



**MOLECULAR IDENTIFICATION OF *LYSINIBACILLUS FUSIFORMIS*
AND FTIR ANALYSIS OF BIODEGRADED PRODUCTS****THONGLIU SK¹ AND MARKANDAN M^{2*}**^{1,2}Department of Zoology, St Joseph University, Chumoukedima, Nagaland, India-797115*Corresponding Author: Dr. Murali Markandan: E Mail: dpimurali2523@gmail.comReceived 18th July 2023; Revised 20th Sept. 2023; Accepted 2nd Dec. 2023; Available online 1st Sept. 2024<https://doi.org/10.31032/IJBPAS/2024/13.9.8315>**ABSTRACT**

Polyethylene terephthalate (PET) is one of the most common plastic available today due to its versatile properties. However, it contributes significantly as a source of environmental pollution. To ensure least possible pollution, we need to develop eco-friendly process. In this paper, biodegradation of PET plastic bottle was studied using *Lysinibacillus fusiformis*. The analysis was carried out after one month incubation. The microbe was identified using 16s rRNA gene sequencing. The effect of microbes on the surface of the PET plastic and biofilm formation of microbes were observed through SEM and chemical changes were confirmed by FTIR analysis. The result shows that *Lysinibacillus fusiformis* have the potential to degrade PET plastic.

Keywords: Polyethylene terephthalate, *Lysinibacillus fusiformis*, biodegradation of plastics, SEM, FTIR**INTRODUCTION**

Polyethylene Terephthalate (PET) is the most commonly used plastic across the globe due to its durability, making it difficult to break down and degrade in the natural environment. It is made by poly condensation of PTA with ethylene glycol. PET is strong stiff synthetic fiber and resin and a member of the polyester family of polymers. The presence of aromatic ring in the PET repeating units gives the polymer the notable stiffness and strength [1]. Due to

its versatile properties, PET plastics are used in thousands of ways. The use of plastic has become an integral part of the modern society, with annual production exceeding 350 million tons [2]. Although it has numerous useful applications and despite being recyclable, it also contributes significant amount of pollution in the nature. PET is used extensively in the food and beverage industry, and for this reason it is the highest thermoplastic producing the

highest amount of waste [3]. PET has an annual worldwide production of over 50 million tons [4], and according to Global cumulative production of plastics since 1950 is forecast to grow from 9.2 billion tons in 2017 to 34 billion tons by 2050 [5].

Enormous amount of pollution has been caused by the excess use of plastic in the environment. Large quantities of PET have been introduced into the environment through its production and disposal, resulting in the accumulation of PET in ecosystem across the globe [6]. PET debris is often eaten by fish and other marine creatures. In this way, PET degradation products and additives are introduced in to food chain, where they have negative impact on human and animal health [7] and also damaging ocean's fauna and slowing down the transition to a circular economy [6]. Everyday people use plastic in every aspect of their life activities, this has now become a global problem. Plastic pollution is becoming increasingly obvious and, together with other environmental matters such as global warming, air and water pollution, deforestation is attracting serious concern globally [8]. Plastic recycling have come a long way to reduce the pollution caused to the environment yet the problem remains the same. It takes more than 50 years for the plastic to degrade in the natural environment, and hundreds of years if

discarded into the oceans, due to their lower temperature and oxygen availability [9].

Exposing the plastic material under the sunlight gradually degrades the plastic but the process is very slow, it takes thousands of years to degrade and it is regarded as an inefficient process. The polymer chain of the plastic disintegrates when UV light acts on it. When the UV light is absorbed by the plastic, the photons in the material get excited which creates free radical from the hydroperoxides. The chain process of light induced degradation of polymers which takes place in the presence of oxygen is called photo-oxidation. Photo-oxidation causes in the breakage of chain polymer leading the material to become weak and brittle [10]. The short chain molecules formed can then be attacked easily by microbial exoenzymes for further degradation and consumption. Moreover, UV radiation can also reduce the plastic hydrophobicity and results in higher colonization [11].

