Bioremediation of texile dye (Magenta MP) using halotolerant marine bacteria

[1]Sumathi S, [2]Shruthy Glory I R, [3]Vinothini S, [4]Jayaprakashvel M, [5]Kalavathy G
[1]Researcher, [2]Member of technical staff -Regulatory Affairs, [3]Research Fellowship, [4]Associate Professor, [5]Assistant Professor
[1]Biozone Research Technologies, Chennai, India
[2]HCL Technologies
[3,4]Department of Marine Biotechnology, AMET University, Chennai
[5] St. Joseph's College of Engineering, Chennai

Abstract - Industrial effluents constitute the major reason for water pollution as the toxic substances in them affect the various water-borne living organisms. Dyes from the effluents of textile industries are one such toxic substance since they are highly carcinogenic. . In this present study, 30 halotolerant bacterial strains were isolated from water samples of Kelambakkam saltpans and they were designated with unique codes as AMETH153 to AMETH182. Experiments were carried out to analyze the hydrolytic enzyme producing ability of the strain such as tannase, lipase and protease. Also the ability to decolorize textile dyes was tested using different dyes namely Magenta MP, Black B, Yellow MR, etc. Finally the strains were screened for the production of important auxin hormone, IAA, and compared. Thus the efficient bacterial strains AMETH 156 and AMETH 182 were chosen for immobilization technique. Dye decolorization studies were also conducted by immobilization of these strains with calcium alginate, which gave a significant improvement in the percentage of dye decolorization. The strain AMETH 156 was found to be the most efficient of all the bacterial strains. The decolorized dye solutions were subjected to plant growth promotion studies by plate and cup assay. They were found to enhance the seed germination of four different crop plants, such as green gram, black gram, paddy and wheat, confirming that there was no phytotoxic effect on these seedlings. The study concluded that halotolerant marine bacterium strain AMETH 156 is a potential and a suitable agent for dye degrading and bioremediation of textile dyes.

Key words - Halotolerant bacteria, hydrolytic enzymes, IAA, immobilization, decolorization, bioremediation.

1 INTRODUCTION

Microorganisms are distributed widely in different environmental conditions including different temperature, pH, pressure, salinity and nutrients. These organisms also survive in extreme conditions such as high salinity, high and low temperature and high pH and these are called as extremophiles. They are classified according to the conditions in which they grow: As thermophiles and hyperthermophiles (organisms growing at high or very high temperatures, respectively), psychrophiles (organisms that grow best at low temperatures), acidophiles and alkaliphiles (organisms optimally adapted to acidic or basic pH values, respectively), barophiles (organisms that grow best under pressure), and halophiles (organisms that require NaCl for growth).[1]

Halophiles are a group of extremophiles that can grow in extreme saline conditions or salty environments. Thus they can balance the osmotic pressure and overcome the denaturing effects caused due to high s alinity. Various stable hydrolytic enzymes, such as proteases, amylases, lipases, tannases, DNAases and gelatinases, are produced by these halophilic microorganisms.[2]. Halotolerant bacteria from the rhizosphere of halophyte were found to produce industrially important extracellular enzymes[3]. Immobilized cells of halotolerant marine bacterium were found to involve in the bioremedation of industrial effluents [4]. Further, halotolerant marine bacteria isolated from coastal sand dune plants are found to be capable of producing Indole Acetic Acid (IAA) [5]. It has also been reported that halotolerant marine bacteria from the salterns were efficient in waste water treatment and plant growth promotion [6].

Major problem faced worldwide is the direct disposal of waste from industries into the soil and water resources. Dyes are one such important toxic chemical that directly pollute the water resources causing damage to the aquatic life. The azo dyes are becoming a great concern due to their visible colour, bio recalcitrance and toxicity to animals and humans.[7] Halophilic microorganisms can be used as a potential agent for bioremediation of these azo dyes due to their ability to degrade toxic substances. Hence, this study was aimed to demonstrate the bioremediation potential of halotolerant marine bacteria with reference to textile dyes pollution. Halotolerant bacteria were isolated from the marine saltpans of Kelambakkam, Tamil Nadu and screened for their ability to degrade azo dye from textile industries. Immobilization of the potential halotolerant bacterium capable of producing proteasae and tannase was carried out to compare the dye degradation potential before and after immobilization. Also the treated dye was used to study the growth promotion of plant seedlings

2 MATERIALS AND METHODS

2.1 Collection of sample and Isolation of halotolerant bacteria

The water and sediment samples were collected from Kelambakkam salterns nearby East Coast of Tamil Nadu, India, using a sterile plastic container. 10g of soil sample or 10 ml of water sample was 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] master dilution was serial diluted upto 10^{-3} dilution. Then 0.1 ml from 10^{-3} dilution was used to spread plate on Halophilic Agar (HA) medium and the plates were left for overnight incubation at 37ºC.[8]

2.1 Composition of Halophilic Agar Media (gm/L)

Peptone	-	5
Yeast extract	-	3
Calcium Chloride	-	0.1
Potassium Chloride	-	5
Magnesium Sulfate	-	6
Sodium Chloride	-	30
Agar	-	20

2.3 Purification of Halotolerant bacterial strains

After the incubation period, the morphologically different bacterial colonies were sub- cultured in HA plates. The pure colonies were isolated by quadrant streaking method and the halotolerant bacterial (HTB) strains were sub-cultured every week by streak plate method in the same HA plates. The strains were named with a prefix of AMETH (indicating the University name and halophilic nature) followed by Arabian numerical in a series from 153. For long term preservation, the culture was stored in double sterile sea water in a microfuge tube at 4°C.

2.4 Optimization of salt tolerance ability of the strain

For optimization one parameter at one time method was followed, the potential HTB were streaked in HA plates with different salt concentrations (0%, 5%, 10%, 15%, 20% and 25%). After the incubation period of 24 hours at 37°C, the growth was checked in all the HA plates.

2.5 Screening of HTB for hydrolytic enzymes

The isolated HTB were screened for their ability to produce extracellular hydrolytic enzymes such as lipase, protease and tannase. The lipolytic activity of the organism was checked using Modified nutrient agar medium with tween 80.[9] Nutrient agar plates were prepared with sea water and the test organisms were streaked and the plates were kept for incubation at room temperature for 48 hours. After the incubation period, the zone of opalascence around the colonies was noted. Nutrient agar was prepared supplemented with Gelatin for the assay of extracellular enzyme protease at a concentration of 1% Test bacterial strains were streaked and incubated at room temperature for 4 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.[7] The nutrient agar medium and 0.5% tannic acid solution were sterilized separately to study the tannase activity of the strains. After the sterilization the two solutions were mixed together and it was poured in Petri plate.[10] The organisms were streaked and the plates were kept for incubation at room temperature for 48 hours and after the incubation period, a dark brown zone around the bacterial culture was noted.

