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Bioremedation of Industrial Effluent using Immobilized Cells of Halotolerant Marine Bacterium

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Dyes are the major toxic substances in all industrial wastewater and are highly carcinogenic to human beings and other soil and water living things. In this present study, 52 halophilic bacterial strains were isolated from water and sediments samples collected from Kelambakkam and Marakkanam salterns, nearby East Coast of Tamil Nadu, India and they were designated with unique codes as AMETH101 to AMETH152. They were screened for their ability to produce hydrolytic enzymes such as protease, lipase and tannase enzymes. On comparison, three strains namely, AMETH111, AMETH132 and AMETH148 have exhibited all of the enzyme activities tested and they were chosen to test their ability in decolorization of five different textile dyes namely Black'B' , Yellow MR , Red BSID , Blue M2R and Torque Blue 'G'. Among the three halophilic bacterial strains, AMETH148 exhibited 93.61% decolorization in Black-B, 68.74% decolorization in Yellow-MR, 72.84% in Torque Blue 'G', 91.27% Red BSID and 92.26% in Blue M2R compared to other two isolates and hence chosen for immobilization and further study. The halotolerant strain AMETH148 was immobilized with calcium alginate and dye decolorization experiments were conducted. After immobilization, there was a good improvement in decolorization percentage by the strain, as compared to their free cell counterparts and it was concluded that AMETH148 was the most efficient of all the bacterial strains in decolorizing all the five textile dyes. Decolorized dye solutions were subjected to plant growth promotion studies to determine to understand the level of bioremediation or detoxification. All the decolorized dye solutions were found to enhance the seed germination and seedling growth parameters of four different crop plants such as, Green gram (*Vigna radiata),* Black gram (*Vigna mungo)*, Wheat (*Triticum aestivum*) and Paddy (*Oryza sativa)*. This confirms that the treated dyes had no phytotoxic effect on crop plant seedlings. This study concluded the potential of halotolerant marine bacterium AMETH148 as a suitable candidate for the decolorization and bioremediation of textile dyes.

Key words: Hypersaline environment, Halotolerant bacteria, bioremediation, immobilization, plant growth promotion.

Halophiles are extremophile organisms that are live in very high concentrations of salt (Roohi *et al*., 2012). Environments with high-salt concentrations are often populated by dense microbial communities. Various applications were found to be halophilic microorganisms which can be isolated from different saline environments and different strains even belonging to the same genus. Wastewater and soil rich in both organic matter

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and salt are difficult to treat using conventional microorganisms typically found in wastewater treatment and soil bioremediation facilities (Zhuang *et al*., 2010). Halophilic bacteria and archaea have found a wide range of distribution in the world and these microorganisms have been isolated hypersaline environments such as saline lakes, saline desert soils, salterns, salt marshes, salt mines, salted hides or foods (Oren, 2002; Ventosa *et al*., 2008; Berrada *et al*., 2012).

In recent years, halophilic microorganisms have been explored for various Biotechnological applications in different area of fields (Mellado and Ventosa, 2003). The applications range from the use of different products, such as the compatible solutes, biopolymers production or carotenoids or enzyme in a variety of industries and in environmental bioremediation processes. In addition, halophilic enzymes offer important opportunities in the field of biotechnological applications, such as food processing, environmental bioremediation and biosynthetic processes (Gomez and Steiner, 2004). Different types of microorganisms are capable of degrading azo dyes due to their high potentials in producing enzymes, organic acids etc. However, many of them cannot be used as dye degrading agents due to the harmful nature of persistent toxic pollutants dye-polluted environments. In this context, halophilic and halotolerant microorganisms can be the best candidates for a practical decolorization process as these microorganisms are able to grow easily at high concentrations of salts. Moreover, some of these organisms can tolerate the presence of other stress factors such as toxic oxy-anions and heavy metals which are so common in several industrial wastewaters. In recent years, studies have been focused on halophilic and halotolerant microorganisms and their abilities for decolorization of azo dyes (Amoozegar *et al*., 2011). The textile industry produces huge volumes of contaminated water; one of the most important contaminants is azo dyes. The presence of azo dyes and their residues in the environment creates a lot of problem due to coloration of natural waters and high toxicity, mutagenicity and carcinogenicity of the dyes and their biotransformation products (Kolekar *et al*., 2008). Microbial processes, which employs whole microorganisms or their products for the treatment of textile wastewater have many advantages such as cost-effective, environment friendly and producing less sludge. In theory and practice, the most promising microorganisms for wastewater treatment are those indigenous organisms isolated from sites contaminated with dyes or from the sludge of treatment plants because they have adapted to survive in adverse conditions. The mechanism of microbial decolouration includes physical adsorption, enzymatic degradation or a combination of both. Both reductases and oxidases are involved in the microbial degradation process. Some oxidoreductase enzymes such as laccases have high potential for dye degradation (Solís *et al*., 2012). Thus the present study was carried out to isolate a halo tolerant bacterium from salterns of Tamil Nadu and test their ability in textile dyes degradation. Attempts were made to study their dye degradation potential after immobilization and also to test the effect of treated dye products on the growth promotion of crop plant seedlings.

