ISOLATION, CHARACTERIZATION, AND IDENTIFICATION OF BACTERIA FROM INDUSTRIAL AND MARKET WASTE AREAS

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Abstract: A study was conducted to isolate and examine the bacterial strains from litter areas. Two soil samples were collected from waste disposal sites, namely, industrial waste sites of Nilon's pickle industry in Dalgaon and market waste sites of Balugaon vegetable market of Kharupetia in Darrang district (Assam). A total of nine bacterial strains were identified at industrial waste dumps, namely *Leclercia adecarboxylata*, two strains of *Pseudomonas putidia, Ralstonia pickettii*, three strains of *Serratia marcescens* and *Stenotrophomonas maltophilia*, and at market waste dumps, four out of five bacterial isolates were identified, namely two strains of *Enterococcus cloacae, Bacillus megaterium* and *Bacillus coagulans* using the Vitek-MS MALDI TOF system. For both soil samples, morphological, biochemical, physicochemical tests were carried out against all bacterial strains to identify a beneficial bacterial strain.

Keywords: Bacterial strains; Garbage area; toxic; Vitek-MS MALDI TOF

1. Introduction:

Huge amounts of waste are generated daily in market areas of Darrang, Assam. The microbial population of soils mainly consists of five major groups including bacteria, actinomycetes, fungi, algae, and protozoa. Among the microbial population, bacteria are the most abundant and important microbes for waste decomposition. In soil, generally, 80 to 99% of microorganisms remain unknown, and it is fundamentally known that these biological communities play a dominant role in maintaining a sustainable biosphere. Of industrial and academic interest, soil bacteria are present in soil due to their unique biologically active metabolites and are able to form novel commercially important products. Continuous efforts have been made to isolate novel bacteria from various environments such as landfills. As the bacteria use waste for their own metabolism, they end up producing some simple and useful compounds that are important for soil health, plant growth, and, most importantly, maintaining the balance of the natural ecosystem [1]. Microorganisms of the genus *Bacillus* and *Enterobacter* preferably by urease activity and calcium carbonate precipitation mechanism may be responsible for waste degradation [2]. Hence, in this study, an attempt has been made to identify beneficial soil bacteria.

2. Materials and methods:

2.1. Collection of soil sample

Two soil samples were collected from two different waste areas. One soil sample was collected from the Balugaon vegetable market garbage area, Kharupetia, and another from Nilon's pickle industrial area, Dalgaon waste dumping site in Darrang, Assam. At each collection point, soil samples were collected at a depth of 2 to 3 cm. The soil samples were first ground with a sterile mortar and pestle to free the attached microorganisms before preparing their suspension [3]. 1 g of each soil sample was weighed and dissolved in 9 mL of sterile distilled water and serial dilution (10-1 to 10-4) was performed.

2.2. Morphological characterization

Isolated colonies of purified bacterial strains from both soil samples were grown on solidified agar plates, observed for colony characteristics and to determine the gram character, the gram staining methodology was followed. The hanging drop method was used to study motility in bacteria. In this method, a drop of the test organism in a salt

suspension was placed on a cover glass, the cover glass was turned over and then placed on a cavity slide and then viewed under the microscope. A sharp, darting movement in different directions across the microscope's field of view indicated a positive motility result.

2.3. Biochemical Characterization

To understand the oxidase activity [4], a filter paper was taken and soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride, after which the paper was moistened with sterile distilled water. The colony to be tested was picked with a platinum loop and streaked onto the filter paper. The inoculated area in the paper was observed for a color change to deep blue or purple within 10 to 30 seconds. Catalase activity [4] was tested by applying H_2O_2 solution to the slides that contained the culture of the isolates separately. The release of O_2 bubbles indicated positive catalase activity. After inoculation of the isolates into nutrient agar with 1% starch at pH 6.06, the culture plates were treated with iodine after the incubation period. A clear zone was observed around the colonies, confirming the amylolytic activity [4]. To test urease activity [5] isolates are grown on the medium containing urea agar. After incubation, the slant is observed to change in color from reddish pink indicating positive urease activity. The slide test method [5] was performed to study the coagulase activity. A loop full of bacteria isolate was smeared with a drop of 0.9% saline water; then, a drop of the plasma was added to the slide. The slide was swirled and observed closely for any visible clumping within 10 sec.