2.6 Quantification of Protease and Tannase activity of HTB

2 ml of 1% casein solution was added to 0.5 ml of culture from nutrient broth which was then incubated for a period of 30 minutes. To this 2.5 ml of 0.4M tricarboxylic acid was added and filtered. 1 ml of this filtrate was mixed with 5 ml of 0.4M sodium carbonate and 0.5 ml of folin's reagent was added. After 10 minutes absorbance was measured at 660 nm.[11] For the quantification of tannase enzyme, 0.5 ml of culture filtrate was added to 2 ml of 0.35% tannic acid solution in 0.05M citrate buffer. Then 20µl of reaction mixture was added to 2 ml of 95% ethanol. Absorbance was measured at 310nm before and after incubation in the water bath.[12]

2.7 Catalase and Oxidase test

Catalase producing ability of strain can be determined by adding 3% Hydrogen peroxide to these cultures. If bubbles of free oxygen gas forms after the addition, then the culture is catalase positive. When there is no gas bubble formation, then he culture is catalase negative.

The oxidase discs that are available commercially check for the presence of indophenol oxidase enzyme. The bacterial culture was placed on this oxidase disc. After 30 seconds, the change in color near the culture was noted. If the disc turns dark purple, then the culture is positive for oxidase enzyme. If there is no color change, then it is taken as oxidase negative.

2.8 Screening of HTB for the production of IAA

The IAA producing ability of the organism was checked using the procedure as in Ahmad *et al*[13]. Test tubes containing 5 ml of nutrient broth medium with 0.3% tryptone was prepared and autoclaved. To this a loopful of bacterial culture was inoculated. The tubes were incubated for 7 days under static condition at room temperature. After the incubation period change in color of the medium was observed when compared with the control (without the organism). Those organisms that showed a color change were noted. To the 2 ml of the culture filtrate, 2 ml of orthophosphoric acid was added and incubated for 10 minutes. Then 4 ml of salkowase reagent was added and mixed well. Absorbance was measured at 530 nm.

2.9 Screening for decolorization of textile dyes

The isolated HTB strains were screened for their ability to decolorize five textile dyes namely Black-B (BB), Yellow-MR (YMR), Magenta-MP (MMP), Orange M2R (OM2R), Brown - BGR (BBGR). Dye decolorizing ability of the organism was checked using nutrient agar medium along with the respective dye. Nutrient agar plates were prepared and the organisms were streaked and the plates were kept for incubation at room temperature for 48 hours. After the incubation period, the zone of clearance around the colonies was noted.

2.10 Quantification of dye degrading ability of the strain

Test tubes containing 5 ml of Half- strength Halophilic broth (HB) medium with 100 mg/L Magenta-MP was prepared and autoclaved. To this a loopful of bacterial culture was inoculated. The tubes were incubated for 9 days under static condition at room temperature.[14]

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

2.11 Identification of the Potential Halophilic Bacterial Strain

Identification and characterization of HTB were carried out according to Javor [15] and Ventosa *et al*.[16] The potential bacterial strain which has the capability to decolorize all the tested five textile dyes and has the maximum % decolorization was chosen. The strains AMETH156 and AMETH182 were identified by simple and Gram staining. Then they were subjected to different biochemical tests such as Indole test, Methyl red test (MR), Voges proskauer test (VP), Citrate utilisation test, Triple sugar ion agar test, Nitrate test and Hydrogen sulfide production test.

2.12 Immobilization of efficient bacterial strains for decolorization of textile dyes

The potential bacterial strains, AMETH156 and AMETH182 which has the capability to decolorize all the textile dyes, were immobilized and its decolorization capacity was checked. The immobilization of cells was performed by using 3% sodium alginate and 0.2 M of calcium chloride. The overnight grown bacterial cells 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.[2] This was followed by few analytical methods and evaluation studies.

2.13 Degradation of textile dye- Magenta MP by immobilized HTB AMETH156 and AMETH182

These immobilized beads from efficient bacteria AMETH156 and AMETH182 were inoculated into two different flasks containing 50 ml of HB medium with Magenta dye (100 mg/L). The flasks were subjected to an incubation period of nine days at room temperature in stationary condition. After the incubation, the cell free supernatant was obtained by obtained by centrifugation at 10,000 rpm for 15 minutes. Decolorization was studied by measuring the absorbance at 480nm (λmax of Magenta MP is 480nm).The dye solution (Magenta MP 100 mg/L) without beads was used as a control.

2.14 Study of bioremediation potential of the efficient bacterial strain

Once the dye has been decolorized, the detoxification ability (end products by dye degradation) of the strain was evaluated by plant growth promotion studies on three different crop plants such as, Green gram (*Vigna radiata)*, Black gram (*Vigna mungo)*, *Oryza sativa* (Paddy) and Wheat (*Triticum aestivum*). Seeds of Green gram, Black gram and Wheat (100 from each) were surface sterilized by washing with sterile distilled water and wiped with a dry tissue paper. These seeds were then soaked in 25 ml of colored Magenta MP (untreated) dye and in two 25 ml of decolorized dye (one by the immobilized AMETH156 and other by AMETH182) for 6 hours. Seeds soaked in water for 6 hours were taken as the control. These seeds were then taken for analysis by plate and cup assay.

2.15 Germination study by Plate assay

Sterile plastic plates were used for the study. A sterile tissue paper was used to cover the plate. 10 seeds from each of the flasks were placed in four petri plates named as untreated, AMETH156, AMETH182 and the control, respectively. The tissue paper soaked from the dye mixtures was placed on the top of the plate. They were subjected to an incubation period of 3days. Water was sprayed to maintain the moisture content on the paper. After 3 days, the germination percentage was calculated using the following formula. Experiment was carried out in triplicates.

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

2.16 Growth study by Cup assay

The growth of these crop plants was also checked in plastic cups. The cups were filled with $3/4^{th}$ of soil (approximately 250g). 5 Seeds from each of the flask were planted in the cups. The seeds were then watered with samples (treated and untreated dye mixture) for the first time. For the control sample water is sprayed. The cups were maintained in a photoperiod of 12hours. The soil was kept moist by spraying water daily. The shoot length, root length and fresh weight of all seedlings were recorded after 7 days.

3 RESULTS AND DISCUSSION

3.1 Isolation of halophilic bacterial strains

Over the last few decades, the fast development of molecular biology techniques has led to significant advances in the field, allowing us to investigate intriguing questions on the nature of extremophiles with unprecedented precision and these findings have made the study of life in extreme environments one of the most exciting areas of research, and can tell us much about the fundaments of life. (Rampelotto, 2013)[1]. A total of 30 bacterial strains were isolated from Kelambakkam salterns and they were named as AMETH153 to AMETH182. In the previous 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 (Jayaprakashvel *et al*, 2014)[4]. The colony morphology of the bacterial strains was recorded (Figure 3.1a, b, c). Some of the isolated colonies were pigmented. However, majority of the colonies were white, small and circular in shape. All the strains were able to grow on HA medium. This indicated that the strains were halotolerant bacteria. The strains were sub-cultured on halophilic agar (HA) medium and preserved.

