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Research Article

ISOLATION AND CHARACTERIZATION OF DYE DEGRADING BACTERIA AND THEIR ROLE IN PLANT GROWTH PROMOTION

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ABSTRACT. Globally rapid industrialization and urbanization is sole responsible for water pollution. Being the backbone of our developing economy, textile industry is main inception of water pollution. Industrial effluent-containing dyes are harmful to both biotic and abiotic components of our surrounding environment. The textile waste contaminated water with dyes results in harmful effects on the plants growth. This study was designed to investigate microbiological removal of dyes by using indigenous microbes. Isolation of indigenous bacterial isolates was done following microbiological techniques and named as WL1, SM2, SM6 and SL4. Growth of isolates was checked at different pH (6, 7 and 8) and varying temperatures (23 ºC, 37 ºC and 50 ºC) in N- broth media supplemented with and without different concentration of dye (0.01µg/ml, 0.02µg/ml, 0.04µg/ml, 0.06µg/ml, 0.08µg/ml). All the isolates showed optimal growth at 37 ºC and 7 pH. It was also observed that the maximum growth of isolates was shown in the presence of 0.02µg/ml malachite green concentration whereas the higher concentration of dye 0.08µg/ml reduced the growth of these isolates WL1, SM2, SM6 and SL4. Efficacy of these bacterial isolate WL1, SM2, SM6 and SL4 were evaluated on the growth promotion of *Triticum aestivum* grown in the presence and absence of dyes containing water and monoculture and co culture inoculation for 15 days in lab under controlled conditions. It was observed that growth of plants in co culture inoculation was higher than those plants inoculated with monoculture both in dye supplemented and dye free conditions. Co-culture of local bacterial isolates showed promising potential to increase the plant growth by reducing malachite green's toxicity.

Keywords: *eco-friendly, decolorize, mono culture, co culture, plant growth promoter*

INTRODUCTION

On a global scale, water pollution is a major problem and conundrum due to rapid Industrialization and urbanization [1]. The main inception of water pollution is textile industries in our developing economy Mondal et al.2017 [2]. Textile industries consume large quantity of water where 90 % appears as wastewater industrial effluent [3]. Due to excessive availability and low cost, dyes are extensively utilized in textile industry and release by product which cause adverse impact on the surroundings [2, 3, 4]. The biotic and abiotic components of the ecosystems are affected by the recalcitrant nature of the dyes and their metabolites [2, 5]. The textiles industry depicts high toxicity, mutagenicity and potentially carcinogenicity [6, 7]. Several physicochemical methods, such as filtration, specific coagulation, electro coagulation, froth flotation, ion exchange have been utilized for the degradation and removal of synthetic dyes in water bodies but these techniques are ineffective, high priced and produce huge quantity of sludge, which further leads to land

pollution Shah et al. 2013 [8]. So, there is an urgency to find a substitute method which is not only inexpensive and eco-friendly [6, 9] but also able to degrade the toxic substances from effluent such as biological or combination systems [5, 10].

The microbial decolorization and degradation of dyes including several taxonomic groups such as bacteria, fungi, and yeast together with algae have been acquired as viable concerns due to their low cost, less sludge productivity and their eco-friendly nature [5, 10]. Bacteria from different trophic groups are considered to be more effective as it shows efficient dye-degradation under optimal conditions [1, 7]. The present study aims to examine the potential of indigenous bacteria to remove dyes from industrial effluents [1, 11, 12]. Current study attempts to segregate and characterize dyes degrading bacteria from industrial effluent [13], followed by the optimization of these dyes degrading bacteria [14, 15], Percentage of malachite degradation [7, 16]. Furthermore, these isolates were investigated to check their efficacy and role in plant growth promotion [13, 17, 18, 19].

MATERIALS AND METHODS

Sample Collection and Physicochemical Characteristics of Sample

The present research was conducted in Biology Laboratory (Lahore Garrison University, DHA phase 6, Lahore. The industrial effluent and adjourning soil samples were collected from the Discharge panel of different dyeing units located in Muridke and Lahore (Pakistan). Two effluent samples (WM) and (WL) were collected in a sterile properly labeled brown bottle while two soil samples (SM) and (SL) were collected in the sterile polythene bag via sterile spatula from the same site for study of the microbial flora in the adjourning area as possible and were subjected for further study. Physicochemical characteristics of each sample pH, temperature and turbidity were determined and results were noted respectively. For physicochemical characterization 2 mL each sample (soil sample 0.2 g of soil was mixed in 2 mL of distilled water) was taken in a microfuge tube, pH was observed using pH meter, temperature was measured using digital thermometer.

Sample Preparation and Isolation of Bacterial Isolates

Isolation of bacterial isolates from the industrial effluent was done by serially diluting 10^{-6} the sample and spreading on L. agar plates supplemented with dyes. Plates were incubated in an incubator for appearance of bacterial growth. Among all the bacterial colonies were 20 isolates were observed and those which scored positive for malachite green were streaked for purification by quadrant streaking method till purified bacterial culture was obtained. For differentiation of bacteria staining technique was used following the method of Cappuccino and Sherman 2013 [20].

Effect of Different Physiological Parameter on Bacterial Growth

To examine the physiological characteristics of the isolated cultures, Luria broth was prepared inoculated with dye degrading bacterial isolates and incubated for 48 hours at different Temperatures (23°C, 37°C, 50°C) different PHs (6,7,8) levels and different concentration of malachite green dye (100 µg/mL, 200 µg/mL, 400 µg/mL, 600 µg/mL , 800

µg/mL). Effects on the bacterial growth of isolates were determined by taking optical density at 600 nm in a spectrophotometer.