MATERIALS AND METHODS

Collection of samples

The water and sediment samples were collected from Marakanam (around 12°14’53.9"N 79°56’10.1"E) and Kelambakkam Salterns (around 12°44’56.8"N 80°12’56.1"E) along the East Coast of Tamil Nadu, India. The surface water samples and sediment soil samples were collected using sterilized plastic bottles, allowing enough air space in the bottles to facilitate thorough mixing and aseptically transferred in to sterile polythene bags and the samples were brought to the laboratory for bacteriological analysis.

Isolation of Halotolerant bacteria (HTB)

Ten gram of soil sample/ 10 ml of water sample were taken in sterile conical flasks and 90 ml of sterile distilled water was added to each flask. The flasks were kept in shaker for approximately 15 minutes at 100 rpm. This 10^{-1} dilution was serially diluted up to 10^{-3}dilution. Then 0.1 ml from 10^{-3} dilution was used to spread plate on Halophilic Agar medium and the plates were incubated for 7 days at 37ºC. The halophilic agar medium containing (gm/L) Peptone - 5, Yeast extract - 3, $CaCl_2$- 0.1, Kcl- 5, $MgSO_4$ - 6, NaCl- 30, Agar - 20.

Purification and storage of bacterial strains

After the incubation period, morphologically distinct bacterial colonies were sub- cultured in Halophilic Agar (HA) plates. The colony characteristics were observed and recorded. The purity of the isolated bacterial strains was tested by quadrant streaking and single colonies were again sub-cultured on the same medium. Then the purified bacterial strains were given a prefix of AMETH (indicating the University name and halophilic nature) followed by Arabian numerical in a series from 101. Then the strains were stored in aged double sterile sea water in 1.5 ml Eppendrof tubes at 4ºC until usage.

Screening of HTB for extracellular hydrolases

All the HTB were subjected to screen for the production of extracellular enzymes such as protease, tannase and lipase in a simple qualitative plate assay. The proteolytic activity of the organism was checked using nutrient agar medium prepared in seawater with casein (0.5%) as substrate. Test bacterial strains were streaked and incubated at room temperature for 7 days. Protease activity was visualized by the clear zone around bacterial patches, after the plates were flooded with saturated ammonium sulfate solution prepared in 0.1 N HCl. The nutrient agar medium was prepared in aged seawater and a 0.5% tannic acid solution was also prepared separately and filter sterilized. After the sterilization, tannic acid was added with the molten medium just before pouring in petriplates. Formation of a dark brown zone around the bacterial culture indicates the positive tannase activity. The lipolytic activity was checked using nutrient agar medium prepared in seawater with Tween 80 (0.5%) as substrate. Lipase activity was visualized as a zone of opalescence around the colonies after 7 days of incubation.

Screening of HTB for IAA production

IAA production was analyzed both qualitatively and quantitatively. Selected HTB strains were also screened for the production of IAA. The halotolerant strains were inoculated in sterilized Nutrient broth prepared in seawater supplemented with tryptophan (10 µg/mL) and incubated at 37ºC for 7 days in shaking conditions. After incubation period, fully grown bacterial broth cultures were centrifuged at 10,000 rpm for 10 minutes. To the supernatant (2 ml), two drops of orthophosphoric acid was added and incubated at room temperature for 10 minutes, followed by addition of 4 ml of Salkowski reagent (50 ml, of 35% sulphuric acid, 1 ml of 0.5M $FeCl_3$). Development of pink color indicates the positive result for IAA production and no color change indicates the negative result for IAA production (Ahmad *et al*., 2008). Culture supernatant was treated with Salkowskis reagent and IAA production was analyzed spectrophotometrically at 545 nm against IAA standard.