Fig 3.1a : Colony Size variation

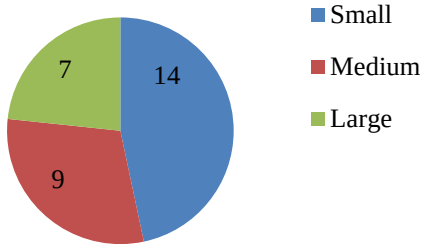


Fig 3.3b: Colony Shape variation

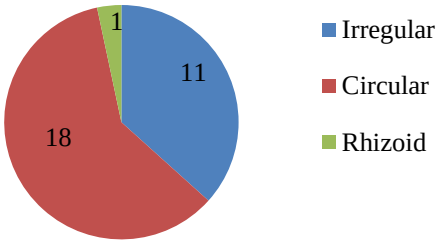
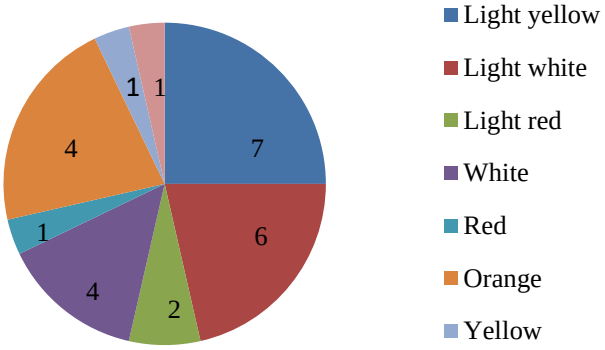


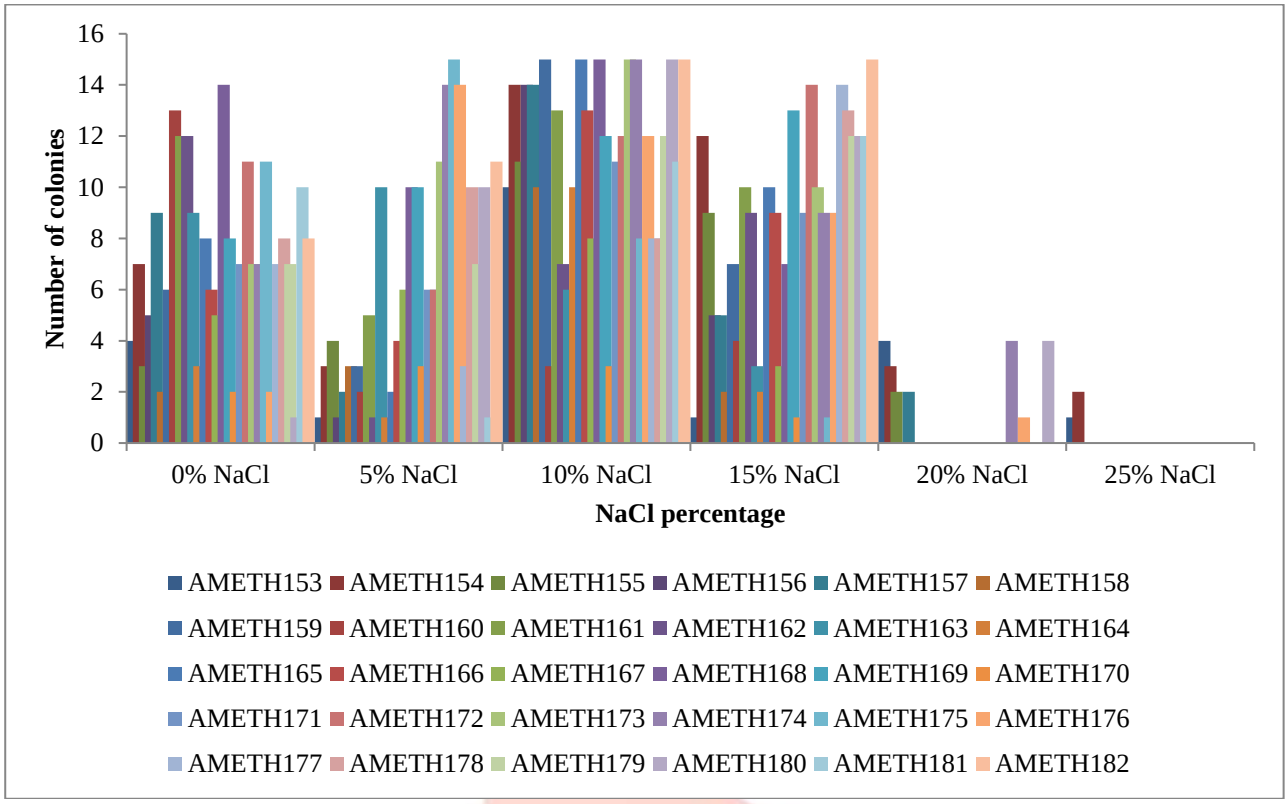
Fig 3.1c: Colony Color variation



3.2 Salt tolerance activity at different salt concentrations:

Halobacteria are generally considered to be extreme halophiles, in most cases requiring at least 12-15% salt for growth[15]. Growth of the microorganisms at different salt concentration was determined. In the work by M. Jayaprakashvel *et al* (2015), all the strains showed growth in 0%, 10% and 20%. However, only 16 strains have grown up to 30% of NaCl. By checking the activity of 30 different halophilic isolates in a halophilic Agar medium (HA), it was found that all the 30 bacterial strains had the ability to grow well in Salt concentrations 0-15%. Thus the results were well clearly demonstrated that the all isolates were belonging to moderate halophilic microorganisms (Figure 3.2)

