Biomedical Letters ISSN 2410-955X DOI: https://doi.org/10.47262/BL/8.2.20220501

Research article
2022 | Volume 8 | issue 2 | Pages 136-143

ARTICLEINFO

Received
March 02, 2022
Revised
April 25, 2022
Accepted
May 30, 2022

***Corresponding Author**
Muhammad Ashraf

E-mail
mashrafmalik11@hotmail.com

Keywords
Low-density polyethylene
Enterobacter cloacae
Polypropylene
Biofilm
Plastic degradation

How to Cite
Khan M, Ashraf M, Ishaque W, Ahsan M, Sajid M, Habib M, Mehmood A. Efficiency of halophilic biofilm producing bacteria towards the degradation of plastic materials at optimum temperature. Biomedical Letters 2022; 8(2):136-143.

Scan QR code to see this publication on your mobile device.

Open Access

Efficiency of halophilic biofilm producing bacteria towards the degradation of plastic materials at optimum temperature

Maham Khan[1], Muhammad Ashraf*[,1], Wajid Ishaque[1], Muhammad Ahsan[2], Muhammad Sajid[3], Mudasser Habib[1], Ansar Mehmood[1]

[1]Soil and Environmental Science Division, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan
[2]Institute of Environmental and Agricultural Sciences, University of Okara, Okara, Pakistan
[3]Department of Biotechnology, University of Okara, Okara, Pakistan

Abstract

The consumption of plastic has drastically increased leads to the plastic waste and became the global issue. In the present study, the screening of bacterial isolates from saline areas along with their microbial and biofilm efficiency in degradation of low-density polyethylene (LDPE), high density polyethylene (HDPE) and polypropylene plastic materials were estimated at two different temperatures (30°C and 37°C). The soil samples were collected from salt-affected lands for the isolation and characterization of bacterial isolates. The isolated strains were characterized by 16S rRNA. Two bacterial strains (*Bacillus subtilis* and *Enterobacter cloacae*) were identified through sequencing (BioEditor Sequence Builder) among the selected bacterial isolates. Effective degradation rate has been observed through *B. subtilis* towards LDPE, HDPE and polypropylene as 18%, 25% and 42% respectively through biofilm, while the degradation rate in TSA media were observed as 32%, 30% and 52% respectively, at 37°C. Similarly, *E. cloacae* degrades the LDPE, HDPE and polypropylene material at 12%, 15% and 30% through biofilm, however 19%, 18% and 38% degradation rate were observed at 37°C respectively. Therefore, both bacterial strains (MK2 *B. subtilis* and MK29 *E. cloacae*) isolated from salt-affected area showed potential to degrade the plastic materials at optimum temperature of 37°C.

Introduction

Plastic has various applications in different fields of life and industries such as packaging, pharmaceutical, agriculture, cosmetics, and chemicals [1, 2]. The most commonly used plastic materials are polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC) [3, 4]. Plastic waste is becoming a global issue and is being addressed on international platforms due to its severity [5]. Plastic degradation is considered a serious problem around the globe. According to a report, almost 311 million tons of plastic produced in 2014 worldwide [6, 7]. Less than 10% of the plastic waste has been recycled however the remaining plastic wastes are still remains in in sea and soil where it pollute the aquatic and soil habitants leads to damage the environment [8].

Various methods for the degradation of plastics are available however the most acceptable eco-friendly method is microbial biodegradation [9]. There are some reported bacterial and fungal species that are associated with the plastic degradation (*Pseudomonas*, *Enterobacter cloacae*, *Bacillus* sp., and *Aspergillus niger*) [10-13]. The production and degradation of organic matter is performed by many microbial communities of biofilm, cycling of sulphuric and nitrogen elements [14]. It is one of the significant bio control agent for plants and have ability to enhance the biodegradability of polyethylene and high molecular weight polycyclic aromatic hydrocarbons (PHA), which are responsible for the contamination of the soil [15].