Screening of selected bacterial strains for decolorization of textile dyes

Selected the potential strains having both the lipase, protease and tannase enzyme producing strains were screened for their ability to decolorize textile dyes namely Black-B (BB), Yellow-R (YMR), Torque Blue 'G' (TQB), Red BSID (RB), Blue M2R (BM). Test tubes containing 10 ml of half- strength Halophilic broth (HB) medium with 100 mg/L BB, YMR, TQB, RB and BM was prepared and autoclaved. To this 100ml of 12hrs old bacterial culture was inoculated. The tubes were incubated for 9 days under static condition at room temperature and read at 600 nm (Silveira *et al.*, 2009).

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Immobilization of efficient bacterial strains for decolorization of textile dyes

The potential bacterial strain which has the capability to decolorize all the tested five textile dyes was chosen to check its decolorization capacity when immobilized. The cell pellets of overnight grown bacterial cells (centrifuged at 10000 rpm for 10mins) were suspended in 3% sodium alginate. The cells- alginate mixture was dripped into cross- linking solution made of 0.2 M $CaCl_2$ to form calcium alginate beads. The diameter of beads was found to be in the range of 3 mm to 4 mm. The beads were left in the calcium chloride solution for 3 hours to attain desirable hardness and uniformity.

Degradation of textile dyes by immobilized HTB AMETH148

The immobilized beads of most efficient bacterium AMETH148 were inoculated into 25 ml of medium containing the respective dye and 0.1 % of yeast extract. This was incubated for 9 days in static condition at room temperature. Then the

supernatant was obtained by centrifugation at 10,000 rpm for 10 minutes. The respective dye solution (100 mg/L) incubated without beads were taken as control for the dyes. These supernatants were used for analytical purposes to determine percent dye decolorization as mentioned previously.

Evaluation of bioremediation potential of the efficient bacterial strain

The evaluation of bioremediation potential was done by plant growth promotion studies to determine the toxic nature of the end products of all the textile dyes that were decolorized by the potential halophillic bacterium. Plant growth promotion studies were carried out on four different crop plants such as, Green gram (*Vigna radiata),* Black gram (*Vigna mungo)*, Wheat (*Triticum aestivum*) and Paddy (*Oryza sativa)*.

Seed germination study (Plate assay)

The experiment was done in disposable plastic petri plates. The top and the bottom of the petri plates were padded with a layer of sterile tissue paper. Surface sterilized seeds of Green gram, Black gram, Wheat and Paddy (40 each) were soaked in 25 ml of colored/untreated dye and 25 ml of decolorized/treated dye for 6 hours. For the controls, seeds were soaked in water for 6 hours. After soaking 40 seeds were placed in each petri plates and the tissue paper was soaked with their respective dye mixtures. The plates were incubated for 3 days. The tissue paper was kept moist by spraying water. After 3 days, the germination percentage was calculated using the following formula. Experiment was done with three replicates.

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total Number of Seeds}} \times 100$$

Seedling growth study

The experiments were carried out in plastic cups. The surface sterilized seeds of Green gram, Black gram, Mustard, Wheat and Paddy (40 each) were soaked in 25 ml of treated dye mixture for 6 hours. Suitable controls were made with water and experiments are conducted in triplicates. 5 seeds from each treatment were added to the plastic cups with 200 g sterile soil. The seeds were then drenched with samples (treated and untreated dye mixture each 20 ml per cup) for the first time. The soil was kept moist by spraying water daily and the cups were maintained in a 12 h photoperiod. The shoot length, root length and fresh weight of all seedlings were recorded after 15 days.

RESULTS AND DISCUSSION

In comparison with the thermophilic and the alkaliphilic extremophiles, halophilic microorganisms have as yet found relatively few biotechnological applications. Halophiles are involved in centuries old processes such as the manufacturing of solar salt from seawater and the production of traditional fermented foods (Oren, 2002). In India, Tamil Nadu is a major state which is having more saltperns for the production of salt (Khalid *et al*., 2008). Solar salterns are the poorly studied habitats on the subject of microbiology. Most commercial solar salterns consist of a series of shallow ponds connected in a series of increasing saline brines. (Wang *et al*., 2009). In this study, a total of 52 Halotolerant bacterial strains were isolated from Kelambakkam and Marakkanam salterns, nearby East Coast of Tamil Nadu, India and they were named as AMETH101 to AMETH152. In a previous study, seven strains of halotolerant, non-pigmented bacteria were isolated from several hypersaline lakes of the Vestfold Hills, Antarctica (James *et al*., 1990). Mageswari et al., (2012) have also isolated several strains of halotolerant bacteria from the sediment sample collected from Marakanam Solar Salterns, Tamil Nadu.