2.4. Effect of pH and temperature on bacterial growth

Temperature and pH are of great importance for bacterial growth. The growth of bacterial strains at different pH and temperature was observed using the nutrient broth media. Optimization and standardization of the growth of isolates on the range of pH [6] and temperature are studied by following the methodology of Pietikäinen et.al. [7]. Isolates are inoculated on nutrient broth media having pH ranges viz., pH 3, pH 4, pH 5, pH 6, pH 8, pH 9, and pH 10. These are then incubated at 37 ℃ for 48 hours. The growth is then determined by taking optical density at 520 nm. Further, to understand the effect of temperature on the growth of isolates the isolates were inoculated in nutrient broth and incubated at different temperatures at 37 ℃, 28 ℃, and 4 ℃, for 48 hours and optical density was measured at 520 nm.

2.5. Identification of bacterial strains through VITEK-MS

A total of 14 isolates were recovered from two different soil samples from Darrang, Assam, and VITEK. MS identification was performed at Nazareth Hospital, Shillong (Meghalaya).

2.6. Plastic biodegradation

For biodegradation of plastic (polythene bags) a minimal media was prepared and streaked on minimal salt agar. Polythene bags of 1x1 cm were cut and placed on the minimal salt agar plates. Sterile polythene pieces are weighed and recorded before inoculating into the culture medium. The control was maintained with plastic in a microbe-free medium. After 1 month of incubation, the growth of microorganisms was observed on the polythene bags strips [8].

3. Results and Discussion:

3.1 Morphological characterization

This study revealed different colored mixed colonies from soil sample 1 (Table 1) and soil sample 2 (Table 1). Macroscopic (Table 1) and microscopic analysis (Table 2) of bacterial strains of soil sample 1 revealed rod cell shape and Gram –ve (Table 2) in nature while S1B6 was found to be Gram +ve (Table 2) in nature. In the motility test, all bacterial strains were found to be motile in nature except S1B1 which was non-motile. All 5 bacterial strains isolated from soil sample 2 (Table 1) were found to be rod-shaped. In the Grams reaction, S2B1 and S2B2 were found as gram –ve whereas S2B3, S2B4, and S2B5 were found as gram +ve in nature (Table 2). S2B1 and S2B2 were found non-motile, whereas the rest of the bacterial strains such as S2B3, S2B4, and S2B5 were found to be motile in nature. The results are in conformity with the reports of several works [9]; where they found same characteristics in *Staphylococcus aureus* (+ve, cocci, in clustered form), *Escherichia coli* (-ve, bacilli), *Pseudomonas aeruginosa* (-ve, bacilli), *Pseudomonas aeruginosa* (-ve, bacilli), *Serratia marcescens* (-ve, rod), *Salmonella enterica* (-ve, rod), *B. cereus* (+ve, bacilli), *B. subtilis* (+ve, bacilli), and *B. megaterium* (+ve, bacilli)

or motility test were like *Staphylococcus aureus* (non- motile), *Escherichia coli* (motile), *Pseudomonas aeruginosa* (motile), *Serratia marcescens* (non- motile), *Salmonella enterica* (motile), *B. Cereus* (non- motile), *B. subtilis* (motile), and *B.megaterium* (non- motile). It has been reported that Gram-positive (+ve) and Gram-negative (-ve) bacteria both degrade plastics which could have important applications in solving plastic waste pollution [10]. Previous studies [11] have shown that polyethylene (PE) degrading isolates belonged to gram-positive Pseudomonas which degrade PE efficiently and utilize it as a carbon source for their survival. The results of this work may agree with earlier reports [12], that Gram-positive (+ve) *Bacillus* species are rod-shaped, aerobic, and non-spore-forming which degraded hydrocarbons of petroleum and polystyrene film.

Table 1. Macroscopic characteristics of isolated bacterial strains of soil sample 1 and sample 2

Soil sample 1						
Isolates	Color	Surface	Opacity	Pigmentation	Elevation	Form
S1B1	Creamy white	Smooth	Opaque	White	Flat	Circular
S1B2	Creamy white	Smooth	Opaque	White	Flat	Circular
S1B3	Creamy white	Smooth	Opaque	White	Flat	Circular
S1B4	Cream	Smooth	Opaque	Creamy white	Flat	Circular
S1B5	Red	Smooth	Opaque	Red	Raised	Circular
S1B6	Cream	Smooth	Opaque	Creamy white	Flat	Circular
S1B7	Red	Smooth	Opaque	Red	Raised	Circular
S1B8	Red	Smooth	Opaque	Red	Raised	Circular
S1B9	Cream	Smooth	Opaque	Creamy white	Flat	Circular
Soil Sample 2						
Isolates	Color	Surface	Opacity	Pigmentation	Elevation	Form
S2B1	Creamy white	Smooth	Opaque	Creamy white	Raised	Circular
S2B2	Creamy white	Smooth	Opaque	Creamy white	Raised	Circular
S2B3	Creamy white	Smooth	Opaque	Creamy white	Flat	Circular
S2B4	Creamy white	Smooth	Opaque	Creamy white	Flat	Circular
S2B5	Creamy white	Smooth	Opaque	Creamy white	Flat	Circular