It is now the basic need to find out the economical and ecofriendly method for the degradation of plastic wastes and still biodegradation seems to be the most effective and promising one [16]. The microbial activity initiates the deterioration of plastic and changes its physical properties including shape and color [17]. The bacterial environment plays an important role through temperature, pH, carbon source and moisture [13]. The breakdown is carried out by the enzymatic activity of the microbes. During this process, the breakdown of polymers to monomers occurred and will ultimately help the microbes to accumulate for degradation [9]. The explicit objectives of this study are to isolate the bacteria, identified through 16S rRNA and evaluate their degradation potential against different kinds of plastic available in market under optimum temperature.to develop an economical and eco-friendly method for plastic degradation.

Materials and Methods

Sample collection

Soil samples were collected from the experimental field of Bio Saline Research Station (BSRS), Pakka Anna, area of Faisalabad and Khewara Salt Mine, Jhelum Pakistan. The selected land have higher salinity level including biofilm-producing bacteria for the degradation of plastic wastes [18]. One gram of the soil sample was mixed with 9 mL of sterile saline distilled water in 50 mL conical flask and placed on shaker for 15 min at room temperature. Serial dilutions of the collected samples were plated on TSA media (tryptic soy agar) and incubated at 37° C for 1-2 days. The colonies were further characterized for identification and purified for processing.

Extracellular polymeric substances (EPS) and bacterial production

The effect of EPS on plastic degradation was also calculated. For this purpose, the bacterial strains were cultivated in RCV-sucrose medium modified from the original RCV media [19]. RCV is a specific media for the growth of EPS producing bacteria. The production was carried out [20]. The RCV-media consists of the following components as (g/L): yeast extract 0.1 g, S-II (nutrient solution) 50 mL, tampon phosphate (T.P) solution 15 mL, NaCl 0.25 M, agar 15 g, sugar (sucrose) 40 g, and distilled water 1000 mL, while the bacterial growth was carried out in TSA media having pH of 6.5. The flasks were incubated on a rotary shaker at 37° C.

Pre-treatment of plastic

Three different types of plastic bags (LDPE, HDPE and polypropylene) were collected from different markets of Faisalabad, Pakistan. Each plastic bag was cut into the pieces of 2 cm strips and washed them with distilled water. The strips were placed in the Petri plates with 70% ethanol for an hour. The strips were transferred to sterilize Petri plates and air-dried. Each strip was weighed on weighing balance. The degradation of the plastic wastes were estimated by using equation 1 [21, 22].

% weight loss = Initial weight – Final weight / Initial weight x 100…… (1)

Inoculum preparation and in vitro biodegradation assay

TSA and RCV(S) media were used to estimate the efficiency of the bacteria and EPS separately towards the degradation of plastic wastes respectively. The isolated strains were separated from the media and placed in 250 mL conical flasks on a shaking incubator at 30° C and 37° C for a week at 150 rpm. The required growth was observed and the plastic strips were added after weighing for a month. Negative control was maintained by adding the strips but without inoculation of bacteria and incubating with the rest of the flasks [21, 23].

Identification of isolated strain

The isolated strains were identified based on various phenotypic, biochemical, and g16S rRNA Polymerase Chain Reaction (PCR) techniques.

Biochemical test

The biochemical tests including gram staining, morphological and other biochemical tests including catalase, oxidase, indole, MR and Voges [24] were performed and gram straining of the selected bacterial isolates were also performed. The isolated strains were grown in plain Nutrient Broth (NB) at 37° C for 24 hours. The freshly grown culture was inoculated in different biochemical media for further analyses.

Results and Discussion

Plastic waste is becoming one of the biggest and life-threatening issues all over the world for human as well as aquatic lives [6, 7, 25]. The biggest problem is to degrade such huge amount of plastic waste, burning plastics releases hazardous gasses in air that could increase the air pollution [26]. According to reports, less than 10 % of plastic waste is recycled while the other 90 % being dumped in soil and rivers that are life threatening for humans as well as for aquatic life. Researchers are focusing on economical and bio-friendly methods for the degradation of plastic wastes [27]. The samples were serially diluted and plated on different media plates as (RCV(S), RCV (G), RCV (F), TSA) in replicates incubated at 37°C for 24 hours. After incubation, the plates exhibited numerous bacterial colonies. Thirty colonies (16 from Pakka Ana and 14 from Khewara soil samples) were selected. The colonies were further streaked on TSA plates to get purified colonies. The colonies were streaked again up to 5 batches. From all the generated colonies, high EPS producing strains under salt stress through spot plate method were selected and 6 (MK1, MK2, MK10, MK22, MK29, and MK33) strains were selected for future studies (**Table 1**).