In recent years, different screening processes have been performed in saline conditions in order to isolate and characterize novel enzymatic activities. Several halophilic hydrolases such as amylases, lipases, proteases etc., were screened and used for novel application in diverse Biotechnological fields. (Moreno *et al*. 2013). In the attempt of exploring enzyme producing halotolerant bacteria, we found that among the 52 HTB strains tested, 38 strains showed protease activity on casein agar plates. Likewise, 52 HTB strains, 27 strains exhibited lipase activity. In case of tannase activity, only 12 strains exhibited positive activity as they showed brown color change around their growth in tannic acid amended medium. On comparison, three strains namely, AMETH111, AMETH132 and AMETH148 have produced all the enzymes tested. In a previous study, Anilkumar *et al*., (2010) have screened

Table 1. Screening of extracellular enzymes from Halotolerant bacteria

Strain No	Zone of clearance (cm)			IAA Production
	Protease	Tannase	Lipase	(OD at 545 nm)
AMETH101	1.2 ± 0.1	-	2 ±0.08	-
AMETH102	-	-	2.1 ±0.2	145
AMETH103	1.6 ± 0.4	-	-	30.5
AMETH104	-	-	1.9 ± 0.1	69.69
AMETH105	-	-	1.4 ± 0.1	-
AMETH106	1.75 ± 0.1	-	1.4 ± 0.1	87.29
AMEH 107	1.5 ± 0.08	-	1.4 ± 0	-
AMETH108	-	-	2.1 ± 0.1	-
AMETH109	1.2 ± 0.1	-	1.1 ± 0.1	120.11
AMETH110	-	-	2.1 ±0.1	-
AMETH111	1.3 ± 0.1	1.8 ± 0.1	2 ± 0.2	85
AMETH112	-	-	2 ±0.2	114.26
AMETH113	-	-	0.5 ± 0.2	60.34
AMETH114	-	-	1.2 ± 0.6	125.30
AMETH115	-	-	-	-
AMETH116	-	-	1.6 ± 0.5	-
AMETH117	1.6 ± 0.1	-	0.6 ± 0.2	90.39
AMETH118	1.2 ± 0.05	-	2.2 ± 0.1	66.38
AMETH119	-	-	2 ± 0.2	21.47
AMETH120	2.1 ± 0.1	2 ± 0.1	2.1 ± 0.2	53.27
AMETH121	-	-	2.1 ± 0.0.8	-
AMETH122	-	-	1.5 ± 0.05	41.27
AMETH123	-	-	1.3 ± 0.08	33.96
AMETH124	-	-	2.1 ± 0.3	20.17
AMETH125	1.5 ± 0.08	-	1.6 ± 0.4	-
AMETH126	-	-	2.1 ± 0.08	-
AMETH127	1.6 ± 0.1	-	1.6 ± 0.4	-
AMETH128	1.5 ± 0.1	-	-	15.9
AMETH129	1.2 ± 0.1	-	2.1 ± 0.08	-
AMETH130	-	-	-	-
AMETH131	-	-	0.5 ± 0.2	-
AMETH132	1.6 ± 0.1	1.6 ± 0.1	0.4 ± 0.1	43.19
AMETH133	1.6 ± 0.1	-	-	-
AMETH134	1.4 ± 0.05	-	-	-
AMETH135	-	-	2.1 ±0.1	-
AMETH136	1.5 ± 0.1	-	0.5 ± 0.1	-
AMETH137	-	-	0.4 ± 0.08	81.06
AMETH138	-	2 ± 0.1	0.5 ± 0.1	+
AMETH139	-	1.8 ± 0.1	0.4 ± 0.08	-
AMETH140	-	-	0.5 ± 0.1	20.06
AMETH141	-	-	2.1 ± 0.1	77.01
AMETH142	1.5 ± 0.1	-	1.5 ± 0.1	-
AMETH143	1.5 ± 0.02	-	1.5 ± 0.1	-
AMETH144	1.3 ± 0.1	-	-	-
AMETH145	1.4 ± 0.08	-	2 ± 0.3	-
AMETH146	2.3 ± 0.1	-	-	-
AMETH147		1.8 ± 0.05	2 ± 0	-
AMETH148	2.2 ± 0.2	1.9 ± 0.1	2.2 ± 0.1	73.45
AMETH149	-	1.6 ± 0.2	-	-
AMETH150	1.6 ± 0.1	-	-	-
AMETH151	-	1.9 ± 0.1	1.5 ± 0.1	-
AMETH152	-	1.8 ± 0.2	2 ± 0	33.08