Malachite Green Degradation Potential

Malachite green degradation potential of isolates was checked Luria broth containing malachite green dye was prepared and autoclaved. Bacterial isolates were inoculated in the test tube and incubated for 37℃ for 48 hours for complete decolorization of malachite green. Cell densities 0.5 were adjusted at 600nm = 10*8 CFU ml-1. Bacterial culture is centrifuged at 14000 for 5 minute for the separation of supernatant, which is utilized for the determination of extent of degradation of malachite green. The supernatant was transferred to cuvette and optical density was measured at 616 nm by using spectrophotometer. The rate of decolorization of malachite green was determined by using the given formula as reported previously. Dye Degradation (%) =Initial OD −Final OD / Initial OD×100

Plant Growth Promotion

For plant growth promotion, the method of Qureshi and Sabri [21] was adopted. Certified seeds of *Triticum Aestivum* (variety wheat Punjab 11) were obtained from Punjab seed corporation, Lahore Pakistan. Seeds were disinfected with 0.1 % $HgCl_2$ solution for 5 minutes and washed with sterile water three to four times to remove traces of $HgCl_2$ Qureshi and Sabri [21]. After sterilization seeds were inoculated with bacterial culture for 20 minutes .Seeds were soaked in sterile distilled water for 20 minutes for control experiment. Seeds were sown in plastic pots containing 400 g sterilized garden soil and five seeds of wheat were added in each pot. Pot in which no dye and no bacterial inoculum was added. In the second experimental setup only dye 200 µg/mL was added whereas in other experimental setup, only bacterial culture 4 ml per gram weight of soil was added. In the fourth and last group, both bacterial suspension of isolates and Malachite green 0.02 µg/ml were added. All the experiments were conducted in triplicates. For evaluating the effect of monoculture on dye degrading bacteria on wheat growth, fresh bacterial culture O.D adjusted 0.5 of isolates was suspended in distilled water and added to each pots of respective group. For the determination of efficacy of co culture of dye degrading bacterial strain in the promotion of plant growth, co culturing of isolates (50:50) was added. These plants were kept for 15 days in controlled temperature conditions and daily observed for their growth. After germination, plants were harvested and growth parameters such as length of shoots and roots, fresh and dry weight, number of leaves and roots were measured.

RESULTS AND DISCUSSION

Physicochemical Characteristics of Samples

Industrial effluents and adjourning soil samples were collected from two dyeing units located in Mureedke and Lahore. Their physicochemical characteristics such as color, temperature and pH of all samples were observed and recorded. The color of effluent 1 is pinkish and 2 is bluish whereas the color of soil 1 is dark brownish. However, the color of soil 2 is brownish. The temperature recorded was 33 ℃ and 37 ℃ of Industrial effluent 1

and effluent 2 respectively. Whereas the temperature of soil 1 and soil 2 was 34℃ and 33℃, respectively. pH is measured by using pH meter and the pH of effluent 1 is 7.8 whereas the PH of effluent 2 is 7.5. In the same way the pH of soil sample 1 is 6.9 and sample 2 is 7.0 as shown in Table 1.

Isolation and Purification of Bacteria

The bacterial isolates were isolated from the industrial wastewater and soil using standard microbiological techniques. All four samples were found positive (100%) showing growth in media. Out of 2 soil samples, 12 bacterial isolates (SM1, SM2, SM3, SM4, SM5, SM6, SM7, SL1, SL2, SL3, SL4 and SL5) were isolated and purified. Out of water samples, 8 bacterial isolates (WM1, WM2, WM3, WL1, WL2, WL3, WL4 and WL5) were isolated and purified. Total 20 (SM1, SM2, SM3, SM4, SM5, SM6, SM7, SL1, SL2, SL3, SL4, SL5, WM, WM2, WM3, WL1, WL2, WL3, WL4 and WL5) isolates were found from industrial water and soil samples collectively shown in Table 2 and further screened.

Table 1. *Physicochemical characteristics of samples*

Sr no.	Sample	Location	Source	Color	Temperature	pH
1	Industrial Effluent	Muridke	Water	Pinkish	37℃	7.5
			Soil	Dark Brownish	33℃	6.9
2	Industrial Effluent	Lahore	Water	Bluish	33℃	7.0
			Soil	Brownish	34℃	7.5

Table 2. *Isolation and purification of bacteria*

Sr no	Sample	Name of colonies	Number of colonies
1	Soil Muridke	SM1	06
2		SM2	05
3		SM3	TNTC
4		SM4	07
5		SM5	05
6		SM6	04
7		SM7	08
8	Water Muridke	WM1	TNTC
9		WM2	07
10		WM3	09
11	Soil Lahore	SL1	08
12		SL2	04
13		SL3	07
14		SL4	06
15		SL5	TNTC
16	Water Lahore	WL1	05
17		WL2	TNTC
18		WL3	08
19		WL4	07
20		WL5	TNTC

Cultural Characterization of bacterial isolates

All the isolated bacteria were subjected to LB agar for cultural characterization, colony shape (circular to filamentous), size (pin –point to moderate), pigmentation (white, Off white, yellow and pale yellow) with entire, lobate, serrate irregular, undulate margins and elevation type (raised, flat, convex and slightly raised) of each isolate was observed and described in Table 3.

Table 3. *Cultural characterization of bacterial isolates*

Sr.no	Isolate	Size	Shape	Texture	Pigmentation	Margin	Elevation
1	SM1	Small	Circular	Mucoid	Pale yellow	Entire	Raised
2	SM2	Small	Circular	Mucoid	Off- White	Entire	Raised
3	SM3	Moderate	Circular	Butyrous	Off- White	Entire	Convex
4	SM4	Small	Circular	Mucoid	Pale yellow	Lobate	Flat
5	SM5	Moderate	Irregular	Butyrous	White	Serate	Convex
6	SM6	Big	Circular	Mucoid	White	Entire	Raised
7	SM7	Small	Circular	Mucoid	Pale yellow	Entire	Flat
8	SL1	Moderate	Circular	Mucoid	Off- White	Serate	Convex
9	SL2	Small	Irregular	Mucoid	Pale yellow	Undule	Flat
10	SL3	Small	Irregular	Butyrous	Off- White	Entire	Raised
11	SL4	Small	Circular	Mucoid	Off –White	Entire	Slightly Raised
12	SL5	Small	Circular	Butyrous	Pale yellow	Undulate	Slightly Raised
13	WM1	Small	Circular	Mucoid	Slightly Yellow	Entire	Raised
14	WM2	Small	Irregular	Mucoid	Pale yellow	Entire	Flat
15	WM3	Small	Irregular	Mucoid	Off –White	Lobate	Flat
16	WL1	Big	Circular	Butyrous	White	Entire	Raised
17	WL2	Small	Circular	Mucoid	Off- White	Undulate	Convex
18	WL3	Moderate	Circular	Mucoid	Pale yellow	Undulate	Flat
19	WL4	Small	Irregular	Non mucoid	Pale yellow	Entire	Raised
20	WL5	Pinpoint	Circular	mucoid	Yellow	Smooth	Flat