The bacterial isolates were also identified through various experiments (**Table 2**). The indole and methyl analyses showed negative results for all the isolated strains except MK22, while Voges Proskauer analyses showed positive for all the selected strains except MK2. Oxidase analyses showed negative results for all the isolated strains except MK29 and reconciled with the Pseudomonas bacterial strain [28].

Polymerase Chain Reaction (PCR) amplification of 16S rRNA region of extracted genomic DNA of bacteria was performed by using 27: 5'-AGAGTTTGATCMTGGTCAG-3'and 1492:5'-CGGTTACCTTGTTACGACTT-3' as forward and reverse primer method. Six bacterial isolates were selected for DNA Sequencing. The generated sequencing data of all the selected isolates were analyzed (bio editor sequence builder) and submitted to the NCBI GenBank database for accession number (**Table 2**).

The bacterial isolates were also studied morphologically, and it was observed that MK1, MK10, MK22 and MK29 have mucoid consistency. The gram staining results showed that all the isolates were gram positive except MK29 which was gram negative. It was also evaluated through 16S rRNA identification that the MK29 was *Enterobacter Cloacae* gram negative bacteria Morphological results showed that all the selected strains have smooth margin except MK10. However, MK1 showed white color, MK10 showed yellowish growth while all other strains were creamy (**Table 3**).

Five bacterial species were used (MK1, MK2, MK10, MK22, and MK29) to evaluate the activity of biofilm and bacteria using RCV and TSA media respectively, at two different temperatures (30° C and 37° C) for two months.

MK29 showed considerable degradation at 30° C and 37° C temperatures for all kind of plastic materials and MK2 showed suitable degradation of plastic (**Table 4**). The graphical representation of the plastic degradation through bacteria in TSA and EPS in RCV are shown in **Fig. 1 and 2** respectively.

There are different standard testing methods to detect the microbial activity in the degrading of plastic materials including morphological changes, cracks, holes and changes in surface and color, formation of biofilm around the material, changes in physical properties such as mass loss and chemical properties

Table 1: Molecular identification of bacterial isolates

Bacterial isolates	Source	Accession no.	Scientific name
MK-1	Khewara Salt Mine	OL468729	*Priestia aryabattai*
MK-2	Khewara Salt Mine	OL630943	*Bacillus subtilis*
MK-10	Khewara Salt Mine	OL468730	*Priestia megaterium*
MK-22	BSRS Pakka Anna	OL629464	*Priestia megaterium*
MK-29	NIAB Research Field	OL468728	*Enterobacter Cloacae*
MK-33	BSRS Pakka Anna	OL630945	*Peribacillus huizhouensis*

Table 2: Biochemical characterization of EPS producing isolates

Biochemical test	MK1	MK2	MK10	MK12	MK22	MK29
Indole test	–	–	–	–	+	–
Methyl red	–	–	–	–	+	–
Voges Proskauer	+	–	+	+	+	+
Citrate Utilization	–	+	+	+	–	–
Lactose utilization test	–	–	+	+	+	–
triple sugar iron test	+	+	+	+	+	+
fructose utilization	+	+	+	+	+	+
Xylose utilization	–	–	+	+	+	+
mannitol utilization	–	–	+	–	–	–
oxidase test	–	–	–	–	–	+
catalase test	+	+	+	+	+	+
starch hydrolysis	+	+	+	+	+	+
gelatin hydrolysis	+	+	+	+	+	+
lipid hydrolysis	–	+	–	+	–	+
casein hydrolysis	+	+	+	+	+	+
urea hydrolysis	–	–	+	+	+	+
ONPG hydrolysis	–	+	+	+	+	–
Arginine Dihydrolase	–	–	–	–	–	–
nitrate reduction	+	–	+	+	+	–