Although the problem of plastics remain unsolved, different approaches are being considered to reduce their impact on environment. Biological plastic degradation, which employs microorganisms and their degradative enzymes, has emerged as one way to address the unforeseen consequences of the waste streams that have resulted from mass plastic production [12]. Therefore, PET biodegradation has attracted more and

more attentions as an environmentally friendly alternative, requiring mild temperature and low energy consumption [13] [14]. Bioremediation involving microorganisms with plastic degrading capacities are regarded as effective and environmental friendly alternatives. Biodegradation of plastics involves excretion of extracellular enzymes by the microorganisms, attachment of enzymes to the surface of plastic, hydrolysis to short polymer intermediates which are ultimately assimilated by microbial cells as carbon source to release CO₂ [14]. UV Pretreatment of plastic is also another way to facilitate the biodegradation rate. Until now, only few species of bacteria and fungi have been described as capable of partially degrading pet to oligomers or even monomers [14]. This present paper reports the use of bacteria *Lysinibacillus fusiformis* having the potential to degrade PET plastic bottle.

2. MATERIALS AND METHODS

2.1. Bacterial isolation and identification

The old PET waste bottle was collected from the waste dumping site (St. Joseph University, Dimapur Nagaland, India). The waste bottle was cut into small pieces and washed properly with distilled water and let it to dry. It was then inoculated in Nutrients broth Media (AI media). The media plate was incubated at 37°C for 24 hour allowing for the bacterial growth. After 24 hours of incubation the bacterial isolates

were identified by the methods described in Bergeys Manual of Determinative Bacteriology [15]. Dominated 10 bacteria were isolated and one has selected for further study and 16srRNA sequences were analyzed from BioEdge Solutions Bangalore, Karnataka. Bacterial Gene sequences were submitted in NCBI to analyze pairwise similarity and submitted to Gene bank to receive accession number.

2.2 Treatment of PET Plastics:

New PET plastic bottle was purchased and cut in small flakes of size about 1cm square. Three categories of PET plastic flakes were maintained: (i) control sample without any treatment (ii) UV treated sample and (iii) UV treated PET sample inoculate with bacteria. The whole culture media was incubated for a period of 1 month at a temperature of 37°C. After completing one month, the PET plastic sample treated with bacterial isolates was sent for further analysis.

2.3 Determination of morphological and structural changes

The morphological surface changes in the sample material was analyzed by using Scanning Electron Microscopy. Analysis was carried out using low vacuum 0.68 torr mode, 10 to 30 kv at different magnification 6.13kx to 500kx and LFD (Large Field Detector) [16].

2.4 FTIR analysis of Treated PET plastics

Chemical changes occurring on the surface of the PET were analyzed using FTIR spectrophotometer Fourier transform infrared (FTIR). Measurements were carried out with the Perkin Elmer Spectrum two (version 10.03.09) in the range of 4000-400 cm^{-1} . FTIR spectra were recorded at a resolution of 2 cm^{-1} and at an accumulation of 32 scans.