Table 2. Microscopic characteristics of isolated bacterial strain of soil sample 1

Soil sample 1									
Isolates	S1B1	S1B2	S1B3	S1B4	S1B5	S1B6	S1B7	S1B8	S1B9
Gram's reaction	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Soil Sample 2					
Isolates	S2B1	S2B2	S2B3	S2B4	S2B5
Grams reaction	-ve	-ve	+ve	+ve	-ve
Cell shape	Rod	Rod	Rod	Rod	Rod
Motility	-ve	-ve	+ve	+ve	+ve

3.2. Biochemical Characterization

Biochemical characteristics of isolated bacterial strains of soil sample 1 (Table 3) showed that all isolated bacterial strains were negative (-ve), except S1B6 and S1B9 which showed positive (+ve) result. S1B2, S1B3, S1B4 and S1B6 bacterial strains showed oxidase +ve result, whereas S1B1, S1B5, S1B7, S1B8, S1B9 showed oxidase –ve result. All nine bacterial strains were found as catalase positive. Urease positive results were observed in all the bacterial strains except S1B9. S1B1, S1B2, S1B3, S1B6, and S1B9 showed coagulase positive result, whereas S1B4, S1B5, S1B7, and S1B8 showed coagulase negative results. Biochemical characteristics of isolated bacterial strain of soil sample 2 (Table 3) for amylolytic activity test showed negative results in S2B1, S2B2, and S2B5 whereas, both S2B3 and S2B4 showed positive nature. All the samples showed oxidase, catalase, and urease positive results. S2B1, S2B2, and S2B3 showed coagulase-positive and S2B4 and S2B5 showed coagulase negative results. Biochemical results of both the soil samples are in corroboration with earlier reports [13], where they found urease, catalase, and oxidase positive results with several bacterial species. Biochemical tests revealed that bacterial isolates that degrade plastics are mostly urease, catalase, and oxidase positive in nature which is also in conformity with this study [13].

Table 3. Biochemical Characterizations of Isolated bacterial Strain of soil sample 1

Soil sample 1					
Isolates	Amylolytic activity test	Oxidase test	Catalase test	Urease test	Coagulase test
S1B1	-ve	-ve	+ve	+ve	+ve
S1B2	-ve	+ve	+ve	+ve	+ve
S1B3	-ve	+ve	+ve	+ve	+ve
S1B4	-ve	+ve	+ve	+ve	-ve
S1B5	-ve	-ve	+ve	+ve	-ve
S1B6	+ve	+ve	+ve	+ve	+ve
S1B7	-ve	-ve	+ve	+ve	-ve
S1B8	-ve	-ve	+ve	+ve	-ve
S1B9	+ve	-ve	+ve	-ve	+ve
Soil Sample 2					
Isolates	Amylolytic activity test	Oxidase test	Catalase test	Urease test	Coagulase test
S2B1	-ve	+ve	+ve	+ve	+ve
S2B2	-ve	+ve	+ve	+ve	+ve
S2B3	+ve	+ve	+ve	+ve	+ve
S2B4	+ve	+ve	+ve	+ve	-ve
S2B5	-ve	+ve	+ve	+ve	-ve

