Microbiology Research Journal International

Volume 34, Issue 8, Page 29-35, 2024; Article no.MRJI.116374
ISSN: 2456-7043, NLM ID: 101726596
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Isolation and Characterization of *Staphylococcus saprophyticus* from Textile Industry Soil for Decolorization of Crystal Violet Dye

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/mrji/2024/v34i81468

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/116374

Original Research Article

Received: 08/05/2024
Accepted: 10/07/2024
Published: 16/07/2024

ABSTRACT

Textile dyes are an important class of synthetic organic compounds and are, therefore, common industrial pollutants. They are produced in large scale and may enter the environment during production or later on during fiber dying. Thus, there is a need for developing treatment methods that were more effective in eliminating dyes from textile waste soil as its source. Staphylococcus

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Cite as: *Ash, Krishna, Sushma Ahalawat, Shailendra Kumar Srivastava, and Sarvesh Kumar Mishra. 2024. "Isolation and Characterization of Staphylococcus Saprophyticus from Textile Industry Soil for Decolorization of Crystal Violet Dye". Microbiology Research Journal International 34 (8):29-35. https://doi.org/10.9734/mrji/2024/v34i81468.*

saprophyticus was isolated from soil of a textile plant and selected as the most active dye degrader of 11 isolates. The important parameters including temperature, pH, carbon and nitrogen source on crystal violet decolorization were investigated. Under the optimum conditions, dye decolorization (92.35%) was successfully achieved within 120 h. at 30°C, pH 8 with sucrose and beef extract as the energy source.

Keywords: Staphylococcus saprophyticus; crystal violet; textile waste; industrial soil; temperature; pH; carbon and nitrogen source.

1. INTRODUCTION

Since the advent of the industrial revolution in the 19th century, the environmental pollution has grown with time and it badly affected the air, water, soil and related ecosystems. Pollution results from different agricultural or urban/industrial sources in the form of fertilizers, pesticides, dyes, etc. The textile and paint industries release large quantities of polluted water containing artificial dyes in the surrounding ecosystem [1]. Dyes are aromatic xenobiotic compounds and are used extensively in the textile industry, todye nylon, wool, silk, and paper, cotton, and leather industries. Dyes are colorant that becomes molecularly dispersed at some points during application to fiber and exhibit some degree of permanence. There are many application classes of dyes, including acid dyes, natural dyes and synthetic dyes, some of the used as a biological stains and in veterinary medicine. The total world textile dye production is estimated to be in the range of 700000 tons per year. During the textile dyeing process, up to 40% of dyes may remain unfixed to the fiber and could contaminate the industrial waste water, which in many cases are flushed directly into water ways. This represents a world dye release ranging from 30000 to 150000 tons per year. These organic colored substances are very stable and difficult to degrade. The textile factory daily discharges millions of liter so fun treated effluents in the form of waste water into public rains that eventually empty into rivers. This alters the pH, increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and gives the rivers intense colorizations Ajayi and Osibanjo [2]. The use of these water resources is limited and the ecosystem is affected. About 10-15% of all dyes are directly lost to waste-water in the dyeing process. Thus, the waste- water must be treated before releasing into the natural environment. Several dye degrading microorganisms have been reported and characterized such as *Bacillus* sp, *Staphylococcus* sp., *Streptomyces* sp., *Arthobacter* sp., *Micrococcus* sp., *Vibreo* sp., *Pseudomonas* sp., *Mycoplasma* sp., *Sarcina* sp. and *E. coli.* Banat et al. [3] & Azmi et al. [4]. Ozonation can degrade such molecules. But the initial investment and subsequent operation costs are high. Therefore, developing a bioremediation process seems essential. Bio-treatment offers a cheaper and eco-friendly alternative for colour removal in textile effluent. The ubiquitous nature of bacteria makes them invaluable tools in effluents bio- treatment. The aim of present study was to isolate and identify potential dye decolorizing bacterial strains from industrial soil samples and to optimize various culture parameters to enhance dye decolorization.

2. MATERIALS AND METHODS

Isolation of bacteria: Bacteria were isolated from industrial soil using serial dilution method described by [5].

Screening of isolated bacteria for dye decolorization: Soils containing industrial effluents were collected from different industries like saw mill, textile mill, bangle mill and cotton mill. After collecting a soil sample serial dilution of each sample up to $10^{-6}$to 10^{-8}level were made. These dilutions were used in pour plate method. After plating, the isolates were inoculated in nutrient broth at 37^0C for 24h. To check the dye decolorizing activity 1ml of inoculated nutrient broth was added to 98ml sterilized nutrient broth and with1ml of dye (crystal violet) the volume was made up to 100ml and it was left on shaker for 24-168h at room temperature. Then the dye decolorization activity was detected by spectrophotometer at 590nm. This method was used for all the isolates [5].