3. RESULT AND DISCUSSION

3.1. Bacterial identification

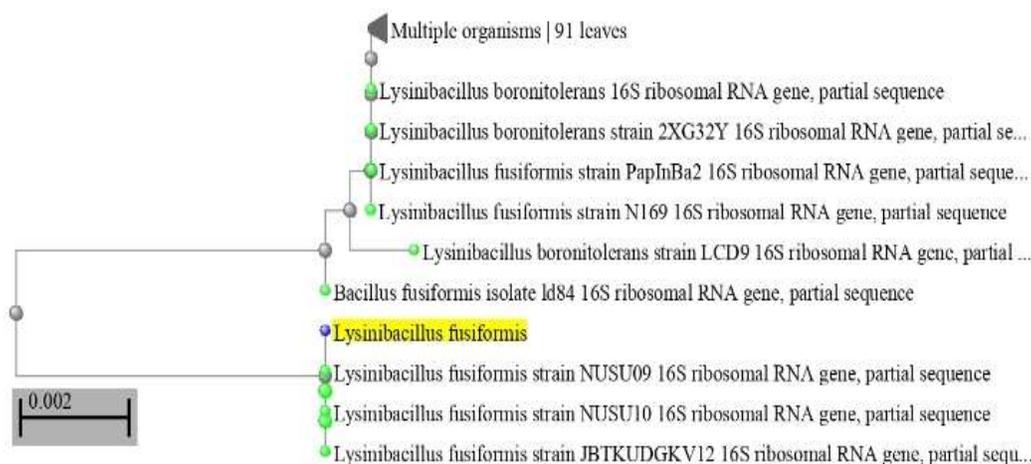


Figure 1: Phylogenetic tree of *Lysinibacillus fusiformis*

3.2 Morphological changes of the treated plastic flakes

In this present study, the bacteria *Bacillus fusiformis* was isolated from the soil and treated with PET plastic. The whole media was incubated for 30 days and the treated PET plastic was observed through Scanning Electron Microscopy.

The biodegradation rate is validated by the morphological changes occurred during Scanning Electron Microscopy [17]. The SEM images of the control PET plastic sample showed that the surface was smooth

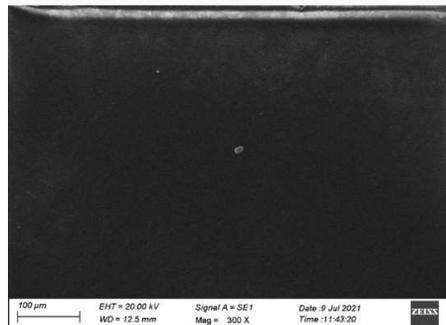
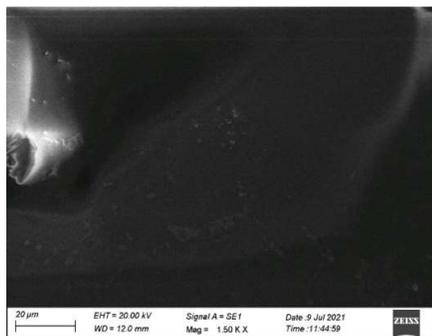
The pure culture on agar-solidified medium was sent for screening. In the **Figure 1** Phylogenetic tree were built by utilizing greatest miserliness criteria with closest neighbor optimization. Sequences with 98.29 % similarity to voucher bacterial specimen (KJ155800.1). Sequence was checked from nucleotide alignment tools of BLAST in NCBI. The bacterial isolate was identified as *Lysinibacillus fusiformis*.

Fig 2(A). In contrast to the control sample, huge number rod shaped colonization and budding stages of bacteria can be clearly seen on the UV treated with Bacterial inoculated PET plastic surface **Fig 2(C)** when compared with the UV treated PET sample **Fig 2: (B)**. The surface of the UV treated sample showed little structural changes as compared to the smooth plain surface of the control sample. Whereas remarkable changes were observed in the UV bacterial treated sample. The formation of biofilm reveals the capacity to utilize PET

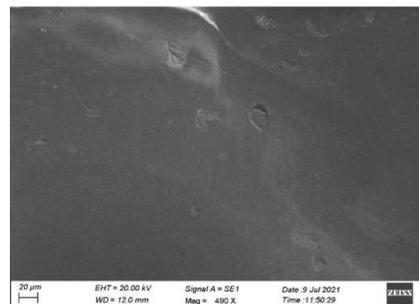
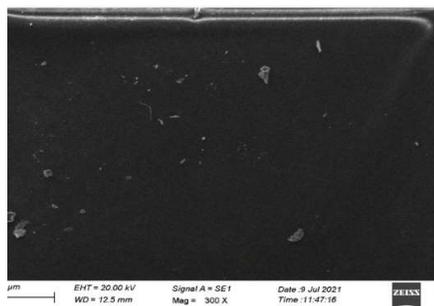
as a carbon source. This observation coincides with Orr *et al.*, 2004 who have reported that bio-film formation determines its biodegradation potential because bio-film causes the bacteria to efficiently utilize the insoluble polymer substrate [18]. The changes in the structure were not uniformly distributed on the entire surface as bacteria attached to only certain regions [17]. From the SEM images it is evident that the colonization of *Lysinibacillus fusiformis* has taken place on the surface. The changes on the surface of PET indicated that biodegradation had occurred. The adherence of the microorganism to the polymeric surface is fundamental for the