3.3. Effect of pH and temperature on bacterial growth

It was observed that the optimum growth of bacterial strains of soil sample 1 (Figure 1) in different pH of bacterial strains viz., S1B1 *Leclercia adecarboxylata* (pH 3 and pH 4), S1B2 and S1B3 both *Pseudomonas putidia* (pH 4 and pH 5), S1B4; *Ralstonia pickettii* (pH 5, pH 6 or pH 8), S1B5; *Serratia marcescens* (pH5, pH 6 and pH 10), S1B7 and S1B8 for both *Serratia marcescens* (pH 3, pH 4 or pH 6) and in case of S1B9 the optimum growth was observed at pH 3. It was seen that the bacterial strains of soil sample 1 (Figure 1) can grow in both acidic and basic environment. Out of 9 bacterial samples, S1B9 can grow in highly acidic conditions and S1B5 can grow in a pH range of 10. The optimum growth of bacterial strains of soil sample 2 (Figure 2), viz., S2B1 and S2B2 both *Enterococcus cloacae* (pH 4 and pH 9), S2B3; *Bacillus megaterium* (pH 5 or pH 6), S2B4; *Bacillus coagulans* (pH 6 or pH 8) and lastly S2B5; unidentified bacterial strain can grow at optimum pH of 6. This study shows similar results with earlier reports [14], where they found the optimum growth of *Pseudomonas putidia* sp. at pH 6. In this study, the optimum growth of bacterial strains of soil sample 1 (Figure 3) revealed optimum growth of S1B1 (*Leclercia adecarboxylata*), S1B2 (*Pseudomonas putidia*), S1B3 (*Pseudomonas putidia*), S1B4 (*Ralstonia pickettii*), S1B5 (*Serratia marcescens*), S1B6 (*Bacillus cereus* group), S1B7 (*Serratia marcescens)* and S1B9 (*Stenotrophomonas maltophilia*) at 37˚C, except S1B8 (*Serratia marcescens*) showed better growth at 28˚C. The optimum growth of bacterial strains of soil sample 2 (Figure 4) of S2B1 (*Enterococcus cloacae*), S2B2 (*Enterococcus cloacae*), and S2B4 (*Bacillus coagulans*) was found to be at 37˚C and in S2B3 (*Bacillus megaterium*) and S2B5 (unidentified) the optimum growth was at 28 ˚C. It has been reported that *Pseudomonas spp*., has the potency of degradation of plastic at 30°C and 37°C temperature and had the potency to degrade polythene [14]. *Enterobacteria* isolated from cow dung showed biodegradation of polypropylene through extracellular enzymes which may be one of the causes of *Enterobacter* degrading plastics in this present study [15]. Earlier reports also showed that the optimal temperature of *Enterobacter* was 40°C and pH 8.0 and caused degradation of polyethylene terephthalate [16].

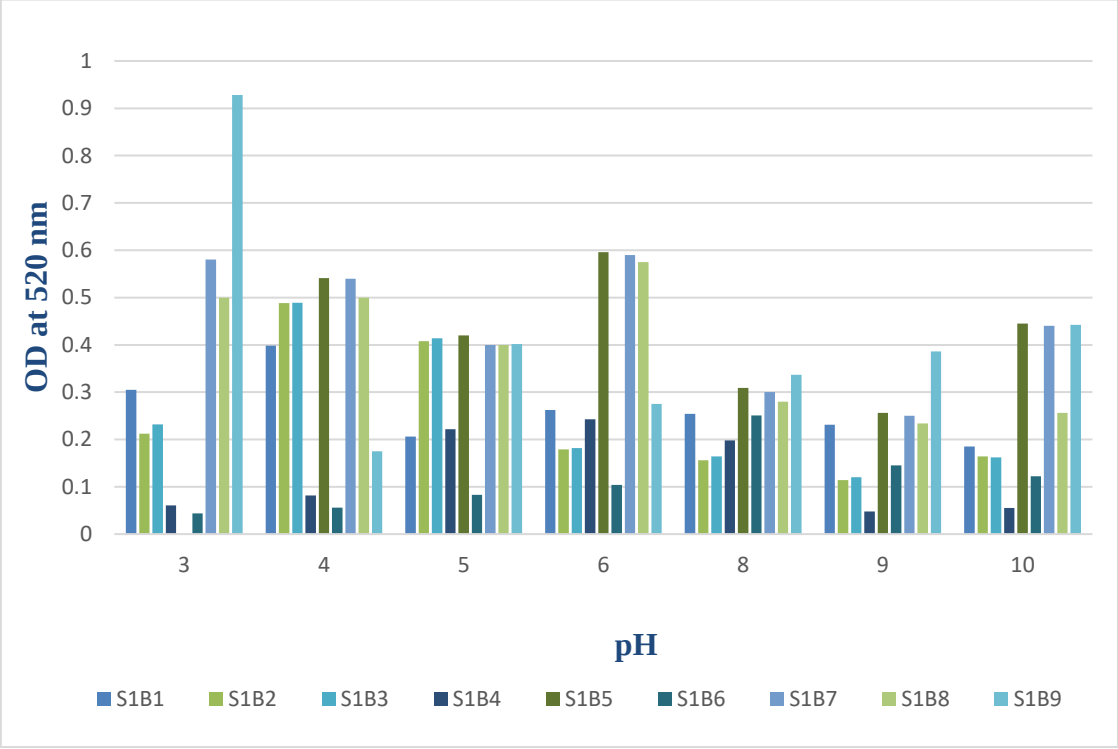

Figure 1: Graphical representation of effect of pH on growth of bacterial strains of soil sample1.