hydrolytic enzymes such as proteases, amylases, xylanases, lipases, esterases, glutaminases, asparaginases and inulinases and also screened the production of therapeutic enzymes such as asparaginase and glutaminase by the strains isolated from Arabian Sea Mumbai, Maharashtra, India. They found a potential extreme halotolerant *Marinobacter hydrocarbonoclasticus* strain AK5 as a very good producer of industrially important enzymes. An extreme halophilic bacterium, strain *Salinococcus* JAS4 isolated from Arabal soil of west coast of Karnataka, India was found to produce the extracellular enzymes such as Amylase, Protease, Inulinase and Gelatinase (Jayachandra *et al*., 2012).

Soil salinity is one of the reasons for the reduced crop productivity globally (Jha *et al*., 2012). Indole-3-acetic acid (IAA) represents one of the most important and applicable phytohormone and also an abundant type of auxin in plants. Synthesis of IAA by plant associated bacteria is most probably important cause for improving the growth and yields of various crops (Ali *et al*., 2009a, b; Akhtar and Ali 2011). Hence, we have subjected all 52 strains isolated for this study in to screening for the production of IAA and found 24 isolates were able to produce IAA. The result clearly indicates that the HTB were able to promote plant growth through the production of IAA like phytohormones. In a previous study, Goswami *et al*. (2014) have isolated a total of 85 strains from the rhizosphere of a halotolerant plant *Suaeda fruticosa* from saline desert of Little Rann of Kutch, Gujarat (India). Out of 85 isolates, 11 isolates produced IAA. Similar to that, a total of 93 halotolerant bacteria were isolated from Sambhar lake, an extreme hypersaline environment of India and while screening 50% of them were found to produce IAA (Sahay *et al*., 2012).

A variety of synthetic dyes released by the textile industry pose a severe threat to environmental safety and public health. Among all, azo dyes account for the majority and are extensively used in many industries such as textile, paper, food, leather, cosmetics and pharmaceutical. Existing effluent treatment procedures are unable to remove recalcitrant azo dyes completely from effluents because of their color fastness, stability and resistance to degradation. Decolorization and degradation of azodyes by employing potential

Table 2. Effect of different treated dye on the growth of four different seedlings

Treatment	Green Gram			Black gram			Wheat			Paddy		
	Shoot Length (cm)	Root Length (cm)	Fresh Weight (cm)	Shoot Length (cm)	Root Length (cm)	Fresh Weight (cm)	Shoot Length (cm)	Root Length (cm)	Fresh Weight (cm)	Fresh Length (cm)	Root Length (cm)	Fresh Weight
Control(WATER)	21.7±0.6	7.5±0.4	4.3±0.4	25.3±0.4	5.7±0.2	3.8±0.1	26.7±0.3	8.4±0.1	4.7±0.3	4.7±0.3	10.3±0.1	0.57±0.03
Black 'B'- Treated	25.6±0.4	8.5±0.2	4.5±0.1	26.5±0.3	6.4±0.1	3.7±0.1	28.2±0.2	10.7±0.2	5.4±0.08	5.4±0.08	11.1±0.1	0.6±0.04
Yellow MR - Treated	28.7±0.9	10.7±0.2	5.4±0.1	28.4±0.4	6.2±0.2	3.6±0.2	27.3±0.4	7.4±0.1	4.2±0.1	4.2±0.1	10.2±0.1	0.5±0.04
Red BSID - Treated	23.8±0.5	7.5±0.3	4.3±0.2	27.4±0.4	7.3±0.08	3.8±0.2	29.1±0.1	11.9±0.1	5.2±0.1	5.2±0.1	11.3±0.2	0.6±0.04
Blue M2R - Treated	29.1±0.2	11.9±0.1	5.6±0.4	29.2±0.3	8.7±0.2	3.9±0.1	31.2±0.2	12.4±0.2	7±0.1	7±0.1	11.1±0.1	0.7±0.005
Torque Blue 'G' - Treated	31.9±0.9	12.5±0.4	7±0.08	31.4±0.4	8.7±0.2	3.9±0.1	31.2±0.2	12.4±0.2	7±0.1	13.4±0.1	11.1±0.1	0.7±0.005