biodegradation to take place [19]. Our results shows good number of bacteria growing on the plastic surface. This is similar to the study conducted by Thi Cam *et al.*, [19] who have reported that PET treated with *Bacillus* sp. showed good result through SEM images like holes, small cracks and cavities can be clearly observed in the treated sample which is absent in the control sample. Similarly in this paper, the microscopic analysis revealed significant surface changes in the UV and bacterial treated sample as compared to the control sample. This confirmed that *Lysinibacillus fusiformis* have been able to degrade PET bottle [20-28].

A



B



C

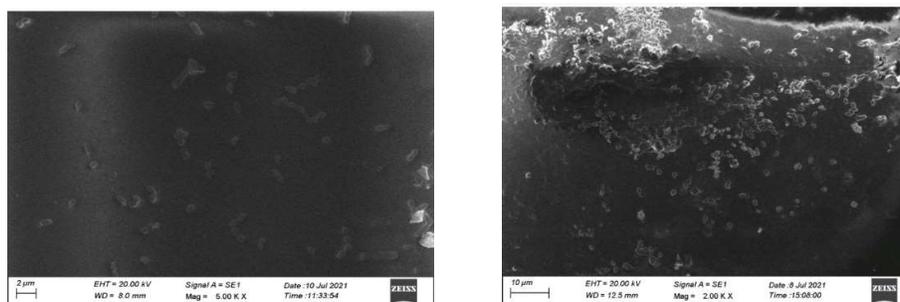


Figure 2: SEM images: A- Control PET plastic; B- UV treated PET plastic; C- UV + *L.fusiformis* treated sample

3.3 FTIR spectra analysis of the UV-bacteria treated plastic flakes

FTIR spectra of PET were performed from 4000 to 400 cm^{-1} wave number region after one month incubation in laboratory condition. The changes in the chemical structure of the PET plastic were determined by studying the spectra observed in through FTIR analysis. Comparison figure of control PET plastic and UV treated Plastics sample is shown in **Figure 3**. From the FTIR spectra, the bands from 3915.84cm^{-1} to 3055.55cm^{-1} corresponds to O-H group, 2969.33cm^{-1} to 2107.25cm^{-1} has been attributed to C-H bond. Bands 1956.55cm^{-1} to 1613.08cm^{-1} corresponds to C=O bond. The bands 1177.02cm^{-1} , 1016.30cm^{-1} and 971.36cm^{-1} corresponds to C-O-C bond. New band was observed at 3915.48cm^{-1} in UV and bacterial treated sample which has been attributed to O-H group bond stretching. Another new band was also observed at 1400.41cm^{-1} and 1074.75cm^{-1} corresponding to CH (CH₂) and C-O-C

bonds respectively. Several band were found to have been vanished when compared to control sample. This implies that the chemical bond is broken down and is no more. From this it is evident that the new bands were formed by the breaking down of some bonds in the plastic polymer chain. The wave numbers of most of the corresponding peaks were shifted remarkably. The changes in the spectrum of the UV bacterial treated sample explicit that the molecular chemical bond structure has been broken thus breaking the polymer chain structure. In comparison to the control sample, the FTIR spectrum of the treated sample showed many changes in their wave number due to the chemical changes. These chemical changes occurred due to the action of the UV light as well as the bacteria acting on the PET surface breaking down of chemical compound present in the plastic sample and it is a proof that the bacteria *Lysinibacillus fusiformis* is able to degrade PET plastic.