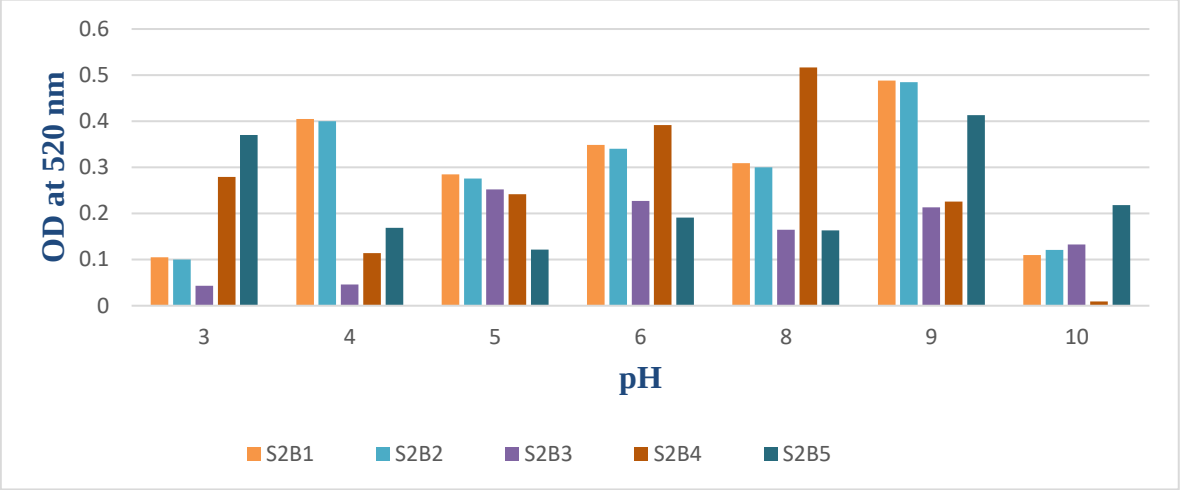

Figure 2: Graphical representation of effect of pH on growth of bacterial isolates of soil sample 2.

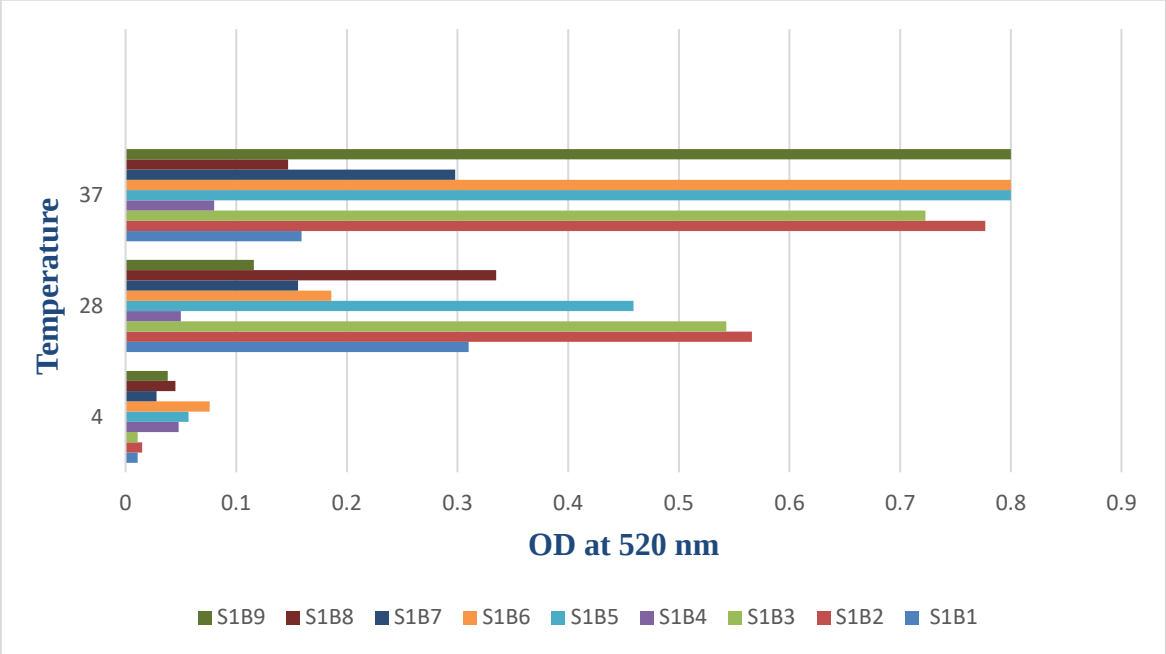

Figure 3: Graphical representation of effect of temperature on growth of bacterial isolates of soil sample 1.