Screening of dye decolorizing bacterial isolate

Out of the 20 isolated bacterial isolates, only 7 (SM2, SM6, SM7, SL1, SL4, WL1 and WL4) isolates showed growth on media supplemented with dyes and screened positive for dye degradation against different textile azo dyes such as Malachite green, Methyl orange, Congo red, Rhodamine pink, Crystal violet and Black shown in table 4. All these selected dyes were obtained from the biology laboratory of Lahore Garrison University. This dye degrading bacterial isolates were further studied microscopically and macroscopically for identification and further utilization purposes.

Table 4. *Screening of dye decolorizing bacterial strain*

Sr No.	Isolates	Malachite green	Crystal Violet	Congo red	Rhoda mine pink	Black	Methyl red	Selected Isolate
1	SM1	+	-	-	-	-	-	
2	SM2	+	-	-	+	-	+	SM2
3	SM3	+	-	-	-	-	-	
4	SM4	+	-	-	-	-	-	
5	SM5	+	-	-	-	-	-	
6	SM6	+	-	-	+	-	+	SM6
7	SM7	+	-	-	+	-	+	SM7
8	SL1	+	-	-	+	-	+	SL1
9	SL2	+	-	-	-	-	-	
10	SL3	+	-	-	-	-	-	
11	SL4	+	-	-	+	-	+	SL4
12	SL5	+	-	-	-	-	-	
13	WM1	+	-	-	-	-	-	
14	WM2	+	-	-	-	-	-	
15	WM3	+	-	-	-	-	-	
16	WM1	+	-	-	+	-	+	WM1
17	WM2	+	-	-	-	-	-	
18	WM3	+	-	-	-	-	-	
19	WM4	+	-	-	+	-	+	WM4
20	WM5	+	-	-	-	-	-	

+Ve indicates degradation
-Ve indicates no degradation

Screening of highly resistant bacterial isolates against Malachite green concentration

For the study of the malachite green dye degrading profile, all purified isolates n=7 (SM2, SM6, SM7, SL1, SL4, WL1 and WL4) were grown in media having different concentration of malachite green (100 µg/mL, 200 µg/ml, 400 µg/mL, 600µg/mL, 800 µg/mL) were incubated at 37°C for 48 hours. Volume of the isolates in tubes showed their growth rate. Out of 7 isolates, four bacterial isolates (SM2, SM6, SL4 and WL1) showed good growth in all concentrations of malachite green. However, the maximum growth was shown in 200 µg/ml, concentration, whereas slightly less growth was observed at 400 µg/mL,600 µg/mL, 800 µg/mL concentration of malachite green.

Table 5. *Screening of highly resistant bacterial isolates against malachite green concentration*

Sr no.	Isolates	100 µg/mL	200 µg/mL	400 µg/mL	600 µg/mL	800µg/mL	Selected isolate
1	SM1	++++	++++	+++	++	-	
2	SM2	++++	++++	++++	+++	+++	SM2
3	SM3	++++	+++	++	+	-	
4	SM4	++++	+++	++	+	+	
5	SM5	++++	+++	++	-	-	
6	SM6	++++	++++	++++	+++	+++	SM6
7	SM7	++++	+++	++	++	-	
8	SL1	++++	+++	+++	++	+	
9	SL2	++++	+++	++	+	-	
10	SL3	++++	+++	++	-	-	
11	SL4	++++	++++	++++	+++	+++	SL4
12	SL5	++++	++	-	-	-	
13	WM1	++++	+++	++	+	-	
14	WM2	++++	++	-	-	-	
15	WM3	++++	+++	+	-	-	
16	WL1	++++	++++	++++	+++	+++	WL1
17	WL2	++++	+++	++	+	-	
18	WL3	++++	+++	+ +	+	-	
19	WL4	++++	+++	++	-	-	
20	WL5	++++	+++	+	-	-	

+ indicates degradation ++ indicates relatively good degradation +++ indicates efficient degradation
++++ indicates highly efficient in degradation - Indicates No degradation

Microscopic Characterization of Isolates

Microscopic characters (shape, color and arrangement) were observed using gram staining. All bacterial isolates were gram positive purple cocci showing different bacterial arrangements (may occur singly, in the form of diplococci, chain and tetrad arrangement) which was displayed in Table 6.

Table 6. *Microscopic characterization of isolates*

Sr.no	Isolate	Gram staining	Microscopic Characters
1	WL1	Gram positive	Cocci
2	SM2	Gram positive	Cocci
3	SM6	Gram positive	Cocci
4	SL4	Gram positive	Cocci

Biochemical Tests

Biochemical tests (MR, VP, Indole test, Catalase, DNASE, Starch agar test) were performed for further confirmation of isolates. Malachite green degrading bacterial isolates WL1, SM2, SM6 and SL4 were positive for Methyl Red, Voges Proskauer and catalase tests.