Table 3: Morphological characterization of EPS producing isolates

Sample	Isolates	Medium	Color	Margin	Shape	Optical char.	Elevation	Size	Consistency	Gram's staining
S1	MK1	TSA	white	Smooth	Round	Opaque	Raised	Small	Mucoid	gram +ve
S1	MK2	TSA	creamy	Smooth	Curled	Opaque	Convex	Moderate	Smooth	gram +ve
S2	MK10	RCV(F)	yellowish	Lobate	Irregular	Iridescent	Convex	Large	Mucoid	gram +ve
S3	MK22	RCV(S)	creamy	Smooth	Curled	Opaque	Convex	Moderate	Mucoid	gram +ve
S4	MK29	RCV(S)	creamy	Smooth	Round	Opaque	Convex	Large	Mucoid	gram -ve
S4	MK33	RCV(S)	creamy	Smooth	Round	Opaque	Raised	Moderate	Smooth	gram +ve

Table 4: Comparison between EPS and bacterial degradation at 30° C and 37° C

	Initial weight (g)	strains	Plastic degradation by EPS in TSA after 2 month (%)		Plastic degradation by bacteria in RCV after 2 month (%)	
			30°C	37 °C	30°C	37 °C
LDPE	0.159	MK1	4.40	12.57	5.66	1.25
		MK2	5.03	**19.49**	10.69	**12.57**
		MK10	5.66	12.57	7.54	9.43
		MK22	2.88	**26.41**	10.69	6.28
		MK29	**8.36**	**32.38**	10.06	**18.86**
HDPE	0.051	MK1	3.84	15.60	12.68	7.84
		MK2	5.52	**18.49**	14.60	**15.05**
		MK10	4.80	11.56	9.80	7.84
		MK22	5.76	10.56	13.68	7.84
		MK29	7.33	**30.52**	14.29	**25.37**
polypropylene	0.075g	MK1	6.66	32.00	8.00	16.00
		MK2	8.00	38.33	10.66	**30.66**
		MK10	12.00	22.00	13.33	18.66
		MK22	9.33	32.00	12.00	**21.33**
		MK29	21.33	**52.00**	18.00	**42.66**

as weight loss [17]. The selected methods were used for evaluation and promising strains for the biodegradation of plastic bags from (**Fig. 1**) represent the change in color and shape by isolated bacterial