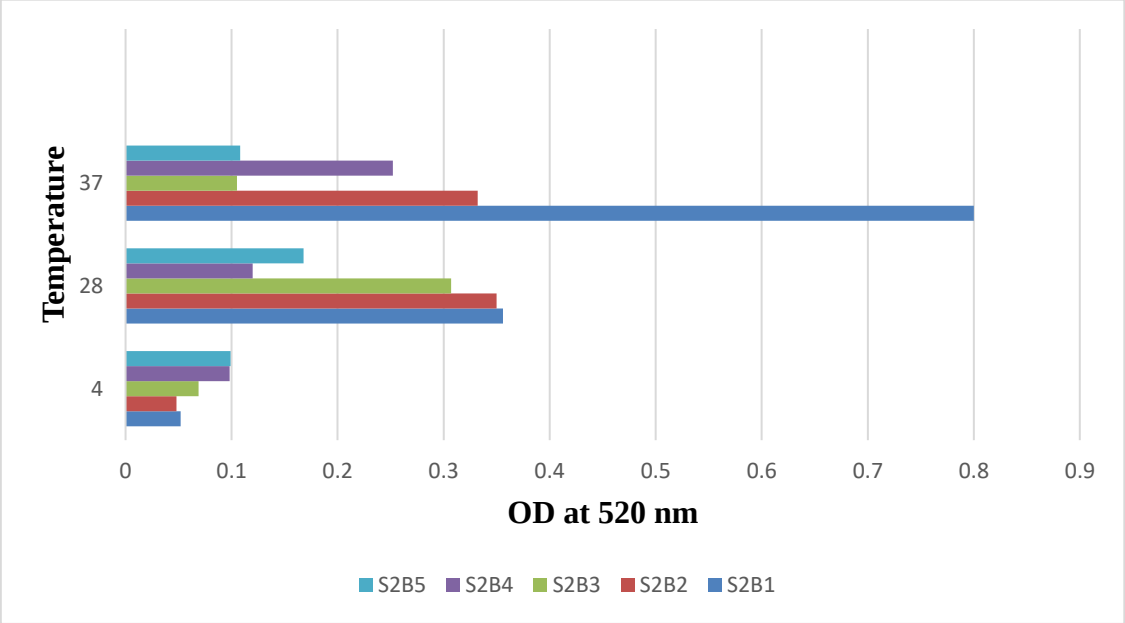

Figure 4: Graphical representation of effect of temperature on growth of bacterial isolates of soil sample 2.

3.4. Identification of bacterial strains through VITEK-MS

From soil sample 1 (Table 4), total 9 bacterial isolates were identified through Vitek-MS MALDI TOF system viz., SIB1 (*Leclercia adecarboxylata*), two strains S1B2 and S1B3 (*Pseudomonas putidia*), S1B4 (*Ralstonia pickettii*), three strains such as S1B5, S1B7 and S1B8 as (*Serratia marcescens*), S1B6 (*Bacillus cereus* group) and S1B9 (*Stenotrophomonas maltophilia*). In case of soil sample 2 (Table 4) total 4 bacterial isolates were identified viz., S2B1 and S2B2 (*Enterococcus cloacae*), S2B3 identified (*Bacillus megaterium*), S2B4 (*Bacillus coagulans*) one bacterial strain was unidentified and the results are in conformity with [17] where they also found bacterial isolates *Pseudomonas sp*., *Bacillus sp*., along with *Aspergillus sp*., *Penicillium sp*., *Streptomyces's sp*. and *Thermo actinomycetes* from different organic wastes. Similarly, *Bacillus subtilis* and *Streptomyces griseus*. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Shigella sp*., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Bacillus anthracis*, *Bacillus subtilis*, and *S. epidermidis* bacterial strains were isolated from agro based industries [18,9]. Detection of soil bacteria is of utmost importance for various aspects. The isolates of *Bacillus* and *Pseudomonas* species were found in both samples in this study. There are reports of isolation of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptococcus*, *Aspergillus niger* species from soil environments which can degrade polyethylene terephthalate (PET) and polystyrene (PS) [19]. Microbes may degrade plastics by the formation of biofilm on the surface of waste by enzymatic activities which needs further study. The largest amount of BPA breakdown was shown by *Leclercia adecarboxylata*, a bacterial strain isolated from municipal waste disposal sites, following four days of incubation at 37 °C [20]. *Bacillus cereus* strain is found to have a higher degrading capacity for plastics [21]. *Staphylococcus aureus*, *Pseudomonas sp*, *Bacillus sp*, *Streptococcus sp* and *Micrococcus sp* degraded plastics with weight loss for their metabolic activity and can be used in bioremediation [12]. Further, they also reported that *Bacillus* degraded plastics upto 31.2%. Earlier reports [22] showed that *Bacillus sp*. and *Ralstonia sp*. accomplished degradation and changes on the polythene and the present study was oriented to isolate soil bacteria that could play a role in plastic degradation. Studies have been reported [23] that *Vibrio harveyi* and *Enterococcus faecalis* formed biofilms on low -density polyethylene and plastic surfaces which is a certainty that *Enterococcus sp*. could be helpful in the biodegradation of plastics and in conformity with this present study.