All bacterial isolate WL1, SM2, SM6 and SL4 were negative to Oxidase, Indole and Starch agar test. Out of 4 isolates, 1isolate SM2 was found positive to Coagulase and DNase tests whereas no coagulation in case of coagulase test and formation of zone around the colonies in case of DNase test were observed in remaining 3 isolates WL1, SM6, SL4 isolates. Biochemical tests showed that isolate SM2 showed relatedness to Staphylococcus aureus and the remaining three isolates WL1, SM6, SL4 with Staphylococcus saprophyticus. The biochemical profile of bacterial isolates was described in Table 7

Table 7. *Biochemical profile of bacterial isolates*

Isolate	MR	Indole	Catalase	DNase	Starch Agar test	Coagulase	Oxidase	VP
WL1	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve
SM2	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve
SM6	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve
SL4	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve

MR =produced red color; Indole – No ring formation; Catalase bubble formation ; DNase + formation of clearing zone around colonies – shows no clearing zone;
Starch Agar – means no formation of clear zones; Coagulase + shows clotting of plasma – shows no clotting; Oxidase – no purple color; VP + formation of brown ring at top

Effect of Different Physiological parameters on the Dye Degrading Ability of Bacterial Isolates

Effects of Different Concentrations of Malachite Green on Isolates

After 48 hours of incubation at different concentrations of malachite green, growth of bacterial isolates was recorded. Isolate SL4 Showed good growth at 100 µg/mL, 200 µg/mL, 400 µg/mL, 600 µg/mL, 800 µg/mL concentrations of malachite green. Maximum growth was of all isolates WL1, SM2, SM6 and SL4 was recorded upto 200 µg/mL concentration of malachite green. Whereas higher concentration of dyes decrease the growth of isolates. Results showed that the growth of isolates SM6 decrease with the increase in the concentration of malachite green displayed in Figure 1.

Effects of Different Temperatures on Screened Isolates

All the isolates showed highest growth at 37°C and less bacterial growth was recorded at 23°C and 50°C which suggest that both low and high temperature did not favourable for the growth of bacterial isolates. In general, temperature 37°C is the optimum temperature for the growth of malachite degrading bacterial isolates SL4 with average growth of 1.79 shown in Figure 2.

Effects of Different pH on Selected Isolates

After following methodology, results indicated that malachite green degrading bacterial isolates required optimum pH 7 for highest growth and varying pH have growth reducing effect on bacterial growth. At low pH 6, the maximum growth was observed by isolate SL4 was recorded in terms of OD 600 nm (1.958) and the least growth was observed by isolate

WL1. Maximum bacterial growth was recorded at 7 pH by isolate SM2 and SL4. At pH 8 the maximum growth was shown by isolate SM2 shown in Figure 3.

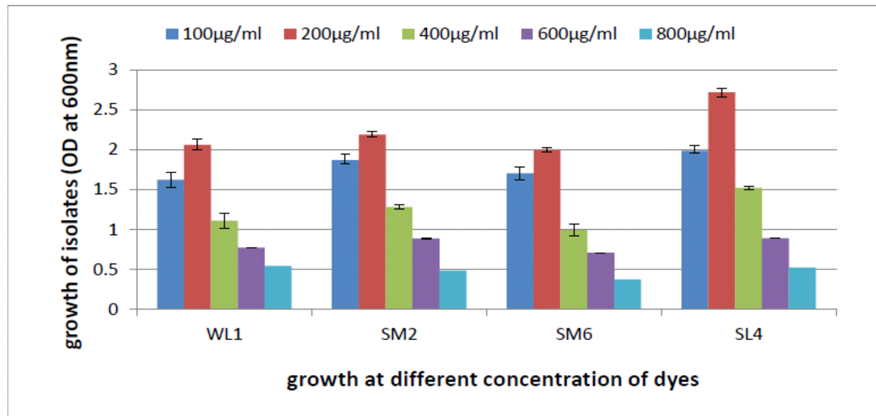

Fig. 1. *Effect of different concentrations of malachite green (100 µg/ml, 200 µg/ml, 400 µg/ml, 600 µg/ml and 800 µg/ml) on the growth of dye degrading bacterial isolates*

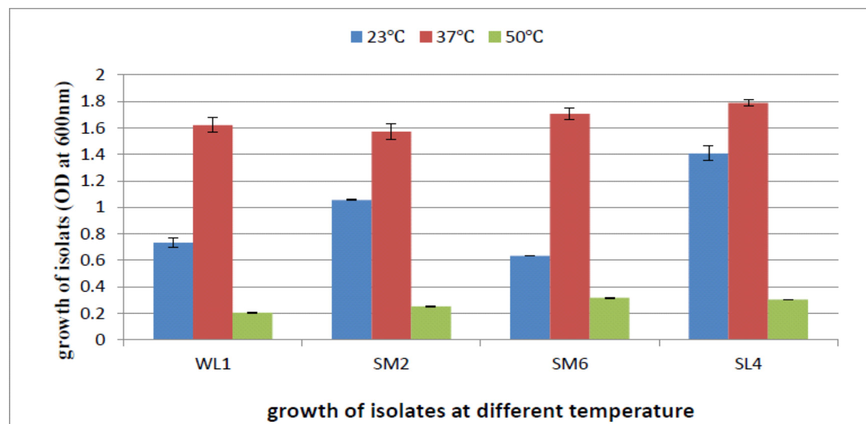

Fig. 2. *Effect of different temperature (23°C, 37°C, 50 °C) on the growth of dye degrading bacterial isolates*

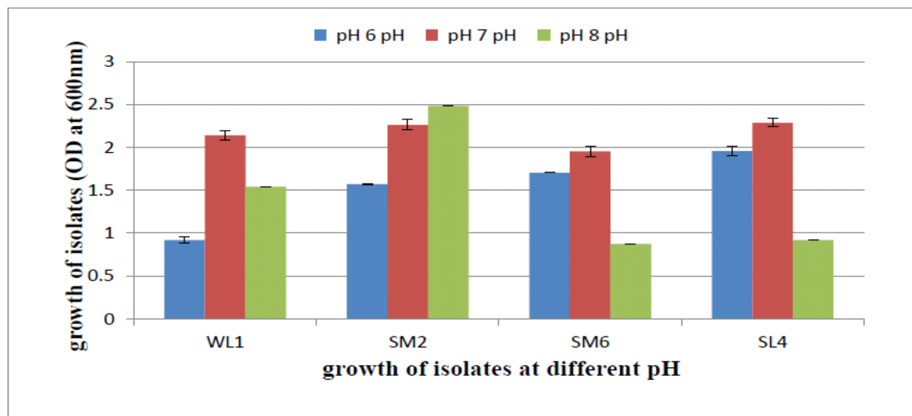

Fig. 3. *Effect of different pH (6pH, 7pH, 8pH) on the growth of dye degrading bacterial isolate*