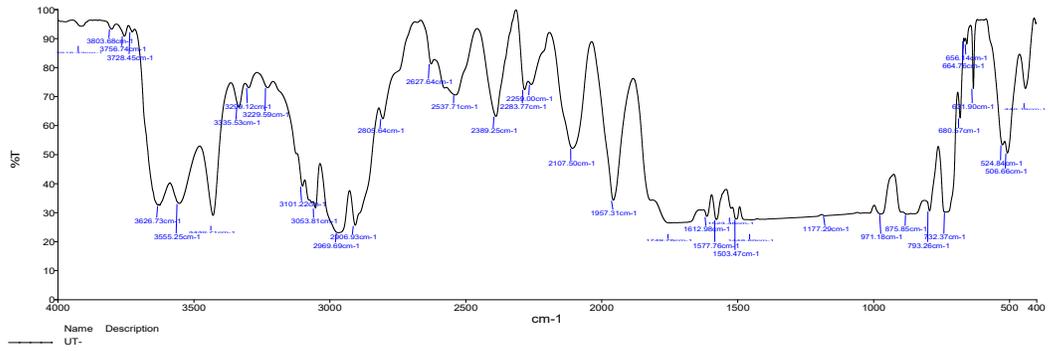


Figure 3: FTIR spectrum of Control PET plastics

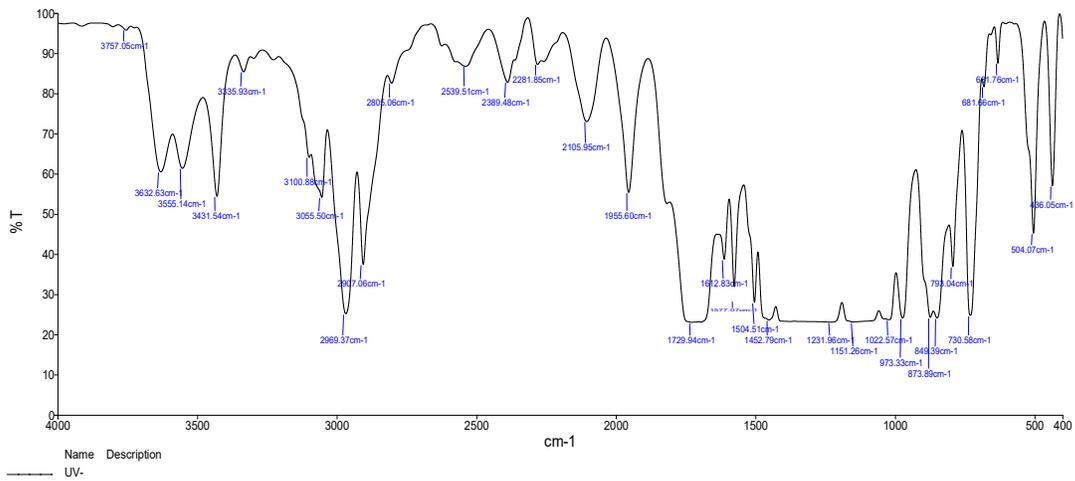


Figure 4: FTIR spectrum of UV Exposed PET plastics

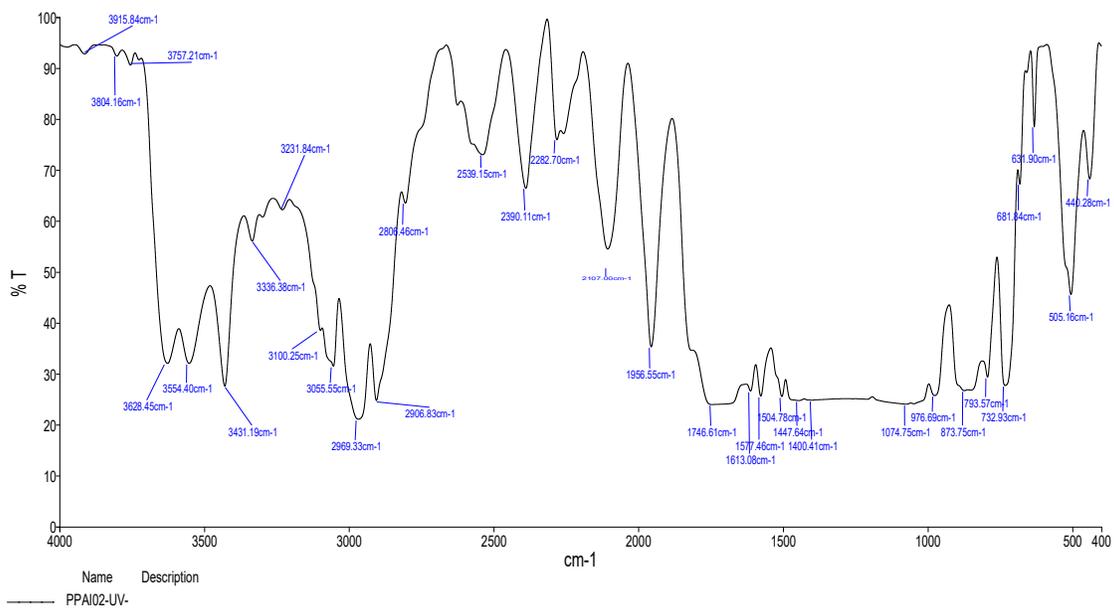


Figure 5: FTIR spectrum of UV Exposed and *Lysinibacillus fusiformis* treated PET plastics

CONCLUSION

PET plastic bottles are one of the major threats to the environment. The studies are about the degradation of PET bottle plastics by biological means and abiotic factor of UV-light. The UV treated PET plastic was treated with *Lysinibacillus fusiformis*. This result showed a huge number of bacterial colonies on the UV and bacterial treated PET plastic surfaces. The result was confirmed through Scanning Electron Microscopy. The chemical changes in UV treated sample displayed new peaks which reveals the positive action of UV light on plastic. The FTIR spectrum confirmed the changed on UV treated sample and Bacterial treated PET plastics. Morphological analysis also shows remarkable surface changes on UV treated sample. Abiotically modified surfaces of plastics promote the growth of microorganisms and thus may accelerate biodegradation. Therefore, this abiotic factor (UV light) and bacteria can be used in the future to degrade PET plastic.