Table 4: Identification of bacterial strains of soil sample 1 and sample 2 results by VITEK-MS

	Isolates	Organisms name	Confidence value
Sample 1	S1B1	*Leclercia adecarboxylata*	75.90
	S1B2	*Pseudomonas putidia*	99.9
	S1B3	*Pseudomonas putidia*	99.9
	S1B4	*Ralstonia pickettii*	99.9
	S1B5	*Serratia marcescens*	99.9

	S1B6	*Bacillus cereus group*	99.9
	S1B7	*Serratia marcescens*	99.9
	S1B8	*Serratia marcescens*	99.9
	S1B9	*Stenotrophomonas maltophilia*	99.9
Sample 2	Isolates	Organisms name	Confidence value
	S2B1	*Enterococcus cloacae*	50.0
	S2B2	*Enterococcus cloacae*	50.0
	S2B3	*Bacillus megaterium*	99.9
	S2B4	*Bacillus coagulans*	99.9
	S2B5	unidentified	-

3.5. Plastic degradation

This study showed that within 15 days, *Enterococcus cloacae* caused 59.02% weight loss of polythene sheet and in mixed culture weight loss was found to be 51.22% (Figure 5). However, in 30 days weight loss increased to 85.25% for *Enterococcus cloacae* containing polythene sheets, and in mixed culture 73.18% degradation was observed, whereas in the control sample without bacterial culture no changes were observed in their weight loss. This study agrees with studies [10,8] where polythene degradation by bacteria was found to increase with the incubation period and there was a dramatic increase in weight loss of polythene bags. In this study plastic degradation took a longer duration by bacteria and further studies can be initiated in the future.

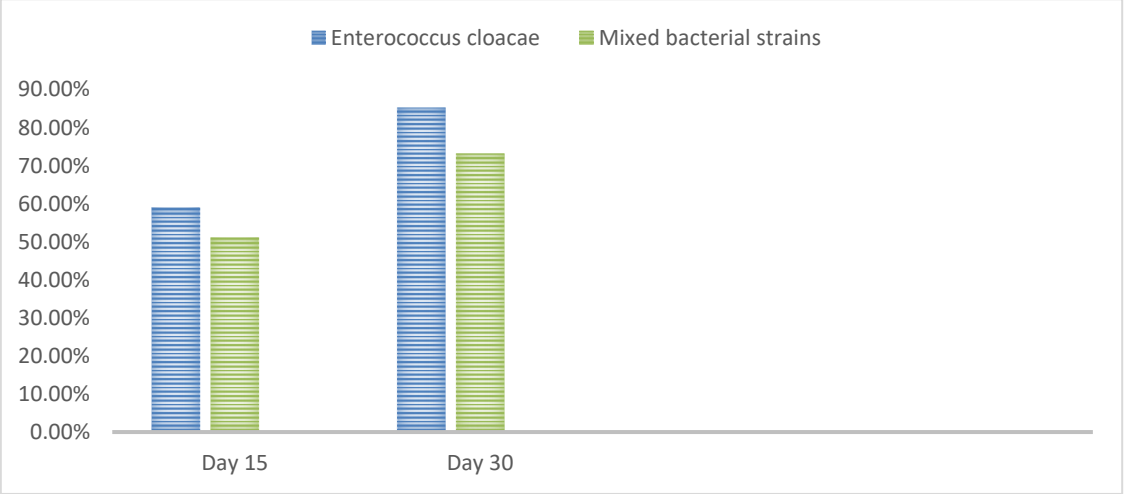

Figure 5: Graphical representation of weight loss of plastic degradation

4. Conclusion:

Nowadays, waste areas are becoming the main source of environmental pollution. A total of nine bacterial strains from Sample 1 and four bacterial strains from Sample 2 were isolated from the waste areas of Dalgaon and Kharupetia which exhibited a range of colony characters. Enzymatic screening through catalase, urease and oxidase activity of bacterial strains gave a better identification of bacterial strains from the garbage areas. Growth curve analysis of the isolates showed the pH and temperature of the isolates. However, to understand the factors that could influence microbial metabolism for waste degradation, future research needs to be conducted in this aspect. Isolates were further confirmed by Vitek MS and the bacterial strains identified from the samples belonged to *Leclercia*, *Pseudomonas*, *Ralstonia*, *Serratia*, *Bacillus*, *Stenotrophomonas*, and *Enterococcus*. In this study, *Enterococcus cloacae* degraded plastics and thus it provides a preliminary step to identify if the isolates of bacteria were able to degrade plastics towards a better future, for a better environment. These bacteria may be useful and promising candidates that may be used to improve environmental problems and human life with their diverse applications in today's world. So, targeted research is required, particularly to improve environmental problems.