Malachite Green Degradation Potential

Varying Concentrations of Dye

Malachite green degradation potential was noted at different concentrations (100 µg/mL, 200µg/mL, 400µg/mL, 600µg/mL and 800µg/mL) of malachite green, isolates were observed, and results were recorded using spectrophotometer. Notable degradation (average percentage=73.29) was observed at 100µg/ml and maximum degradation was determined at 200µg/ml (average percentage =85.84%), concentration of malachite green followed by 400µg/ml (average percentage =61.75%) whereas degradation decrease with the increase of concentration of malachite green under 600 µg/ml and 800 µg/ml (average percentage= 47.4% and 35.25%, respectively). Result indicates that the dye degradation rate increases with dye concentration upto 200 µg/ml beyond which the degradation rate is lowered. The optimum dye concentration was hence found to be 200 µg/ml shown in Fig. 4.

Varying Temperatures

To determine the degradation of malachite green at different temperature the selected isolates were inoculated in L-broth tubes containing 200 µg/mL concentrations of malachite green dye and incubated at different temperature ranges (23℃, 37℃, 50℃) for 24 hours. Following incubation, the bacterial cell density was adjusted by adjusting bacterial O.D 600 nm (0.5) using spectrophotometer. Results showed that maximum degradation was recorded at 37 ℃ (average percentage =84.25%) whereas at low and high temperature degradation efficiency was reduced. Hence results showed that optimum temperature for the degradation of malachite green was 37 ℃ (Fig. 5).

Varying pH

Results indicated that there was a general trend of maximum dye degradation observed at optimum pH7 (average percentage =84.75%) while at low or High pH the rate of degradation was changed. So optimum 7 pH was required for maximum degradation of malachite green. Variably (Fig. 6).

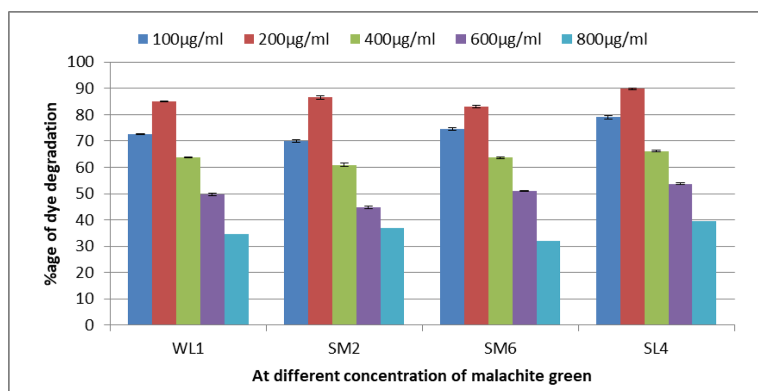

Fig. 4. *Effect of different concentrations of malachite green (100 µg/ml, 200 µg/ml, 400 µg/ml, 600 µg/ml and 800 µg/ml) on the percentage of dye degradation potential of dye degrading bacterial isolates*

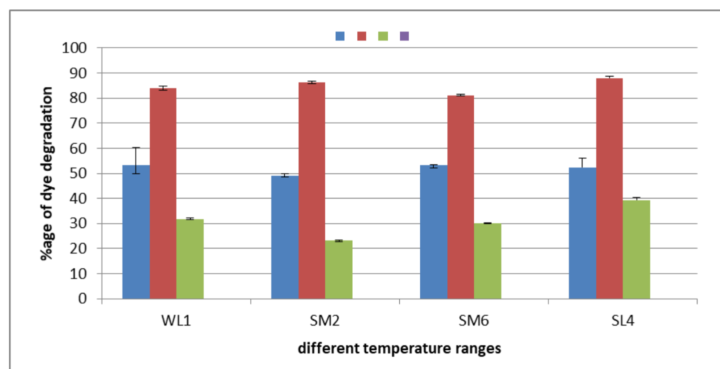

Fig. 5. *Effect of different temperatures (23°C, 37°C, 50°C) on the percentage of dye degradation potential of dye degrading bacterial isolates*

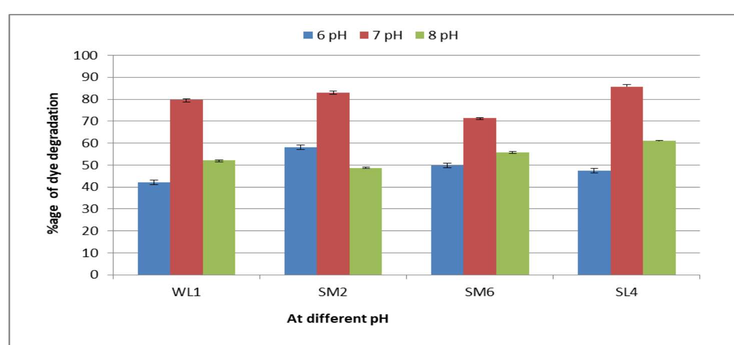

Fig. 6. *Effect of different pH (6pH, 7pH, 8pH) on the percentage of dye degradation potential of dye degrading bacterial isolates*

Result of Plant Microbial Interaction

The impact of monoculture and co culture dye degrading bacterial isolates supplemented with malachite green was observed on Triticum aestivum and different parameter such as shoot length, root length, fresh weight, dry weight, number of leaves and number of roots was observed and recorded.