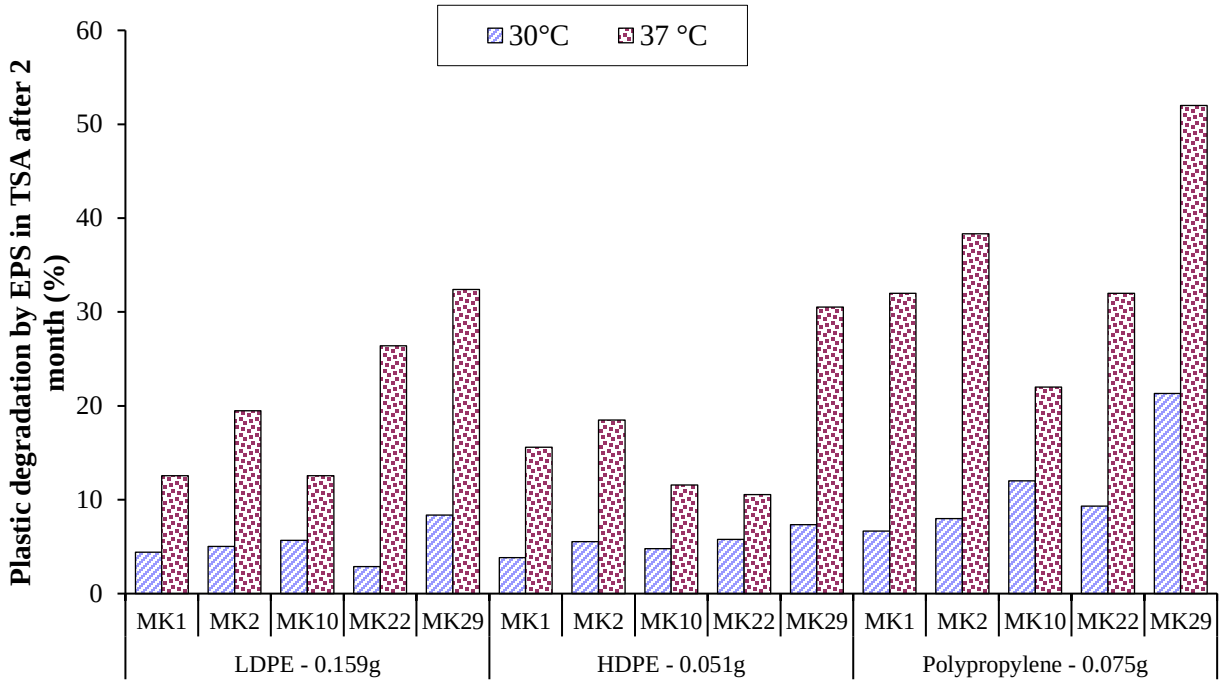

Fig. 1: Showing the % of degradation by bacteria in TSA media at two different 30°C and 37°C. MK29 showed the best results among all strains for all kinds of plastic material at both temperatures.

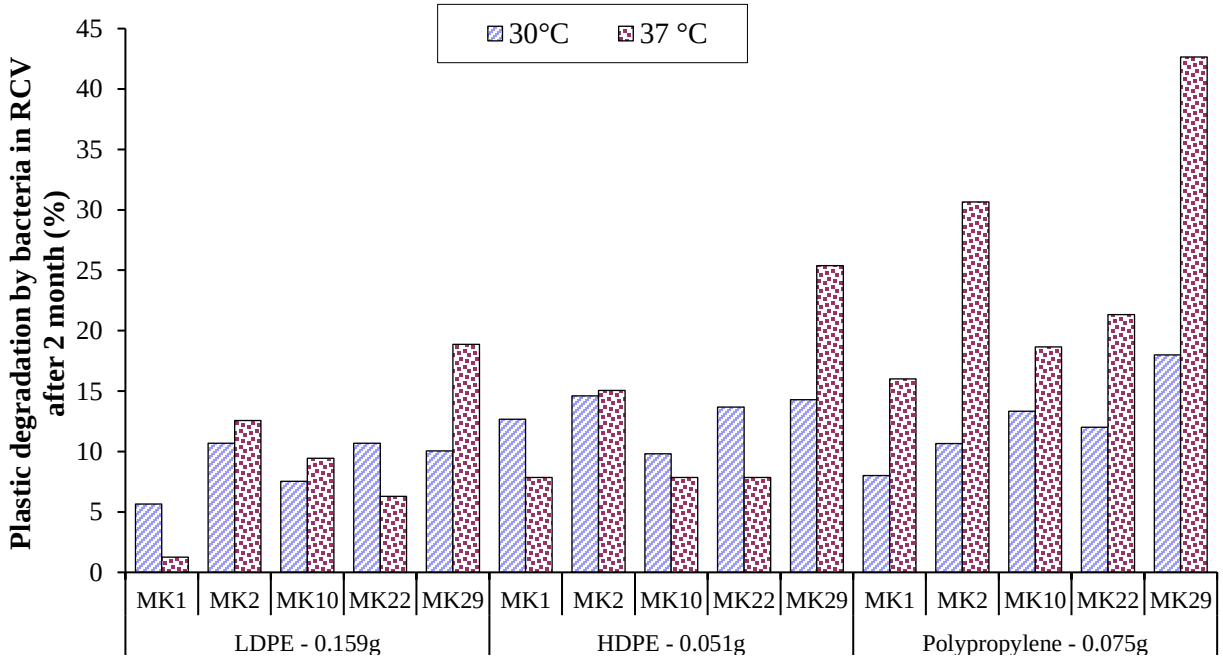

Fig. 2: Showing the % of degradation by biopolymer in RCV media at two different 30° C and 37° C. EPS by MK29 showing best results in degrading plastic material.

strains. It was observed that the MK29 in both RCV and TSA media showed a hole and alter the shape of plastic through the degradation process respectively, while other stains also showed promising results in degrading plastic material.