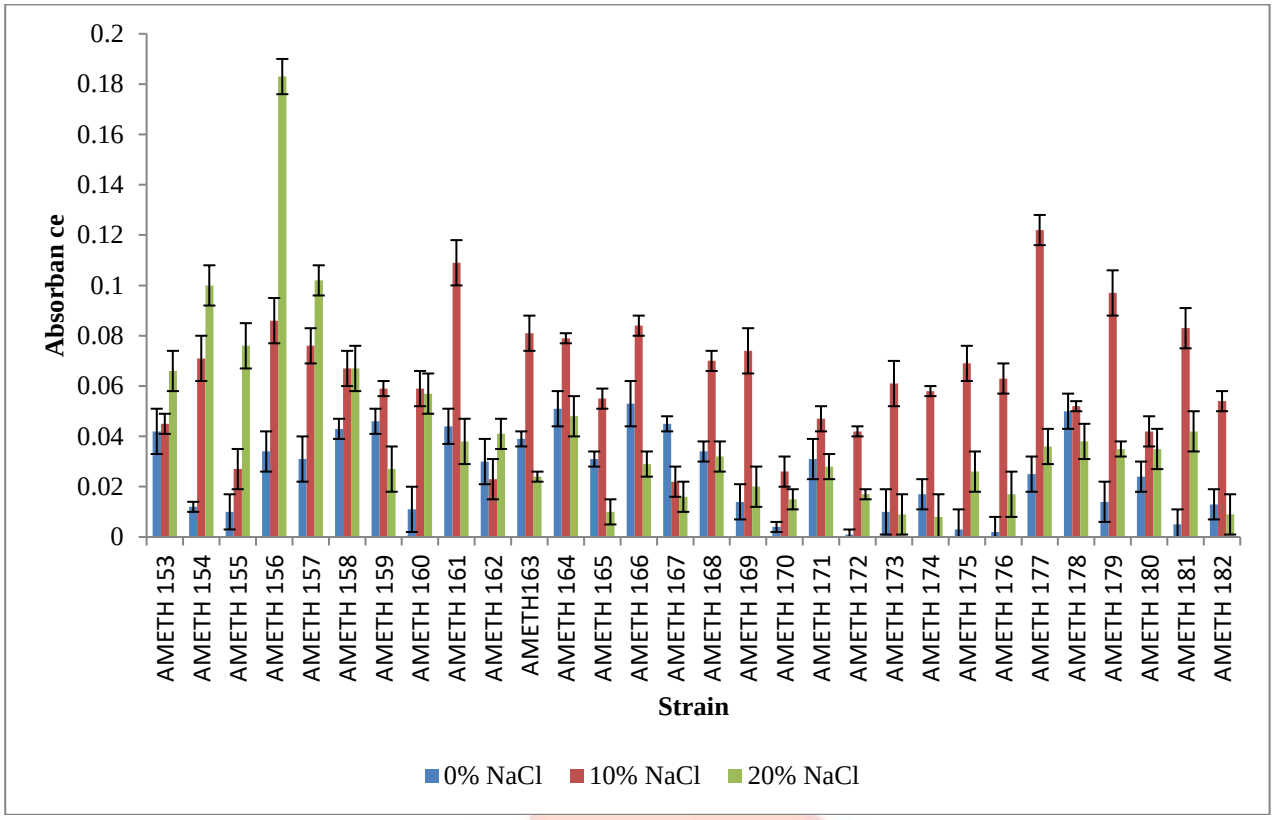
Figure 3.2: Salt tolerance ability of halotolerant strains



3.3 Quantification of Salt Concentration

From the above 6 different Salt concentrations, quantification of growth in salt concentration was proceeded with three concentrations such as 0%, 10% and 20%. In the present work the absorbance was read at 600nm and the results are tabulated below as Optical density (Figure 3.3).

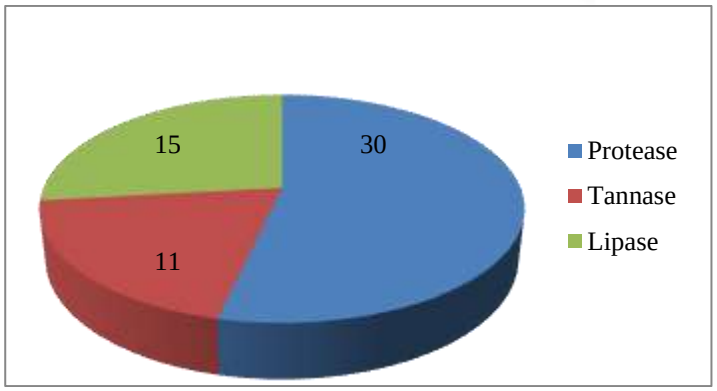
Figure 3.3: Quantification of Salt Concentration



3.4: Screening of bacterial strains by production of protease, lipase and tannase enzyme

Protease activity was visualized by the clear zone around the bacterial patches, flooding the plates with saturated ammonium sulfate solution prepared in 0.1 N HCl and from that it was concluded that all 30 strains showed positive result. Lipase is an enzyme that hydrolyses fats to free fatty acids and glycerol. The oxidative deterioration of fats involves the reaction of unsaturated fatty acids with oxygen to yield hydroperoxides (Mielmann, 2006). Lipase activity was visualized by opalescent zone and 15 strains were found to be positive. Tannase activity was visualized as a brown color halo zone around the bacterial patch and 11 strains were found to be positive. Radius of these zones was measured and tabulated. Fig 3.4 represents the ability of all the 30 strains to produce various degradative enzymes.

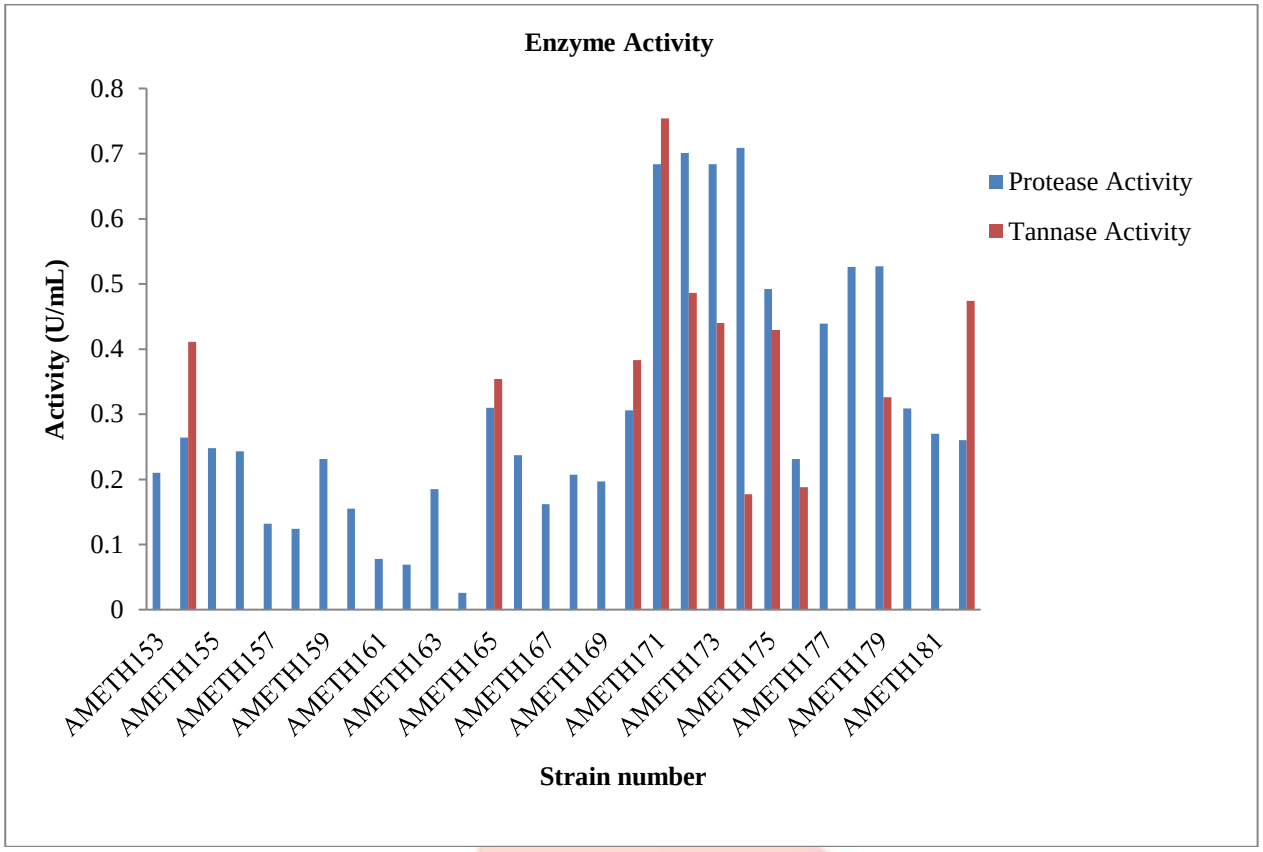
Figure 3.4: Enzyme producing ability of the strain



3.5 Quantification of Bacterial strain producing Protease and Tannase activity

All the 30 strains with positive protease activity were subjected to quantitative assay. Absorbance was measured and the activity was calculated. 11 strains with positive protease activity were subjected to quantification activity was calculated [11]. Quantification of tannase enzyme was carried out by Iibuchi *et al*, 1967[12].