Determination of decolorization activity: Colour was measured at the dye's optimum wavelength (590 nm). For this purpose, samples were centrifuged at 3000rpm for 15min and absorbance of supernatants was determined. The decolorization efficiency was expressed as a following equation described by Chong et al.[6].

Decolorization (%) = (I-F)/I*100

Where I= initial absorbance and F= absorbance of decolorized medium

Selection of dye decolorizing bacteria: The strain, which was showing highest decolorizing percentage on Nutrient Broth, was selected for further study.

Effect of temperature: Arrange of temperature (20-60°C) was used to study the effect of the temperature on dye decolorization. In this medium 10ml inoculums (10%v/v) was added with 1ml dye (0.50 mg/1000 ml) along with 89 ml sterilized nutrient bro that pH7 to make a volume of 100 ml then it was in cubated for different time intervals (24- 120h), respectively. After every time interval i.e. 24, 48, 72, 96 and 120h, the dye decolorization activity was detected by centrifuging the supernatant at 3000rpm for 15min in spectrophotometer at 590nm. This method was used for all the parameters.

Effect of pH: Effects of pH for decolorization of industrial soil containing dyes (0.50 mg/1000 ml) were studied over a pH range 5 to 10 by adjusting pH with HCL and Na0H. In this process 10ml inoculums (10%v/v) was used with 1ml dye (0.50 mg/1000 ml) along with 89 ml sterilized nutrient broth at 30^0C to make a volume of 100ml then it was incubated over a period of 24-120h.

Effect of different carbon sources on decolorization: Effect of various carbon sources on decolorization of industrial soil was assessed by growing the isolate in the sterilized 79ml nutrient broth with 10ml inoculums and 1ml dye at 300C and pH8 along with different carbon source such as glucose (1%), mannitol (1%), sucrose (1%), maltose (1%) and lactose (1%), respectively then incubated for different time intervals i.e. 24-120h.

Effect of different nitrogen sources on decolorization: Effect of various nitrogen sources on decolorization was assessed by growing the isolate in the 69 ml sterilized nutrient broth with 10 ml inoculums, 1mldye and 10 ml carbon source i.e. sucrose (1g/100ml) at 300C, pH 8 and different organic nitrogen source such as peptone (1%), beef extract (1%), malt extract (1%), yeast extract (1%) to make a volume of 100ml. Same procedure was used for different inorganic nitrogen sources such as NaNO3 (1%), NH4NO3(1%), KNO3(1%), NH4Cl (1%) and (NH4)2SO4(1%), respectively.

Statistical analysis: The data recorded during the course of investigation was statistically analyzed using analysis of variance ANOVA (two way classification), correlation and t-test at 5% significance level.

3. RESULTS AND DISCUSSION

Incidence of dye decolorizing bacteria in industrial soil samples: In the present study total 12 bacteria were obtained from the soil sample of saw mill among them three (25%) isolates showed positive dye decolorization activity.

Table 1. Sample collection sites

Sample No. Sample site	Total No. of isolates	Total No. of positive isolates
1 Sawmill (Naini)	12	3(25%)
2 Textile mill (Naini)	15	3(20%)
3 Bangle mill (Ferozabad)	10	3(30%)
4 Cotton mill (Prayagaraj)	09	2(22.22%)

Significant t cal(6.52)>t tab(5%) (2.44); dof=6

Almost same observation was obtained from textile mill, where total 15 isolates were obtained in which 3 (20%) showed positive result. From bangle mill, number of isolated bacteria was 10 in which 3 (30%) isolates have dye decolorizing capacity. From cotton mill 9 bacteria were isolated among them 2 (22.22%) isolated showed positive test i.e. showing dye decolorization potential. The result was found to be statistically significant ($p<0.05$) there wasa significant difference in the incidence of dye decolorizing bacteria from different industrial soil samples (Table 1).

These observations were similar to the findings of Meehan et al. [7] who found an azo dye reducing, endospore forming bacterium isolated from textile industry. *Staphylococcus saprophyticus* was isolated from textile soil and selected as the most active azo dye degrader of 19 isolates by Seesuriyachan et al., [8]. Most of the results were found in textile industries, the reason was that textile industry contains azo compounds which were very toxic in nature and very hard to degrade but there was an enzyme called laccase was present not in every bacteria but most of them. This enzyme had ability to degrading dyes, Joseph et al., [9].