Shoot Length

Effect of isolated monocultures and co culture was checked on the shoot length of *Triticum aestivum* in the presence as well as in the absence of malachite green dye. It was observed that presence of dye in the soil caused remarkable reduction in the shoot and root length as compared to the plants growing in dye free conditions. In non-stress condition (in the absence of malachite green) the shoot length of WL1 and SM6 was reduced by 8.4% and 6.2% respectively whereas the shoot length of SM2 and SL4 was increased by 0.4% and 9.6% in case of monoculture of bacterial isolates. In dye free condition, stimulation in shoot length was observed by co culture WL1SM2, WL1SM6, SM2SM6, SM2SL4, SM6SL4, WL1SM2SM6, WL1SM6SL4, WL1SM6SL4, SM2SM6SL4, WL1SM2SM6SL2 4 %, 11.3 %, 7.2 %, 8.8 %, 16.4 %, 4.6 %, 18.15 %, 26.02 %, 14.37 % and 35.40 % respectively. The shoot length of control of non-inoculated seeds is 13.22 larger as compared to the shoot length of control 11.38 which was inoculated with malachite green. In case of mono culture

shoot length of seeds, inoculated with WL1, SM2 and SM6 isolates, supplemented with malachite green decreased by 12.68%, 10.42% and 4.84 respectively. Whereas shoot length of seeds, inoculated with SL4 isolate, supplemented with malachite green was increased by 4.36%. In the case of co culture, the shoot length of seeds, inoculated with WL1SM2 isolate, supplemented with malachite green was decreased by 14.05%. Shoot length of seeds inoculated with WL1SM6 isolate in the soil supplemented with malachite green was decreased by 11.38%. The shoot length of seeds inoculated with WL1SL4 isolate in the soil supplemented with malachite green was increased by 12.43%. The shoot length of seeds, inoculated with SM2SM6 isolate grown in soil supplemented with malachite green was decreased by 10.25 %. Shoot length of seeds, inoculated with SM2SL4 isolate, supplemented with malachite green was increased by 7.75%. Shoot length of seeds, inoculated with SM6SL4 isolate, supplemented with malachite green was increased by 14.70%. Shoot length of seeds, inoculated with WL1SM2SM6 isolate 75 supplemented with malachite green was increased by 18.57%. Shoot length of seeds, inoculated with WL1SM2SL4 isolate, supplemented with malachite green was increased by 22.94%. The shoot length of seeds, inoculated with WL1SM6SL4 isolate, supplemented with malachite green was decreased by 1.21%. The shoot length of seeds, inoculated with SM2SM6SL4 isolate supplemented with malachite green was increased by 3.95%. Shoot length of seeds, inoculated with WL1SM2SM6SL4 isolate, supplemented with malachite green was increased by 10.95% as shown in Fig. 7.

Root Length

In non-stress condition (in the absence of malachite green) the root length of WL1 and SL4 was increased by 2.75% and 1.24% respectively as compared to control whereas the root length of SM2 and SM6 was decreased by 17.26% and 9.6% as compared to control in case of monoculture of bacterial isolates. In dye free condition, stimulation in shoot length was observed by co culture WL1SM6, WL1SL4, SM2SM6, SM2SL4, SM6SL4, WL1SM2SM6, WL1SM2SL4, WL1SM6SL4, SM2SM6SL4 and WL1SM2SM6SL by 1.73%, 3.34%, 2.27%, 4.90%, 0.06%, 2.45%, 3.46%, 8.93% and 0.31% respectively as compared to the control. The root length of control of non-inoculated seeds is 15.89 as compared to the shoot length of control 11.23 which was inoculated with malachite green. In case of mono culture shoot length of seeds, inoculated with WL1and SM6 isolates, supplemented with malachite green decreased by 4.54% and 4.5% respectively. Whereas shoot length of seeds, inoculated with SM2 and SL4 isolate, supplemented with malachite green was increased by 0.62% and 4.09% respectively. In case of co culture, the root length of seeds, inoculated with WL1SM2, SM6SL4, WL1SM2SM6 and WL1SM6SL4 isolates, supplemented with malachite green was decreased by 7.7%, 2.22%, 1.60% and 2.22% respectively. Root length of seed, inoculated with WL1SM6, WL1SL4, SM2SM6, SM2SL4, WL1SM2SL4, SM2SM6SL4 and WL1SM2SM6SL4 isolate, supplemented with malachite green was increase by 1.51%, 1.15%, 8.01%, 8.72%, 11.57%, 0.71% and 4.98% respectively as compared to control as shown in Fig. 7.

Fresh Weight

In dye free condition (in the absence of malachite green) the fresh weight of plant inoculated WL1, SM2 and SM6 was reduced by 3.39%, 7.5% and 12.2% as compared to control, respectively whereas the fresh weight of SL4 was increased 0.04% in case of monoculture of bacterial isolates. In dye free condition, in case of co culture, the fresh weight of seeds inoculated with WL1SM2, WL1SM6, SM2SM6, SM2SL4, SM6SL4, WL1SM2SM6 and WL1SM6SL4 was decreased by 10. 27%, 4.2%, 5.3%, 3.7%, 6.2%, 2.4%, and 3.7% as compared to control respectively. However the fresh weight of seeds inoculated with WL1SL4,WL1SM2SL4, SM2SM6SL4 and WL1SM2SM6SL4 was increased by 4.18%, 4.18%, 0.26% and 6.20% as compared to control respectively. In dye supplemented condition, the fresh weight of plant inoculated with WL1, SM2 and SM6 was reduced by 14.47%, 20.73% and 12.37% as compared to control, respectively whereas the fresh weight of SL4 was increased 6.36 % in case of monoculture of bacterial isolates. In stress condition (in the presence of malachite green), in case of co culture, the fresh weight of plants inoculated with WL1SM2, SM6SL4, WL1SM2SM6 and WL1SM6SL4 was decreased by 3.32% , 5.5% and 1.4% as compared to control respectively. However, the fresh weight of seeds inoculated with WL1SM6, WL1SL4, SM2SM6, SM2SL4, WL1SM2SL4, SM2SM6SL4 and WL1SM2SM6SL4 was increased by 4.29%, 11.07%, 3.16%, 5.23%, 1.55%, 6.87% and 13.27%as compared to control respectively as displayed in Fig. 8.