Figure 3.5: Enzyme activity of the strains



3.6 Catalase test

Bubble formation was observed in all the 30 halotolerant bacterial strains which indicated a positive catalase activity.

3.7 Oxidase test

Change in color from yellow to purple within 10-15 seconds, indicated that 28 halotolerant strains showed an immediate positive response for oxidase enzyme and two strains AMETH 166 and AMETH 182 showed a delayed response.

3.8 Screening of bacterial strains for the production of IAA

In present study, tryptone was used as the basic substrate to find out the presence of IAA in the isolated organisms similar to the study carried by Jayaprakashvel *et al*, 2014[5]. In presence of tryptophan, the microbes release greater quantities of IAA and related compounds. In the work of Ahmad *et al* [13], 11 selected isolates were tested for the quantitative estimation of IAA in the presence of different concentrations of tryptophan while in our study out of 30 halotolerant strains, 15 strains showed a color change compared to the control which indicates that these organisms have the ability to produce IAA. Quantification was done using these 15 organisms (Figure 3.6).

Figure 3.6: Ability to produce IAA

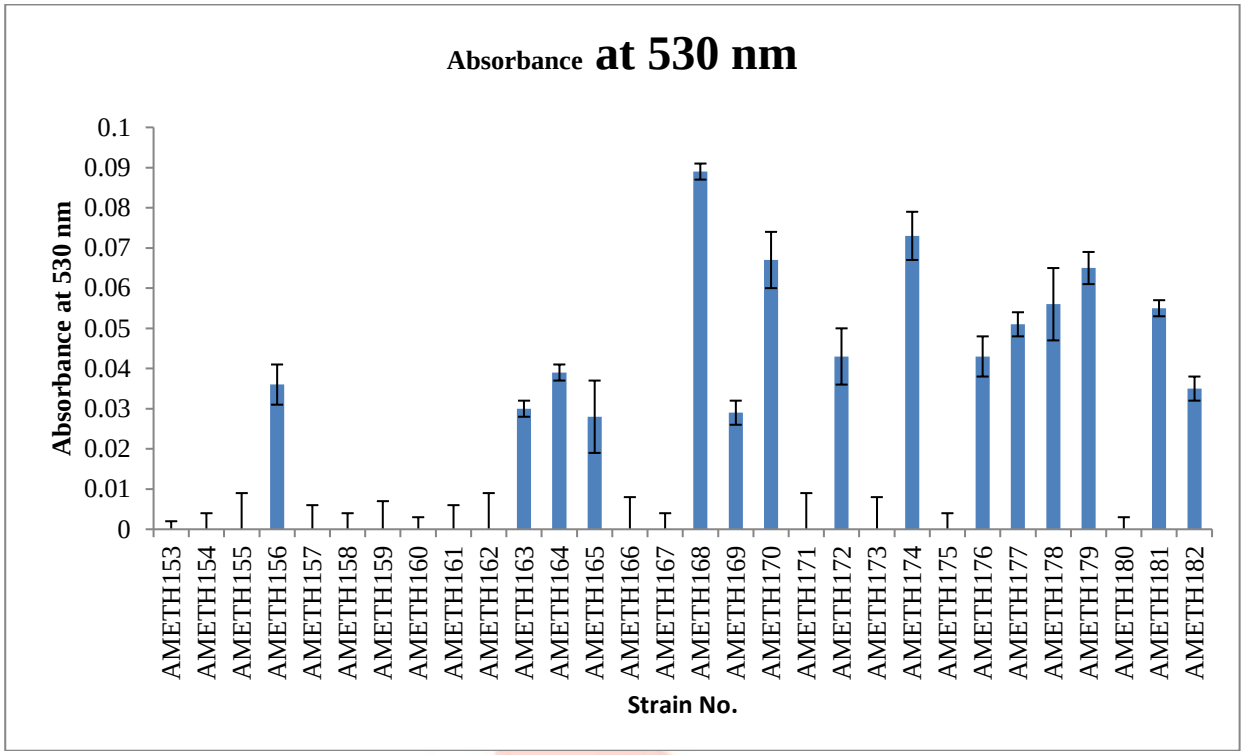
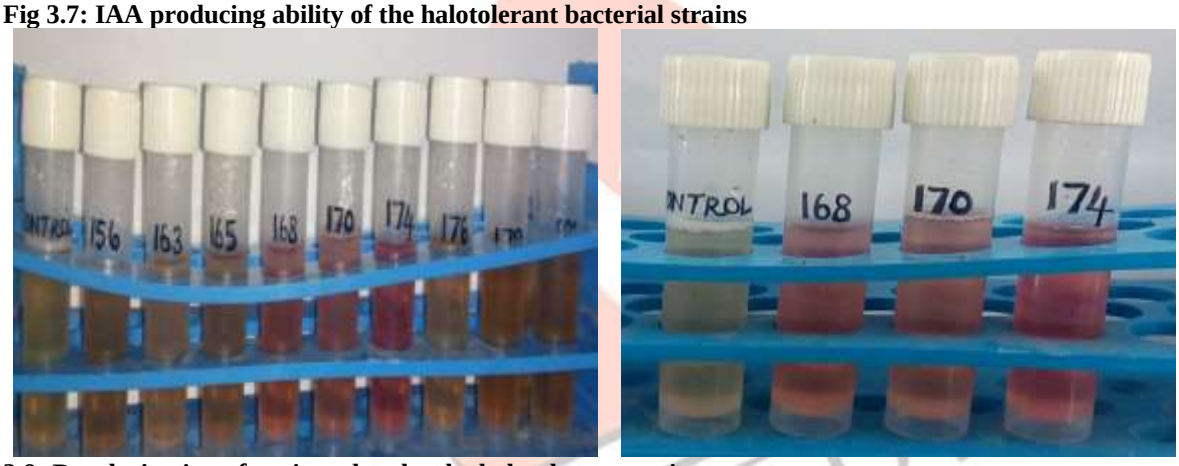