Screening of potential isolate for dye decolorization: The isolate showed random increased decolorization % between 24-120h, after that the increasing percentage was nearly steady. Total 12 isolates from saw mill soil samples were obtained out of which 3 isolates i.e. SM1, SM2 and SM3 showed dye decolorizing ability.

When the isolates were studied at different time intervals, it was observed that SM1, SM2 and SM3 showed maximum dye decolorizing activity after 120h i.e. 48.48, 47.45 and 73.39%, respectively. Similar percentage activity was showed by three isolates of textile mill namely TM1, TM2 and TM3. Maximum was at 120h with 29.16% (TM1), 40.19% (TM2) and 84.17% (TM3) activity. Almost similar observations were seen in the soil samples collected from bangle mill. Three isolates were obtained that possess dye decolorization ability and were designated as BM1, BM2 and BM3. On comparing BM1, BM2 and BM3 isolates, maximum activity was observed with BM1 after 120h. of incubation. In the case of cotton mill isolates namely CM1 and CM2, the maximum decolorization was observed at 120h i.e. 43.66% (CM1) and 42.99% (CM2).

From all the data obtained it was clearly observed that TM3 was showing most decolorizing percentage at 120h after that time is seems to be constant. It was observed that the rate of removal of acidic dyes increases with increase in contact time to some extent. Further increase in contact time does not increase the uptake due to deposition of dyes on the available adsorption site on adsorbent material, Sumanjit et al., [10]. So all the parameters were optimized at 120h and this strain was selected for further studies. The results were found to be significant ($p<0.05$) means the decolorization was supported by the time duration (Table 2). These observations were similar to the findings of Chong et al., [6] who found 53.60% decolorization of direct blue 15 at 120h.

Table 2. Percentage dye decolorization by isolated bacteria

			Decolorization%						
Sl.	**Sample no.**	**Isolate no.**	**24h.**	**48h.**	**72h.**	**96h.**	**120h.**	**144h.**	**168h.**
1	Sawmill	SM1	21.44	25.12	34.01	42.24	48.48	48.64	48.00
		SM2	17.63	22.72	24.36	39.27	47.45	47.63	47.81
		SM3	42.59	62.06	66.95	69.12	73.39	73.46	73.54
2	Textile mill	TM1	18.16	24.18	27.65	28.72	29.16	30.44	32.17
		TM2	18.70	28.32	37.23	39.56	40.19	40.64	40.73
		TM3	30.97	40.17	55.57	76.93	**84.17**	84.42	84.59
3	Bangle mill	BM1	32.54	41.38	57.58	59.35	60.53	60.67	60.82
		BM2	19.17	28.02	37.46	44.24	51.90	52.21	52.50
		BM3	14.35	23.58	30.51	35.89	42.82	43.33	44.10
4	Cotton mill	CM1	17.09	21.60	28.70	36.64	43.66	44.27	44.73
		CM2	20.42	26.84	36.34	40.61	42.99	43.46	44.18

Significant for hour:
Fcal (55.45)>Ftab(5%)(2.17)
S.E. = 2.72
C.D. =5.9024

Significant for isolates:
F cal (46.6)>F tab (5%) (1.91)
S.E. =2.17
C.D. =4.1447

Table 3. Effect of temperature on decolorization of crystal violet

Temperature	Decolorization%	Variation%
20^0C	54.83	–29.34
30^0C	80.36	–3.81
40^0C	52.80	–31.37
50^0C	39.44	–44.73
60^0C	27.76	–56.41

Non-significant tcal (3.15) <t tab(5%) (3.18);r=-0.7628; dof=3

Table 4. Effect of pH on decolorization of crystal violet at 30⁰C

pH	Decolorization%	Variation%	Temperature
5	40.58	–43.59	30°C
6	42.33	–41.84	
7	66.77	–17.4	
8	82.28	–1.89	
9	33.22	–50.95	
10	28.01	–56.16	

Non-significant t_{cal} (0.3875)<t_{tab} (5%)(2.776);r=-0.1902; dof=4

Table 5. Effect of carbon source on decolorization of crystal violet at 30⁰C and pH8

Carbon Source (1%)	Decolorization %	Variation %	Temperature	pH
Mannitol	58.77	–25.4	30°C	8
Maltose	52.81	–31.36		
Glucose	47.19	–36.98		
Sucrose	89.66	+5.49		
Lactose	45.39	–38.78		

Significant t_{cal}(7.28) >t_{tab}(5%) (2.13);dof=4

Microbial decolorization of acid black 24 (dye conc. 0.1g/l) a water soluble, benzidine based azo dye was studied by Ozdemir et al., [11]. They found decolorization was 74% under shaking conditions within 336h, although dye reduction was stable between 144h and 336h. In our study the dye decolorization was stable between 120-168h. In shaking conditions the dye decolorization was due to adsorption of dye by viable or died cells.