Dry Weight

In dye free condition (in the absence of malachite green) in the case of mono culture all strains WL1, SM2, SM6 and SL4 showed increased in dry weight by 1.21%, 11.83%, 8.06% and 3.02% as compared to control respectively. In dye free condition, in case of co culture, the dry weight of seeds inoculated with WL1SM2, SM2SM6, SM2SL4 and SM6SL4 was decreased by 14.69%, 13.3%, 15.49% and 11.88% as compared to control respectively. However, the dry weight of seeds inoculated with WL1SM6, WL1SL4, WL1SM2SM6, WL1SM2SL4, WL1SM6SL4, SM2SM6SL4 and WL1SM2SM6SL4 was increased by 17.8%, 2.77%, 2.01%, 4.65%, 6.96%, 18.13% and 3.10% as compared to control respectively.In dye supplemented condition, the dry weight of plant inoculated with WL1, SM2 and SM6 was reduced by 17.42%, 23.71% and 18.69% as compared to control, respectively whereas the dry weight of SL4 was increased 0.05 % in case of monoculture of bacterial isolates. 77 In stress condition (in the presence of malachite green) in case of co culture, the dry weight of plants inoculated with WL1SM2, WL1SM6, SM2SL4 and SM2SL4 and SM6SL4 was decreased by 13.67%, 12.81%, 4.27% and 0.57% as compared to control respectively. However, the dry WL1SL4, SM2SM6, WL1SM2SM6, WL1SM2SL4, SM2SM6SL4 and WL1SM2SM6SL4 was increased by 5.13%, 1.90%, 0.75%, 1.78%, 4.50%, 9.29% and 12.63% as compared to control respectively as shown in Fig. 8.

Number of Leaves

In dye free condition (in the absence of malachite green) the number of leaves of plant inoculated with WL1 and SM6 was increased by 40% and 25% as compared to control,

respectively whereas the number of leaves of SM2 and SL4 was 20% in case of monoculture of bacterial isolates. In dye free condition, in case of co culture, the number of leaves of plant inoculated with WL1SL4, SM6SL4, WL1SM2SM6, WL1SM2SL4 was increased by 30%, SM2SM6 was increased by 45% and SM2SL4, SM2SM6SL4 and WL1SM2SM6SL4 was increased by 35% as compared to control respectively. The number of leaves was reduced in only two co-cultures WL1SM2 and WL1SM2SL4 by 15 %. However the number of leaves of plant inoculated with WL1SM6 was similar to control. In dye supplemented conditions, the number of leaves of plants increased as compared to the control in case of WL1, SM2 and SL4 by 15 % where as in SM6 it was increased by 20 % in case of mono culture. In dye supplement condition, in case of co culture ,the number of leaves of plant inoculated with SM2SM6SL4 and WL1SM2SM6SL4 was increased by 15%, WL1SM6SL4 and SM2SM6SL4 was increased by 10% as compared to control as displayed in Fig. 9.

Number of Roots

In dye free condition, the number of roots of plant inoculated with WL1, SM2 and SM6 was reduced by 2.77%, 8.33% and 5.55% as compared to control, respectively whereas the number of roots of SL4 was increased 2.77 % in case of monoculture of bacterial isolates. In dye free condition, in case of co culture, the number of roots of plant inoculated with WL1SL4, WL1SM6 and SM2SM6 was decreased by 2.77%, 11.11% and 11.11% as compared to control respectively. In dye supplemented condition, the number of roots of plant inoculated with SM6 was reduced 3.03% as compared to control, whereas the number of roots of WL1and SM6 was increased by 3.03 % and 9.09% respectively as compared to the control in case of monoculture of bacterial isolates. In dye supplement condition, in case of co culture, the number of roots of plant inoculated with WL1SL4, WL1SM6SL4 was decreased by 15.15% and 6.06% as compared to control respectively. The number of roots of plant inoculated with WL1SM2, SM6SL4, WL1SM2SM6, WL1SM6, SM2SM6 was increased by 3.03% WL1SM2SL4 was increased by 12.12%, SM2SL4, SM2SM6SL4 and WL1SM2SM6SL4 was increased by 6.06% as compared to control as shown in Figure 9.

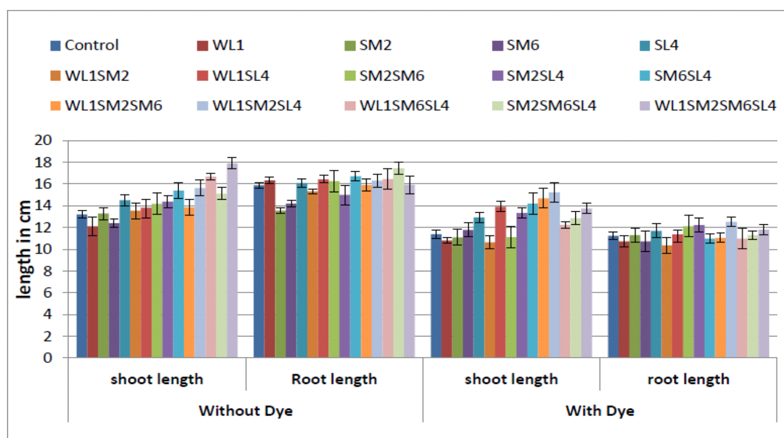

Fig. 7. *Effect of mono and co cultures of dye degrading bacterial isolates on the shoot* and root length of plants in the presence and absence of dye

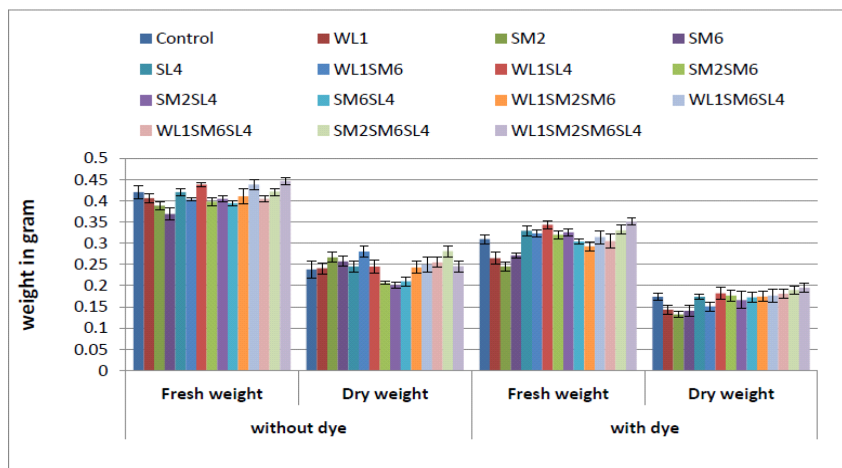

Fig. 8. *Effect of mono and co cultures of dye degrading bacterial isolates on fresh weight and dry weight of plants in the presence and absence of dye*