strains of bacteria under specified environmental conditions has gained momentum as a method of treatment, as these are inexpensive, eco-friendly and can be applied to wide range of such dyes (Saratale *et al*., 2011). In this context, the present study has concentrated on identifying potential HTB strains for the decolorization and degradation of five azo dyes used in textile industry. Selected three strains namely AMETH111, AMETH132 and AMETH148 which were found to produce all the enzymes tested and also exhibited IAA production were subjected to experiment their dye decolorization potential against five commercially used textile dyes (Black 'B' , Yellow MR , Red BSID , Blue M2R and Torque Blue 'G'). Among the three, the strain AMETH148 have showed 93.61% decolorization in Black-B, 68.74% decolorization in Yellow-MR, 72.84% in Torque Blue 'G', 91.27% Red BSID and 92.26% in Blue M2R. So it has been concluded that AMETH148 as the most efficient

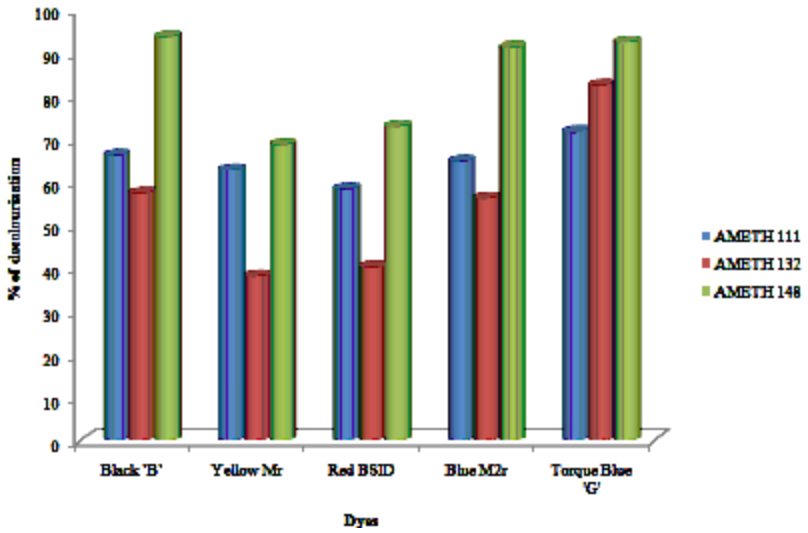

Fig. 1. Percentage of dye decolorization by selected halotolerant bacterial strains

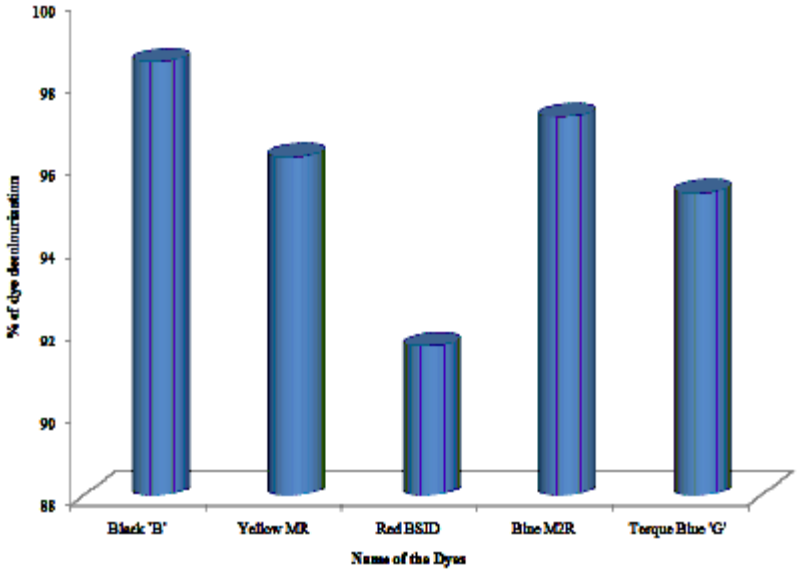

Fig. 2. Percentage of dye decolorization by the potential halophilic bacterial strain AMETH148 (a-e)

of all the bacterial strains in decolorizing all the five textile dyes. So, the strain was chosen for immobilization study (Figure 1). Similarly, Asad *et al.*, (2007) have carried out studies on the decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Among the 27 strains of halophilic and halotolerant bacteria isolated from effluents of textile industries, three showed remarkable ability in decolorizing the widely utilized azo dyes.