Identification of bacterial strain: Based on cultural, morphological and biochemical characteristics tests the TM3 was identified as Staphylococcus saprophyticus [12].

Effect of temperature: The decolorization of crystal violet was most efficient at 30^0C, with 80.36% of colour removal, followed by 20, 40, 50 and 60^0C. The decline of microbial activity on decolorization could be due to the loss of cell viability and denaturation of enzyme Pearce [13]. The results were found to be non-significant (p>0.05). Our findings were similar to the findings of Ilhan et al., [14] who discovered a bacterial strain Staphylococcus saprophyticus which has ability to degrade or remove chromium, lead and copper ions from industrial waste water at 30^0C with 66, 91 and 48% degradation at 120h, respectively.

Effect of pH: The optimum pH for decolorization in this study was pH8, in which decolorization efficiency was 82.28%. Amongst pH, pH8 was used for subsequence experiments as it was slightly basic and suitable for industrial application. The results were found to be non-significant (p>0.05) (Table 4). Similar findings are reported by Sumanjit et al. [10] who tested the dye decolorizing activity was maximum at 30^0C and pH8 which is slightly basic against *Staphylococcus saprophyticus* with 78% decolorization of acid red 119. These results indicated that dye decolorization was due to the degradation or conversion of complex substances into smaller substances by particular strain.

Effect of carbon source: In an attempt to enhance decolorization performance with extra supplements of carbon source in a medium 89.66% decolorization with sucrose (1%) was found in 120h with pH8 and temperature30^0C. Results were found to be statistically significant (p<0.05) (Table 5).

The decolorization process requires carbon substrates as an energy source. Sucrose (1%) was the best energy source tested with Methyl Orange. This particular dye was degraded to 39% of the normal level in the presence of sucrose by *Lactobacillus casei* as described by Seesuriyachan et al. [8].

Effect of organic nitrogen source: In an attempt to enhance decolorization performance with extra supplements of organic source in a medium we found 92.35% decolorization with beef extract (1%), in 120h with carbon source sucrose, pH8 and temperature 30°C. The results were statistically significant (p<0.05) (Table 6).

Table 6. Effect of organic nitrogen source on decolorization of crystal violet at 30^0C and pH8 with carbon source sucrose

Organic nitrogen Source (1%)	Decolorization %	Variation %	Temperature	pH	Carbon source
Peptone	78.28	–5.35	300C	8	Sucrose
Beef extract	92.35	+8.18			
Malt extract	55.09	–29.08			
Yeast extract	83.46	–0.71			

Significant tcal(9.72) > t tab(5%) (2.35); dof=3

Table 7. Effect of inorganic nitrogen source on decolorization of crystal violet at 30°C and pH8 with carbon source sucrose

Inorganic nitrogen Source (1%)	Decolorization %	Variation %	Temperature	pH	Carbon source
NaNO3	70.00	–14.17	30^0C	8	Sucrose
NH4NO3	80.97	–3.2			
KNO3	88.95	+4.78			
NH4Cl	14.28	–69.89			
(NH4)2SO4	20.11	–64.06			

Significant t cal (3.39) > t tab (5%) (2.13); dof=4

Effect of inorganic nitrogen source: In an attempt to enhance decolorization performance with extra supplements of inorganic nitrogen source in a medium we found 88.95% decolorization with potassium nitrate (1%), in 120h with carbon source sucrose, pH8 and temperature 30°C. The results were found to be statistically significant ($p<0.05$) (Table 7).

Wong and Yuen [15] studied the decolorization of n,n-dimethyl-p-phenylenediamine by *Klebsiella pneumoniae* and *Acetobacter liquefaciens* with adding nitrogen source as ammonium sulphate (1%) they found 80 and 45% decolorization by both the strains [16,17].

The study revealed that maximum decolorization was obtained against crystal violet (0.50mg/l) at 30^0C, pH8; sucrose (1%) and beef extract (1%). Using all these modified parameters the decolorizing activity was increased up to 92.35% otherwise it is 84.17%.

4. CONCLUSIONS

- *Staphylococcus saprophyticus* showed the most promising decolorization activity among the eleven isolates.
- The presence of carbon and nitrogen sources, such as sucrose and beef extract, was crucial for biomass maintenance and enhanced decolorization.
- The study suggests that *Staphylococcus saprophyticus* could potentially decolorize other dyes, contributing to the reduction of soil and water pollution.
- Advanced techniques like HPLC, NMR, and GC-MS can be employed to detect biodegradation products and understand the decolorization mechanism in detail.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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