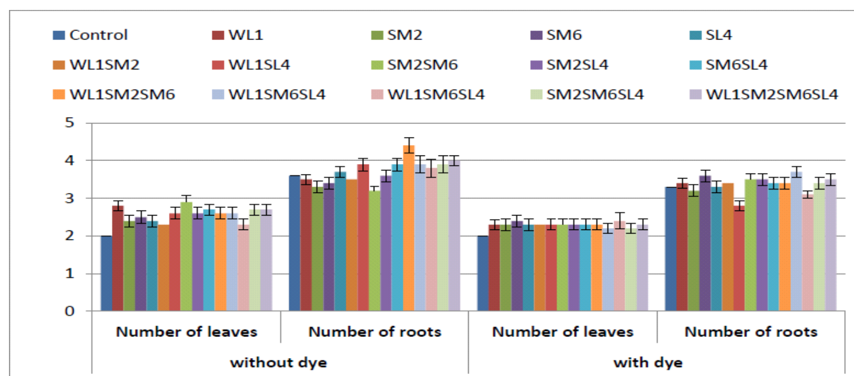

Fig. 9. *Effect of mono and co cultures of dye degrading bacterial isolates on the number of leaves and number of roots of plants in the presence and absence of dye*

Rapid industrialization is at forefront of global water pollution because it usually released wastewater into the surrounding area [4]. Synthetic chemicals are widely utilized as dyes in textile industries and their effluent cater severe problem for the environment [22]. Bioremediation is very effective and eco-friendly methodology to encounter the hazardous dyes [7, 9, 10]. Biological consortium can be utilize as bioreactor for effective bioremediation and decolorization of azo dyes together with the utilization of these isolates for plant growth promotion [23]. The aim of current study was to isolate the dye degrading bacteria and utilization of these isolated bacterial isolates in plant growth promotion. It has been indicated previously that textile effluent is a best habitat for the bacteria that can degrade and easily decolorize the dyes by their natural abilities [6, 12, 14]. Total 20 isolates were isolated from the collected samples shown in Table 3.4. In the present study, four bacterial isolates SL1, SM2, SM6 and SL4 showed effective dye degradation as shown in Table 5. The identification of bacterial isolates was done by cultural and microscopic characterization. Following Cappuccino& Sherman, 2013 [20] all isolates WL1, SM2, SM6 and SL4 appeared as Gram positive cocci in Table 6. The result in the present study showed

that SM2 isolate resembles *S.aureus* spp and bacterial isolates WL1, SM6 and SL4 resemble Staphylococcus *spp*. Our results are in line with previous research findings and showing similarity to *S.saprophyticus* spp shown in Table 3.7 [4,13,19]. Environmental conditions affect the dye degradation efficiency of bacterial isolates. All these isolates showed maximum growth at 37℃ and optimum pH was 7 and its clearly depicted in figure 2 and 3 respectively [7, 24]. The result showed that maximum growth of isolates were shown in the presence of 0.02µg/ml whereas the higher concentration of dyes reduced the growth of these isolates shown in figure 1 [22, 25]. Different researches had shown that the biodegradation processes were highly dependent on pH value. The result of the current study concluded that neutral pH i.e pH 7 is an optimum pH for bacterial growth, at which each bacterial isolates can decolorize malachite green effectively [11, 25]. Previous study also investigated that pH 7 is more encouraging for complete degradation of textile dyes shown in Fig. 7 [26].

Different temperatures (23℃, 37℃, 50℃) were provided to the bacterial isolates and it was observed that maximum degradation of staining dyes was achieved at 37℃ in Figure 6 [26, 27]. Dye concentration plays a vital role in the bioremediation of dye wastewater Kumar et al 2006. [16]. Result indicates that the dye degradation rate increases with dye concentration at 200µg/ml beyond which the degradation rate is lowered. Hence, optimum dye concentration was found to be 200µg/ml in Fig. 5 [24]. The effect of these bacterial isolates was observed on the Triticum *aestivum* growth plant in which monoculture and co culture of these isolates was prepared and their effect on growth parameters was observed. It is found that the shoot length, root length, fresh weight, dry weight, number of leaves and number of roots were high in plants which grew in the absence of dyes as compared to the dye treated plants as shown in Figure 9, 10 and 11. These bacteria isolates, increase plant growth, accelerate seed germination, improve seedling, increase plant growth and protect plants from abiotic stress, by utilizing different strategies [15, 17, 19, 26]. During the plant microbial interaction several isolates can release indolic compounds such as the auxin phytohormone indole-3-acetic acid (IAA), which is growth regulator in plant and control the developmental processes such as cell division and elongation, tissue differentiation, apical dominance [17, 27]. It was observed that the growth of plants having co culture of bacterial strains are larger than those plants which contain monoculture bacterial strains in both dye supplemented and dye free condition dyes as shown in Fig. 9, 10 and 11. This is because of mutual association of these bacterial strains which supports each other and help to flourish and sustain in stress conditions [23]. Several microbial strains showed dual characters for the degradation of azo dye and plant growth promotion by adopting several strategies such as production of indole acetic acid, phosphate solubilization and halo ring formation [11]. The present study relates to a previous report showing biological treatment that causes reduction in toxicity of dyes and promotion of plant growth [11, 15, 28].

CONCLUSION

It is important to control the release of recalcitrant compounds into the environment because it is causing devastating effects on the plants thus affecting the economy of Pakistan. So an alternative pollution free environment is required to enhance our agricultural yield. The aim of present study is to provide indigenous bacterial isolates that are capable of dye

degradation and producing less harmful by-products, used by plants as metabolites. Utilization of co culture as compared to monoculture of bacterial isolates is more effective in dye degradation and plant growth promotion. These findings are helpful for controlling the environmental pollution caused by industrial effluents and can be further used in future research.

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