Immobilized microbial cells have been used extensively in various industrial and scientific endeavours. However, immobilized cells have not been used widely for environmental applications (Cassidy et al., 1996). Hence, we have tested the effect of a selected HTB AMETH148 on dye decolorization. After immobilization of AMETH148 there was a good improvement in decolorization percentage by the strain, as compared to their free cell counterparts. Immobilized cells of AMETH148 have showed 98.51% decolorization in Black-B, 96.20% decolorization in Yellow-MR,

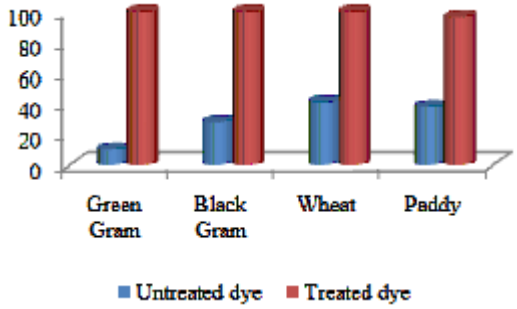

Fig. 3(a). Black 'B'

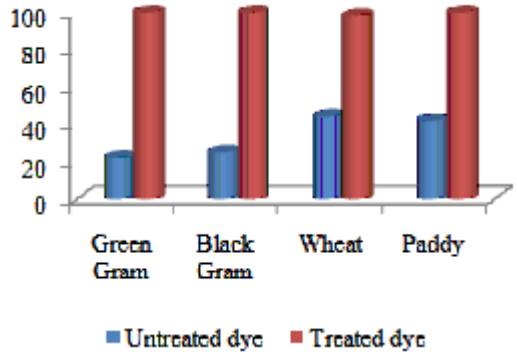

Fig. 3(b). Yellow MR

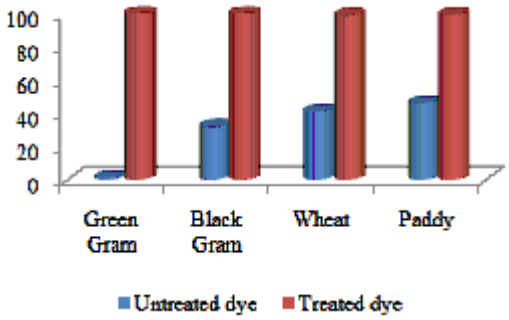

Fig. 3(c). Red BSID

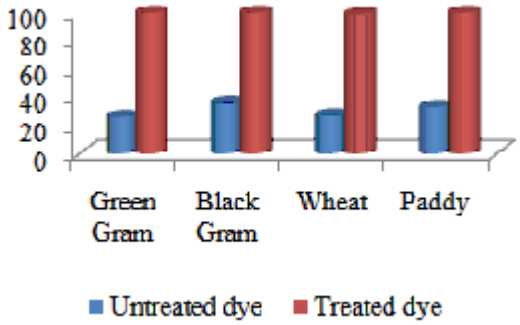

Fig. 3(d). Blue M2R

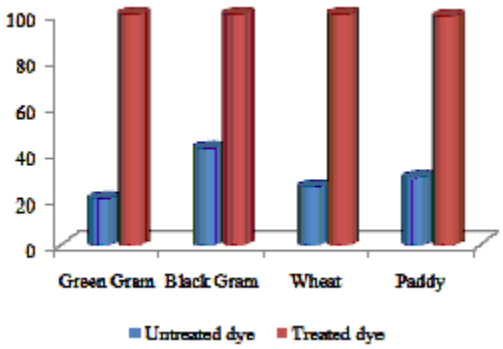

Fig. 3(e). Torque Blue 'G'

91.62% in Torque Blue 'G', 97.18% Red BSID and 95.33% in Blue M2R. Therefore, it was concluded that AMETH148 was the most efficient of all the bacterial strains in decolorizing all the five textile dyes (Figure 2). Immobilized microbial cells create opportunities in a wide range of sectors including environmental pollution control. Compared with suspended microorganism technology, cell immobilization shows many advantages, such as resistance to toxic chemicals (Martins et al., 2013). He *et al.*, 2004, stated that, by immobilization, the toxic effect of the recalcitrant dyes on the growth rate of the bacteria could be nullified. It might also be attributed to the increase in microbial activity inside the immobilized beads or creation of low oxygen micro environment due to diffusion resistance. The trend of our results were also similar to the studies by Su *et al.*, (2009) who found that the anthraquinone and quinine- reducing bacterial consortium increase the dye degradation after immobilization in calcium alginate. Further, alginate-silicate immobilized *Pseudomonas luteola* exhibited increased decolorization of azo dye-Reactive Red 22.