There are different factors affecting the degrading process such as temperature, pH, salinity, availability of oxygen, water, light and culture [16], and temperature is the significant factor. The thermophilic bacteria are considered potentially affected by degradation

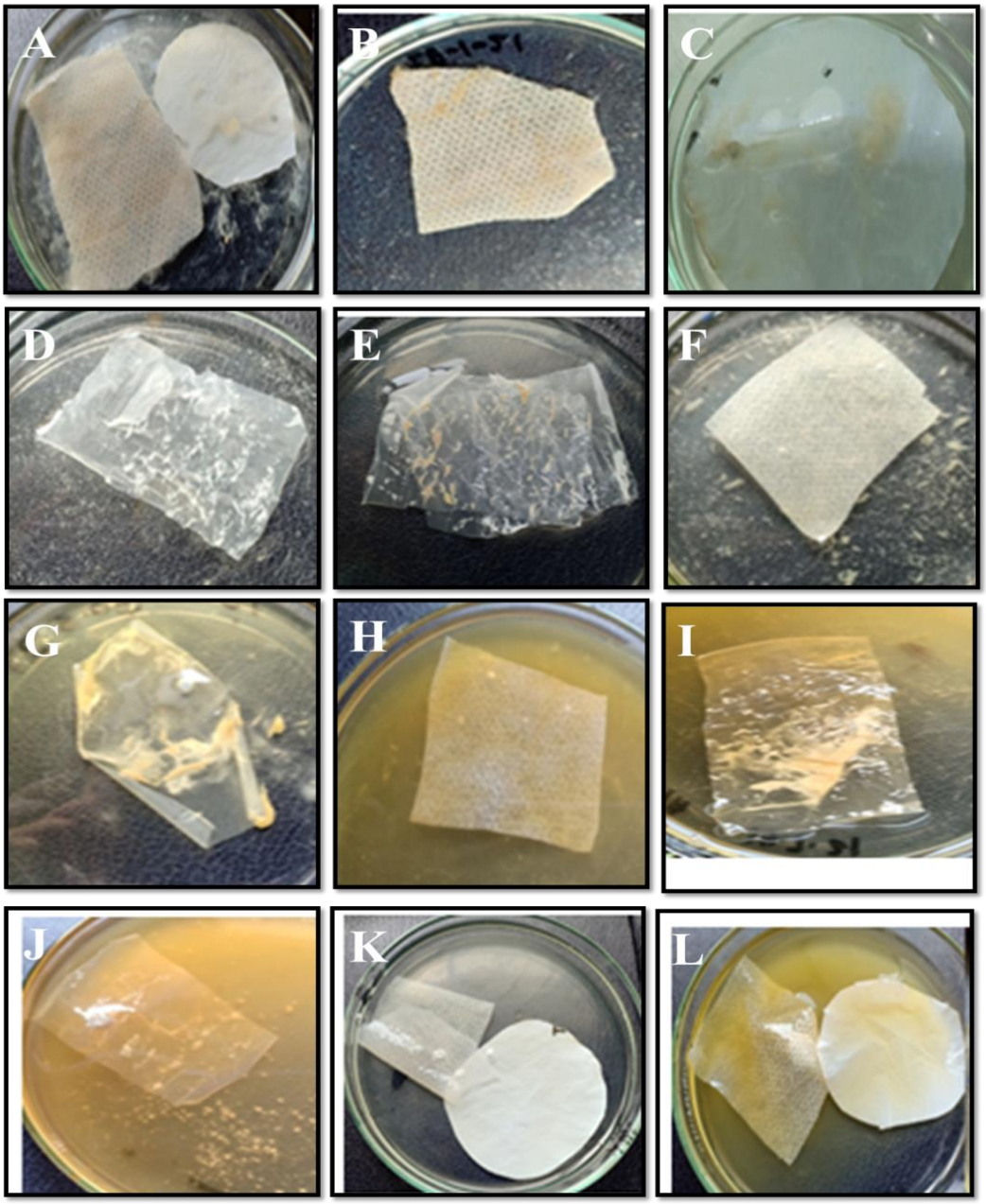

Fig. 3: The degradation of plastic through EPS and bacteria at 37° C **(A)** showing the degradation of polypropylene and HDPE by MK2 in RCV media through EPS **(B)** polypropylene degradation by MK22 **(C)** HDPE degradation by MK29 **(D)** LDPE degradation by MK29 **(E)** LDPE degradation by MK2 **(F)** polypropylene degradation by MK29 **(G)** LDPE degradation by MK29 in TSA through bacterial growth **(H)** polypropylene degradation by MK29 in TSA **(I)** LDPE degradation by MK2 in TSA **(J)** LDPE degradation by MK22 in TSA **(K)** Negative control for RCV **(L)** Negative control for TSA.

process [29]. The studies showed that *Bacillus sp.* is one of the most promising strains in the degradation of plastic [30, 31]. The present study was conducted to differentiate between the EPS and bacterial activity in degrading plastic at two different temperatures at 30° C and 37° C. It was observed that the MK2 *Bacillus subtilis* and MK29 *Enterobacter Cloacae* were the best strains in degrading at 37° C, at 30° low and negligible degradation was observed. MK29 at 30°C showed 8% while 32% at 37° C of degradation of LDPE was observed in TSA media. MK2 showed 5% at 30° C and 19% at 37° C degradation of LDPE in TSA, while degradation through biofilm MK2 and MK29 showed 12% and 18% degradation of LDPE at 37° C respectively. It is concluded that the MK29 showed considerable degrading of LDPE in both TSA and RCV media at 37° C. In degrading HDPE, MK2 showed 15% while MK29 showed 31% of degradation