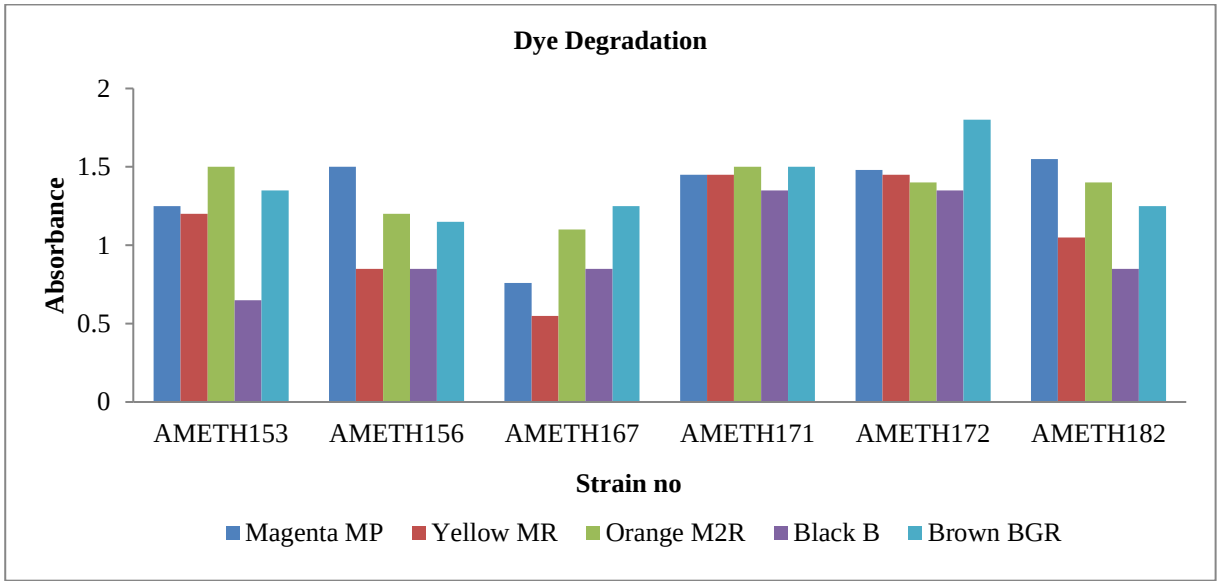
Fig 3.7: IAA producing ability of the halotolerant bacterial strains



3.9: Decolorization of various dyes by the halotolerant strains.

Bacteria isolated from high polluted environments have well proven to have very good bioremediation potentials because of their diverse adaptable characteristics (Jayaprakashvel *et al*, 2014). The isolated organisms were inoculated in the medium with dyes. After the incubation period, a zone was formed in all the plates. From the qualitative assay, zone of clearance (radius in cm) was found to be maximum for 6 strains AMETH 153, 156, 167, 171, 172 and 182. Radius in cm of the zone formed on the plate was measured and the values were tabulated.

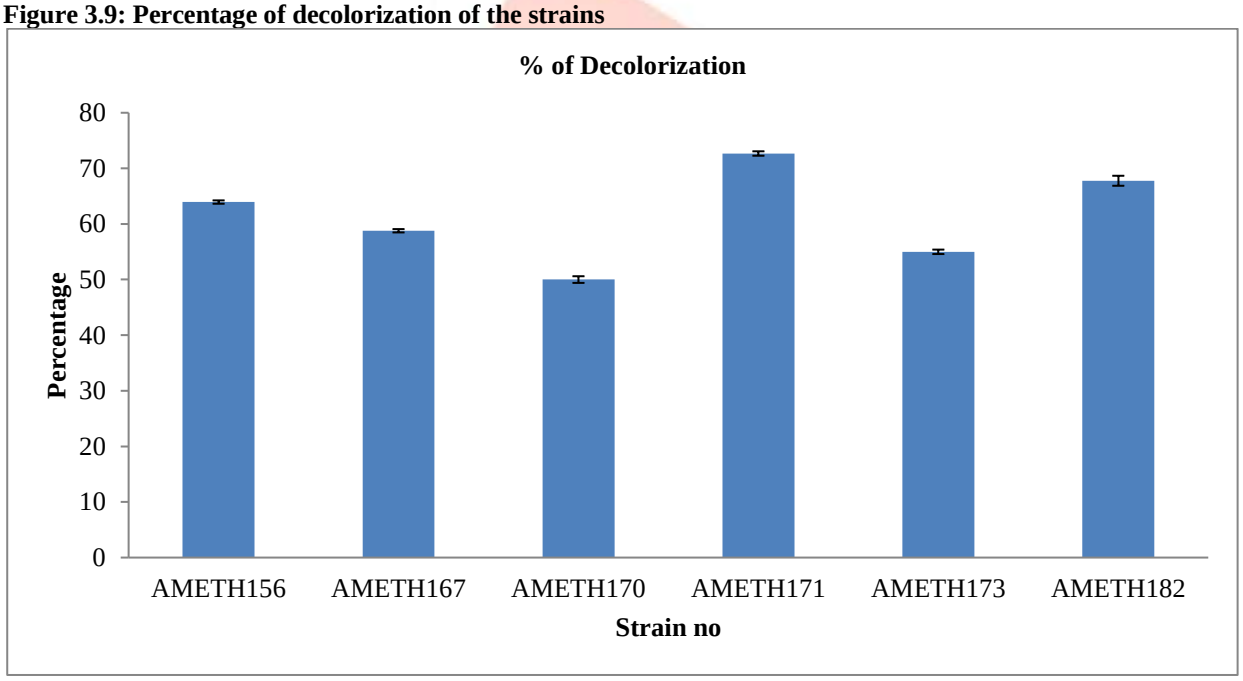
Figure 3.8: Dye degrading ability of the strain



3.10 Quantification of dye decolorization of bacterial strains

The decolorization rate of the azo dyes also depends on the oxidation potential of the azo dyes (Senan *et* al, 2004). Quantification of dye degradation was done using the dye Magenta MP in the broth for all the strains and then % of decolorization was calculated. The decolorization capacity was found in six of the isolated strains namely AMETH 156, 167, 170, 171, 173 and 182.

Figure 3.9: Percentage of decolorization of the strains



3.11 Selection of the efficient strain for dye degradation and plant growth promotion

The previous study undertook with the ultimate aim of identifying potential halotolerant bacterial strains which has the ability to degrade textile dyes and enhance the plant growth by the production of hydrolases and oxidoreductases along with plant growth promoting hormone IAA. The phytohormone IAA production offers great promise for sustaining the increased crop productivity (Vinothini *et al*, 2014). On comparing the various properties such as enzyme producing ability, plant growth promotion property and dye degrading capacity of the isolated halotolerant strains, two strains namely AMETH156 and AMETH182 were selected.

3.12 Biochemical characterization

Various biochemical tests were carried out for selected strain AMETH 156 and AMETH 182 and the following results (Table 3.1) were obtained.