Testing of phytotoxicity is one of the standard protocols in bioremediation studies. Phytotoxicity studies were carried out using *Triticum aestivum*, *Hordeum vulgare*, and *Lens esculenta* to conclude the detoxification of the dyes following degradation by *Aeromonas hydrophila*, a novel bacterial strain isolated from a textile wastewater treatment plant in Greece and capable of decolorizing triarylmethane dyes (Ogugbue and Sawidis, 2011). In the present study, four crop plant seeds such as, Green gram, Black gram, Wheat and Paddy were used to study the phytotoxicity or plant growth promotion effect of untreated dyes and treated dyes on the seed germination. The decolorized dye solutions after the treatment of immobilized cells of selected HTB strain AMETH148, have increased the germination percentage of all the tested crop plant seeds. All the treated dyes have effected 100% germination in Green gram and Black gram seeds while in control (untreated dye solutions) it was only 88.66% and 87.60% respectively for green gram and black gram. Wheat and paddy germination percentage were found to get increased 93-100% where as control showed 100 and 87% respectively in wheat and paddy. The different germination percentage of seeds after treatment with untreated and treated dyes is represented in Figures 3 and 4 respectively.

Auxins play a major role in plant growth promotion. The bacteria that can promote plant growth, that is, PGPB, include those that are free-living, those that form specific symbiotic relationships with plants, bacterial endophytes and cyanobacteria may promote plant growth directly usually by either facilitating resource acquisition or modulating plant hormone levels (Glick 2012). In a previous study, 14 halotolerant bacterial strains isolated from coastal soils and having found to exhibit multiple plant growth promoting traits including production of IAA were subjected to ameliorate salt stress in canola plants where they increased the root length between 5.2% and 47.8%, and dry weight between 16.2% and 43%, in comparison with the uninoculated positive controls. In particular, three of the bacteria, *Brevibacterium epidermidis* RS15, *Micrococcus yunnanensis* RS222, and *Bacillus aryabhattai* RS341, all showed more than 40% increase in root elongation and dry weight when compared with uninoculated salt stressed canola seedlings (Siddikee et al., 2010). In our study, we found that the selected strain AMETH148 is efficient in decolorization of textile dyes. Since the strain AMETH148 is also capable of producing IAA, a plant growth promotion experiment was conducted to find the effect of AMETH148 treated textile dyes. Four crop plant seeds were subjected in the study and shoot length, root length and fresh weight of all the seedlings were recorded after 10 days of treatments (Table 1). Compared to the control groups, all the five treated dyes were very efficient in enhancing the plant growth promotion parameters of all the seedlings. Among the treated dyes Torque Blue 'G' and Blue M2R have exhibited maximum plant growth promotion activity compared to the other treated dyes. Very recently, Nabti et al., (2014) have demonstrated the plant growth promoting effect of bacteria from salt-affected agricultural rhizospheric soil from Bejaia, Algeria, on barley seedlings as well as biological control abilities of these isolates against phytopathogenic fungi. A similar significant positive effect of treated dye wastewater amended with different co-substrates on the seed germination index, root and shoot length and biomass was observed (Khalid et al., 2013). Further

they concluded that dye-degrading microbial cultures could be used to enhance the treatment efficiency of dye-contaminated wastewater that can be utilized for irrigation of crops and biomass production. Since our strain AMETH148, an efficient strain in decolorization and detoxification of textile dyes, is also producing IAA, it can be very well used for the bioremediation of textile dyes and plant growth promotion which is a likely combination.

CONCLUSION

Normally, the microorganisms especially bacteria from new environments like extreme conditions with salt and heavy metal tolerating ability will always withstand for many applications. A halotolerant marine bacterial strain AMETH148 isolated from the Kelambakkam and Marakkanam Salterns of Tamil Nadu proved to be an efficient strain in decolorization and detoxification of commercially used textile dyes which can be very well used for the bioremediation of textile dyes and plant growth promotion.

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