in RCV at 37° C. In TSA media, MK2 and MK29 showed 18% and 30% degradation at 37° C respectively. In degrading polypropylene, MK2, MK22 and MK29 showed potential degradation in RCV media with 30%, 21% and 42% at 37° C, while in TSA MK29 showed 52% degradation at 37° C. The isolated strains showed potential at 37° C as compared to 30° C. According to [21], *Pseudomonas* at 30° C degrade 8.9% while at 37° C degrade up to 9% also showed that higher temperature plays an important role in the plastic degradation process.

According to [32, 33], biofilm is attached to the surface and starts degrading the plastic material by utilizing polymers as carbon sources. The bacterial and EPS efficiency on plastic degradation have potential degradation and bacterial degradation is higher as compared to biofilm such as MK29 degrade LDPE 18% by biofilm while 32% by bacteria directly after two months of incubation. It was reported [34], that 5% of LDPE degradation in 45 days through *Pseudomonas.* AKS_2 biofilm, whereas [35] showed that some species of *Pseudomonas* directly degrade LDPE up to 15% in 40 days. Both bacteria and biofilm showed degradation of plastic (**Fig. 3**).

Conclusion

The isolated strains from salt-stress environments have the potential for the degradation of plastic. MK2 and MK29 are considered potential strains in degrading all kinds of plastic bags at 37° C among all other isolates. This study revealed the potential of the microorganism and their activity in different media such as TSA and RCV in plastic degradation. The result also exposed the differences in three kinds of biodegradable plastic bags to develop a more environmentally friendly product.

Acknowledgments

The authors are thankful to Muhammadan Laboratory of Computational Biology, Pakistan to provide the platform and support for this project.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Council NR. Polymer Science and Engineering: The Shifting Research Frontiers. Washington, DC: The National Academies Press; 1994. 192 p.

[2] Oehlmann J, Schulte-Oehlmann U, Kloas W, Jagnytsch O, Lutz I, Kusk KO, Wollenberger L, Santos EM, Paull GC, Van Look KJ. A critical analysis of the biological impacts of plasticizers on wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences 2009;364(1526):2047-2062.