Table 3.1: Biochemical characterization of the strains AMETH 156 and AMETH 182

S.No	Tests	Results

		AMETH 156	AMETH 182
1	Simple Staining	Rod shaped cells	Rod Shaped cells
2	Gram Staining	Gram Negative	Gram Negative
3	Indole	Negative	Negative
4	Methyl red	Positive	Positive
5	Voges proskaver	Negative	Negative
6	Citrate utilization	Positive	Positive
7	Triple sugar ion	Negative	Negative
8	Nitrate	Negative	Negative
9	Hydrogen sulfide	Negative	Negative

3.13 Immobilization of the potential bacterial strain for decolorization of textile dyes

The potential halophilic bacterial strain AMETH 156 & AMETH 182 was immobilized and compared for their ability to degrade the textile dye (Table 3.2) while in the work of Prabhakar in 2013, the two strains AMETH72 and AMETH77 were immobilized and compared for their ability to decolorize the textile dyes. After immobilization, there was a good improvement in the decolrization property by the strain, as compared to their free cell counterparts. Therefore, it was concluded that AMETH 156 was the most efficient of all the bacterial strains in decolorizing all the textile dyes.

Percentage of decolorization was calculated using control (broth with dye alone). Halotolerant bacterial strain AMETH 156 had the maximum value of 74.28% degradation.

Table 3.2: Percentage of decoloriation of the immobilized selected strains.

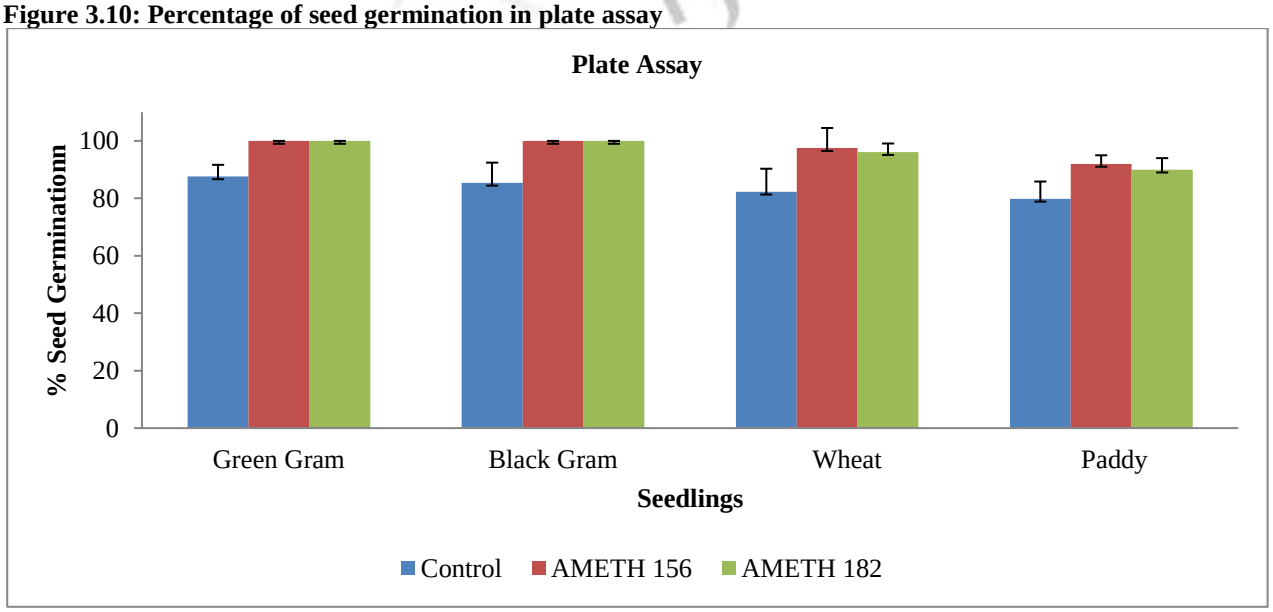
Strain.No	% of Decolorization
AMETH 156	74.28
AMETH 182	69.34

3.14 Evaluation of bioremediation potential of the efficient bacterial strain

3.14.1 Seed germination study (Plate assay)

The seeds used in this study are paddy, wheat, green gram and black gram. The effect of untreated dye and treated dye on the seed germination of these seeds was noted. In this study, all the seeds were well grown and good germination was observed in treated dyes as compared to untreated dyes. The percentage of seed germination in both the treatments was calculated and recorded. Halotolerant bacterial strain AMETH 156 showed the maximum percentage of seed germination.

Figure 3.10: Percentage of seed germination in plate assay



The graph shows the effect of treated dye on plant growth promotion. X-axis denotes the various seedlings (green gram, black gram, wheat and paddy) and Y-axis denotes the percentage of seed germination.

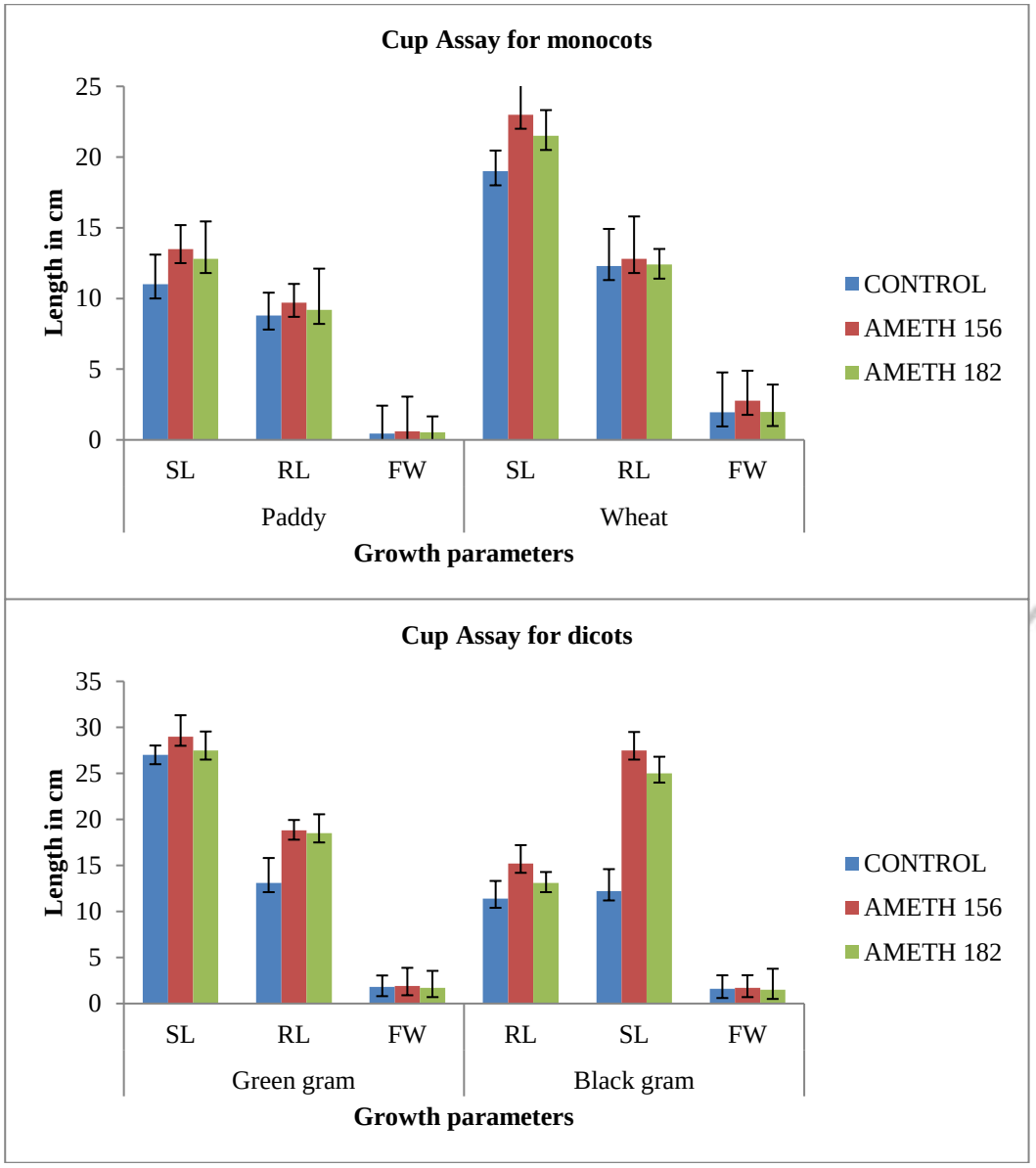
3.15 Estimation of Shoot length, Root length and Fresh weight of the crop plant seedlings