Acknowledgements

Special thanks to Vincent Sagayaraj ACIC Tiruchirappalli for carrying out the Scanning Electron Microscopy and FTIR test.

REFERENCES

[1] Britannica, T. Editors of Encyclopaedia (2020). Polyethylene

Terephthalate. Encyclopedia Britannica.

<https://www.Britannica.Com/Science/Polyethylene-Terephthalate>.

- [2] Danso D, Chow J, Streit Wr. (2019). Plastics: Environmental And Biotechnological Perspective On Microbial Degradation. Applied And Environmental Microbiology, 85.
- [3] Baris, S., Uygunoglu, T., Korucu, H., Kocakerim, M. (2019). Performance Of Dioctyl Terephthalate Concrete. Use Of Recycled Plastics In Eco-Efficient Concrete. Woodhead Publishing Series in Civil and Structural Engineering, 249-267.
- [4] Bornscheuer, Uwe T (2016). Microbiology. Feeding On Plastic. Science (New York, N.Y.) Vol.351,6278):1154-5. Doi:10.1126/Science.Aaf2853
- [5] Geyer, R. (2020). Production, use, and fate of synthetic polymers. Plastic Waste and Recycling, 13-32.
- [6] Len, N., Fabienne, S., Ruth S., Terry, G. (2016). The New Plastics Economy: Rethinking the Future of Plastics, World Economic Forum.
- [7] Sax L. (2010). Polyethylene Terephthalate May Yield Endocrine Disruptors. Environmental Health