References:

[1] H. K. Zaved, M. M. Rahman, M. M. Rahman, A. Rahman, S.M.Y. Arafat, M. S. Rahman, Current Applied Science and Technology, 8(2), 44-85, 2008.
[2] J. R. D Santos, C. Effting, M. P. Serbent, & J. B Matsuda. Biomineralization of calcium carbonate in concrete by the action of Bacillus pumilus. Engenharia Sanitaria e Ambiental, 2023, 28, e20220277
[3] R. C. Onyeze, G. T. Onah & C. C. Igbonekwu, International Journal of Life Sciences Biotechnology and Pharma Research, 2(3), 438-445, 2013.
[4] R. M. Gupta, P. S. Kale, M. L. Rathi, Nikhil N. Jadhav, Pelagia Research Library Asian Journal of Plant Science and Research, 5(6), 36-43, 2015.
[5] K. R. Aneja, P. Jain, and R. Aneja. A Textbook of Basic and Applied Microbiology, New Age International Publishers, New Delhi, India, 1st edition, 2008.
[6] D. Fernández-Calviño, & E. Bååth, FEMS microbiology ecology,73(1), 149-156, 2010.
[7] J. Pietikäinen, M. Pettersson., & E. Bååth, FEMS microbiology ecology, 52(1), 49-58, 2005.
[8] S. Dey, A. K. Singh, & G. Singh, European Journal of Biomedical and Pharmaceutical Sciences, 3(10), 272-277, 2016.
[9] A. K. Ullas and A. K. Isolation, International Journal of Advanced Information in Engineering Technology (IJAIET), 5 (4), 7-13, 2018.
[10] J. Sharma, T. Gurung, A Upadhyay, K. Nandy, P. Agnihotri, A.K. Mitra, International Journal of Advanced and Innovative Research, 3(3), 2278-7844, 2014.
[11] A. Amal, I. K. Hussein, A. S. H. Khudeir, Mesopotamia Environmental Journal, 4, 1- 14, 2015.
[12] A. Hilda, E. Akerele, P. O. Akinyemi1, O. E. Igbogbo-Ekpunobi, Advances in Environmental Technology, 1, 59-71, 2022.
[13] S. A. Soud, Journal of Pharmaceutical Sciences and Research, 11(4), 1333-1339, 2019.
[14] G. Badahit. Screening of plastic degrading Pseudomonas spp. from soil (Doctoral dissertation, Department of Microbiology, 2018.
[15] S. Skariyachan, N. Taskeen, A. P. Kishore, B. V. Krishna, & G. Naidu, Journal of Environmental Management, 284, 112030, 2021.
[16] L. Qiu, X. Yin, T. Liu, H. Zhang, G. Chen, & S. Wu, Journal of Basic Microbiology, 60(8), 699-711, 2020.
[17] M. R. K. Rao, S. K. Sivagnanam, F. B. Syed, Scholars Research Library Der Pharmacia Lettre, 8 (5), 79-86, 2016.
[18] D. Kumar, A. Srivastava, N. Chandra, A. Pandey, S. Kumar, American Journal of Biomedical Sciences, 10 (1), 9-17, 2018.
[19] A. Kamble, T. Shubhamsingh and S. Tejashree, International Research Journal of Environment Sciences, 4(3), 77-85, 2015.
[20] K. Prasad, D. K. Pariha, Chhattisgarh Journal of Science and Technology, 18(1), 85-101, 2021.
[21] J. Sharma, T. Gurung, A Upadhyay, K. Nandy, P. Agnihotri, A.K. Mitra, International Journal of Advanced and Innovative Research, 3(3), 2278-7844, 2014.
[22] S. P. Biki, S. Mahmud, S. Akhter, M. J. Rahman, J. J. Rix, M. A. l. Bachchu, M. Ahmed, Environmental Technology & Innovation, 22, 101495, 2021.
[23] I. Hchaichi, F. Bandini, G. Spini, M. Banni, P.S. Cocconcelli, & E. Puglisi. Enterococcus faecalis and Vibrio harveyi colonize low-density polyethylene and biodegradable plastics under marine conditions. FEMS Microbiology Letters, 2020, 367(15), fnaa125.
[24] M. A. Begum, B. Varalakshmi and K. Umamagheswari, International Journal of Current Microbiology and Applied Sciences, 4(11), 674-680, 2015.