In this study, all the collected four different crop plant seeds, 5 seeds from each were added to the pots. The seeds were then watered with samples (treated dye mixture) for the first time. The soil was kept moist by spraying water daily. The shoot length; root length and fresh weight of all the seedlings were recorded after 10 days. Moreover, compared to the control groups, all the five treated dyes were shown some maximum and minimum activity in plant growth promotion of all the seedlings.

3.15.1 Effect of different treated dye on the growth of four different see.

After a period of 7 days, growth of the seedlings was determined and the various growth parameters such as root length, shoot length and fresh weight of the seedlings were calculated and tabulated (Figure 3.11).

Figure 3.11: Growth parameters in Cup assay



3.15 Identification of the potential halophilic bacterial strain AMETH156

The identification and characterization of halophilic bacteria were carried out according to Javor (1989)[15] and Ventosa *et al* (1998)[16]. AMETH156 bacterial strain is rod shaped, gram negative and motile. It is a non sporulating and oxidase and catalase positive. Based on the colony morphology, Gram's staining, biochemical characteristics and compared with the standard description of the Bergey`s Manual of Determinative Bacteriology[17], the strain AMETH156 was confirmed that, it is belonging to *Halomonas* sp.

4 CONCLUSION

Microorganisms growing in extreme environmental conditions, such as high salt, temperature and heavy metal concentration, can be used widely for many industrial and environmental applications since they have excellent tolerance ability. Thus the study concluded that the halotolerant marine bacterial strain AMETH156 isolated from the Kelambakkam salterns of Tamil

Nadu proved to be an efficient strain since it was able to decolorize and detoxify the various commercially used textile dyes especially azodyes. By the biochemical characterization, the strain was identified to be belonging to *Halomonas* sp. Moreover, a molecular level identification is needed to identify the potential halophilic bacterial strain at genus level. Hence this strain can be very well used for the bioremediation of textile dyes at the same time promoting plant growth.

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6 REFERENCES

[1] Rampelotto Pabulo Henrique, "Extremophiles and extreme environments," Life (Basel), 3(3):482-5, Aug 7, 2013.
[2] M. Jayaprakashvel, N. Imran Sheriff, M. Venkatramani, S. Vinothini and A. Jaffar Hussain, "Plant growth promotion potential of heavy metal resistant bacteria from the sewage contaminated coovum river, Chennai, India," biosciences biotechnology research asia, Vol. 11(Spl. Edn. 1), p. 149-158, 2014.
[3] Jayaprakashvel, M., Rajan, A. and Hussain, AJ, "Bioprospecting of halotolerant bacteria from the rhizosphere of a halophyte for the production of industrial enzymes," Int. J. Pure App. Biosci 3 (4), 193-201, 2015.
[4] Jayaprakashvel, M., R. Divyalakshmi, M. Venkatramani, S. Vinothini, R. Muthezhilan and A. Jaffar Hussain, "Bioremedation of industrial effluent using immobilized cells of halotolerant marine bacterium," Biosciences Biotechnology Research Asia, Vol. 11(Spl. Edn. 1), p. 69-79, 2014.
[5] Jayaprakashvel, M., K. Abishamala, A. Jaffar Hussain, and M.C. Vanitha, "Isolation and characterization of Indole Acetic Acid (IAA) produced by a halo tolerant marine bacterium isolated from coastal sand dune plants," Biosciences Biotechnology Research Asia, Vol. 11(Spl. Edn. 1), p. 263-269, 2014.
[6] Vinothini, S., A. Jaffar Hussain and M. Jayaprakashvel, "Bioprospecting of halotolerant marine bacteria from the Kelambakkam and Marakkanam salterns, India for wastewater treatment of plant growth promotion," Biosciences Biotechnology Research Asia, Vol. 11(Spl. Edn. 1), p. 313-321, 2014.
[7] Senan, R.C and Abraham E.T, "Bioremediation of textile azo dyes by aerobic bacterial consortium," Biodegra. 15: 275-280, 2004.
[8] Pandu Krishna Compala Prabhakar, "Bioremediation of textile dyes and improvement of plant growth by marine bacteria," Thesis submitted to the University of Boras, 2013.
[9] Mielmann A, "Food spoilage characteristics of Chryseobacterium species," University of the Free State, 2006.
[10] Kiiskinen LL, Ratto M and Kruus K., "Screening for novel laccase-producing microbes," Journal of Applied Microbiology, 97:640-646, 2004.
[11] Cupp-Enyard C, "Sigma's non-specific protease activity assay - casein as a substrate," Journal of Visualized Experiments : jove. (19):899, 2008.
[12] Iibuchi S, Minoda Y, and Yamada K, "Studies on tannin acyl hydrolase of microorganisms," Agric. Biol. Chem, 32, 513 – 518, 1967.
[13] F. Ahmad, I. Ahmad, M.S. Khan, "Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities," Microbiol. Res., 163, pp. 173-181, 2008.
[14] Silveira E, Marques PP, Silva SS, Lima-Filho JL, Porto ALF and Tambourgi EB, "Selection of *Pseudomonas* for industrial textile dyes decolourization," Int. Biodeterior Biodegradation, 63:230–235, 2009.
[15] Javor, Barbara, "Hypersaline environments: microbiology and biogeochemistry," 1989.
[16] Ventosa, A., Nieto, J. J. and Oren, A, "Biology of moderately halophilic aerobic bacteria," Microbiol Mol Biol Rev 62, 504–544, 1998.
[17] D H Bergey, Robert S Breed, "Bergey's manual of determinative bacteriology," American Society for Microbiology, 1957.