- Perspective, 118, (4), 445-448. Doi: 10.1289/Ehp.09091253.
- [8] Fawai K. (2021). The Current State of Research on PET Hydrolyzing Enzymes Available for Bio Recycling. Catalyst 11(2)206. <https://doi.org/10.3390/Catal11020206>
- [9] Nisha, M., Zahra, M., Parveen, K., David, B. (2020) Microbial and Enzymatic Degradation of Synthetic Plastics. Front Microbial,11,580709. <https://doi.org/10.3389/Fmicb.2020.580709>
- [10] Bottino, F.A., Cinuegrani, A.R., Pasuale, G.D., Leonardi, L., Pollicino A. (2004) Polymer Testing 23(4), 405-411.
- [11] Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008). Biological degradation of plastics: a comprehensive review. Biotechnology advances, 26(3), 246-265. <https://doi.org/10.1016/j.biotechadv.2007>
- [12] Carr, C.M., Clarke, D.J., & Dobson, A. D. W. (2020). Microbial Polyethylene Terephthalate Hydrolases: Current And Future Perspective. Frontiers In Microbiology, 11, 571265.
- [13] Zimmermann, W., & Billig, S. (2011). Enzymes for the Biofunctionalization of Poly (Ethylene Terephthalate). Advances In Biochemical Engineering/Biotechnology,15,97-120.
- [14] Wei, R., & Zimmermann, W. (2017). Microbial Enzymes For The Recycling Of Recalcitrant Petroleum-Based Plastics: How Far Are We?. Microbial Biotechnology 10,6, 1308-1322. Doi:10.1111/1751-7915.12710
- [15] Sneath P.H.A., Mair S.N., Elisabeth Sharp M. and Holt G.J., Bergeys Manual of systematic Bacteriology, Williams ailkines, Baltimore, USA (1994)
- [16] Flavia, P., Giovanna, P., Antonella, M. (2006). Biodeterioration of Paper: A SEM Study of Fungal Spoilage Reproduced Under Controlled Conditions, Macromol. Symp. 238, 57-66
- [17] Aishehrei, F. (2017). Biodegradation of Synthetic and Natural Plastic By Microorganisms. Journal of Applied and Environmental Microbiology,5(1),8-19.
- [18] Orr, I.G., Hadar, Y., & Sivan, A. (2004). Colonization, Biofilm Formation and Biodegradation of Polyethylene by a strain of *Rhodococcus ruber*. Applied Microbiology and Biotechnology, 65, (1)97-104.
- [19] Choi, H.S., Shin, M.S., Kim, J. H. (1999). Enhancement of Microbial

- Adhesion on the Chemically Modified Polyethylene Surface. *Environmental Engineering*,4,127-133.
- [20] Tania, V., Gerardo, C. S., M. Gutierrez, R., Angel, M. (2001). Thermally Treated Low Density Polyethylene Biodegradatyion by *Penicillium pinophilum* and *Aspergillus niger*. *Journal of Applied Polymer Science*, 83.
- [21] Thi Cam Ha Dang *et al.* (2018). Plastic Degradation By Thermophilic *Bacillus* Sp. BCBT21 Isolated From Composting Agricultural Residual In Vietnam. *Advances in Natural Sciences: Nanoscience and Nanotechnology*,9 (1).
- [22] Waqas, M., Haris, M., Asim, N., Islam, H, U., Abdullah, A., Khan, A., Khattak, H., Waqas, M., & Ali, S. (2021). Biodegradable potential of *Bacillus amyloliquefaciens* and *Bacillus safensis* using Low density Polyethylene Thermoplastic (LDPE) substrate. *European Journal of Environment and Public Health*, 5(2), em0069. 2021, 5(2).
- [23] Ghosh, S. K., Pal, S., Ray, S. (2013). Study of microbes having potentiality for biodegradation of plastics waste. *Environment Science Pollution Research*,0,4339-4355.
- [24] Webb, H., Arnot, J., Crawford, R., Ivanova E. (2012). Plastic degradation and its environmental Implications with special Reference to Poly(ethylene terephthalate). *Polymers*, 5, 1-18.
- [25] Priyanka, N., Archana, T. (2011). Biodegradability of polyethylene and plastic by the help of microorganism:A way for brighter Future. *Journal of Environmental and Analytical Toxicology*,1.
- [26] Kawai F. (2021). Emerging Strategies in Polyethylene Terephthalate Hydrolase Research for Biorecycling. *Chem. Sus. Chem*,14(14),4115-4122.
- [27] Urbanek, A.K., Kosiorowska, K.E., & Mironczuk, A. M. (2021). Current knowledge on Polyethylene Terephthalate Degradation by Genetically Modified Microorganisms. *Frontiers in bioengineering and biotechnology*, 9, 771133.
- [28] Lear, G., Kingsbury, J.M., Franchini, S., Gambarini, V., Maday, S. M., Wallbank, J.A., Weaver, L., & Pantos, O. (2021). Plastics and the microbiome: impacts and solutions. *Environmental microbiome*, 16(1), 2.