	Treatment plant	Creamy White	Irregular	Dry	Gram -ve
35.	IEKB17	Sludge	Treatment plant	Non-Pigmented	Irregular	Mucoid	Gram -ve
36.	IEKB18	Sludge	Treatment plant	Pale Yellow	Irregular	Moist	Gram -ve
37.	IEKB19	Sludge	Treatment plant	Creamy white	Rhizoid	Moist	Gram -ve
38.	IEKB20	Sludge	Treatment plant	Pale Yellow	Irregular	Dry	Gram -ve
39.	IEKB21	Sludge	Treatment plant	Non-Pigmented	Irregular	Moist	Gram +ve
40.	IEKB22	Sludge	Treatment plant	Light Orange	Irregular	Mucoid	Gram +ve
41.	IEKB23	Sludge	Treatment plant	Non-Pigmented	Irregular	Dry	Gram -ve
42.	IEKB24	Sludge	Treatment plant	Creamy White	Irregular	Moist	Gram -ve
43.	IEKC1	Effluent	Treatment plant	Non-Pigmented	Spherical	Moist	Gram -ve
44.	IEKC2	Effluent	Treatment plant	Non-Pigmented	Spherical	Dry	Gram +ve
45.	IEKC3	Effluent	Treatment plant	Non-Pigmented	Spherical	Dry	Gram -ve

46.	IEKC4	Effluent	Treatment plant	Light Orange	Irregular	Mucoid	Gram +ve
47.	IEKC5	Effluent	Treatment plant	Non-Pigmented	Irregular	Moist	Gram -ve
48.	IEKC6	Effluent	Treatment plant	Non-Pigmented	Irregular	Dry	Gram +ve
49.	IEKC7	Effluent	Treatment plant	Non-Pigmented	Spherical	Dry	Gram -ve
50.	IEKC8	Effluent	Treatment plant	Non-Pigmented	Spherical	Dry	Gram -ve
51.	IEKC9	Effluent	Treatment plant	Non-Pigmented	Irregular	Dry	Gram -ve
52.	IEKC10	Effluent	Treatment plant	Tan Colour	RodType	Smooth	Gram -ve
53.	IEKC11	Effluent	Treatment plant	Non-Pigmented	Irregular	Moist	Gram +ve
54.	IEKC12	Effluent	Treatment plant	Non-Pigmented	Irregular	Mucoid	Gram -ve
55.	IEKC13	Effluent	Treatment plant	Half White	RodType	Dry	Gram +ve

The bacteria were isolated from the textile effluents collected in different areas of Komarapalayam (random sites S1, S2, S3, S4 and S5) and were proven to be effective for the bioremediation of heavy metals from textile dye effluents. Two colonies were Gram- negative, straight rods, motile, circular, smooth and colour less , moist, and translucent and remaining two colonies were Gram-positive, rods, motile, circular, entire and transparent (Basha and Rajaganesh, 2014). A similar attempt was done by Barathi and Arulselvi (2015) to isolate bacterial strains from textile effluent contaminated soil samples in the neighbouring of textile industry of Tirupur region. Among the 40 bacterial strains isolated, 6 strains showed maximum potential (Barathi and Arulselvi, 2015).

Ten colonies isolated from soil (numbered as HKS1-10) and twelve colonies from water (numbered as HKW1-12) were selected for the determination of dye decolourization activity on Acrolite Fast Green PGN at the concentration 50 mg/l. All the isolates were identified as Gram-negative and rod shape, except for one isolate which was round in shape (Jana *et al.*, 2015). The number of microbial cultures were less in the ground water containing soil than the ones in the wastewater and dye contaminated soil (Khan and Malik, 2018). Till date researches in the textile dye bioremediation field relies on native bacteria for their processing.

Enumeration of bacterial colonies:

The colonies morphological characteristics analyzed, based on their physical appearance namely colour, shape and texture are given in

Table 1. Majority of bacterial isolates produced non-pigmented colonies on nutrient agar. Of the 55 isolates, 26 (47.3%) were pigmented and 29 (52.7%) were non-pigmented isolates. Pigments include light yellow, reddish orange, half white, creamy white, green, yellow, and orange. 21 colonies were gram +ve and the remaining 34 were gram -ve. Shapes vary from irregular, spherical rhizoid and rod shape. Colony forming units (CFU) of each sample in three dilution range 10^{-4} , 10^{-5} and 10^{-6} were enumerated in Table 2.

Diversity in micro-organisms is the most integral part of the ecosystem as it represents the biological wealth of the environment. Warm and humid places are highly diversified and are hence called as the region of mega biodiversity. The bacterial population in SFT sample was 4×10^4 CFU/ml, DCSK sample was 21×10^4 CFU/mg, IEKB was 68×10^4 CFU/mg, and IEKC was 15×10^4 CFU/ml. Among all the samples collected IEKB had the highest diversity and IEKA the population were the lowest in 10^{-4} dilution rate. The high bacterial counts indicates that the textile effluents are good sources of nutrients to facilitate the growth of bacteria. Total viable bacteria recorded in the 8 samples collected from different spots of outlet of textile effluent, Gazipur, Dhaka, Bangladesh. ranges from 6.8×10^4 - 2.8×10^8 CFU/ml and characterized by gram-positive, rod shaped with single, paired or short chain arrangement bacteria (Mahbub *et al.*, 2012).

Likewise, Khan and Malik, (2018) recorded the aerobic heterotrophic bacterial population as the highest (7.70×10^6 CFU/ml), and next highest as asymbiotic nitrogen fixing bacterial population (4.18×10^3 CFU/ml), actinomycetes population

Table 2: Colony forming units (CFU)

S.No.	Dilution	SFT	DCSK	IEKA	IEKB	IEKC
1.	10 ⁻⁴	4	21	-	68	15
2.	10 ⁻⁵	2	3	1	20	5
3.	10 ⁻⁶	-	1	-	14	-

were 3.50×10^4 CFU/ml and fungi were 0.70×10^4 CFU/ml which is an evidence for good biodiversity of micro-organism in textile effluents.

Conclusion

This study highlighted the isolation of 55 bacterial strains from textile effluent samples and their characterization goes much with the previous literatures available. This part of our research further insists that bacteria inhabiting in textile industry effluent were using the dyes as their source of nutrition and energy and implies their crucial role in bioremediation of textile industries effluents.

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