[3] Kupolati WK, Sadiku ER, Ibrahim ID, Adeboje AO, Kambole C, Ojo OOS, Eze AA, Paige-Green P, Ndambuki JM. 16 - The use of polyolefins in geotextiles and engineering applications. In: Ugbolue SCO, editor. Polyolefin Fibres (Second Edition): Woodhead Publishing; 2017. p. 497-516.

[4] Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K. A bacterium that degrades and assimilates poly (ethylene terephthalate). Science 2016;351(6278):1196-1199.

[5] Wilkes R-A, Aristilde L. Degradation and metabolism of synthetic plastics and associated products by Pseudomonas sp.: capabilities and challenges. Journal of applied microbiology 2017;123(3):582-593.

[6] Rujnić-Sokele M, Pilipović A. Challenges and opportunities of biodegradable plastics: A mini review. Waste Management & Research 2017;35(2):132-140.

[7] Schwab K. World economic forum. Global Competitiveness Report (2014-2015) Retrieved http://www3 weforum org/docs/WEF_GlobalCompetitivenessReport_2014-15 pdf 2015.

[8] Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. Science advances 2017;3(7):e1700782.

[9] Usha R, Sangeetha T, Palaniswamy M. Screening of polyethylene degrading microorganisms from garbage soil. Libyan Agric Res Cent J Int 2011;2(4):200-4.

[10] Swift G. Non-medical biodegradable polymers: environmentally degradable polymers. Handbook of biodegradable polymers Hardwood Academic, Amsterdam 1997:473-511.

[11] Sarker RK, Chakraborty P, Paul P, Chatterjee A, Tribedi P. Degradation of low-density poly ethylene (LDPE) by Enterobacter cloacae AKS7: a potential step towards sustainable environmental remediation. Archives of Microbiology 2020;202(8):2117-2125.

[12] Bhatia M, Girdhar A, Tiwari A, Nayarisseri A. Implications of a novel Pseudomonas species on low density polyethylene biodegradation: an in vitro to in silico approach. SpringerPlus 2014;3(1):1-10.

[13] Gu J-D. Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. International biodeterioration & biodegradation 2003;52(2):69-91.

[14] Sharma P. Role and significance of biofilm-forming microbes in phytoremediation -A review. Environmental Technology & Innovation 2022;25:102182.

[15] Eich A, Mildenberger T, Laforsch C, Weber M. Biofilm and diatom succession on polyethylene (PE) and biodegradable plastic bags in two marine habitats: early signs of degradation in the pelagic and benthic zone? PloS one 2015;10(9):e0137201.

[16] Kale SK, Deshmukh AG, Dudhare MS, Patil VB. Microbial degradation of plastic: a review. Journal of Biochemical Technology 2015;6(2):952-961.
[17] Atanasova N, Stoitsova S, Paunova-Krasteva T, Kambourova M. Plastic degradation by extremophilic bacteria. International Journal of Molecular Sciences 2021;22(11):5610.
[18] Vague M, Chan G, Roberts C, Swartz NA, Mellies JL. Pseudomonas isolates degrade and form biofilms on polyethylene terephthalate (PET) plastic. BioRxiv 2019:647321.
[19] Weaver PF, Wall JD, Gest H. Characterization of Rhodopseudomonas capsulata. Archives of Microbiology 1975;105(1):207-216.
[20] Ashraf M, Hasnain S, Berge O, Mahmood T. Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biology and Fertility of Soils 2004;40(3):157-162.
[21] Badahit G, Kumar J, Singh A. Screening of Plastic Degrading Pseudomonas spp. From Soil. Int J Sci Eng Res 2018;9:484-489.
[22] El-Shafei HA, Abd El-Nasser NH, Kansoh AL, Ali AM. Biodegradation of disposable polyethylene by fungi and Streptomyces species. Polymer degradation and stability 1998;62(2):361-365.
[23] Bhatia M, Girdhar A, Tiwari A, Nayarisseri A. Implications of a novel Pseudomonas species on low density polyethylene biodegradation: an in vitro to in silico approach. SpringerPlus 2014;3(1):497.
[24] Forbes BA, Sahm DF, Weissfeld AS. Diagnostic microbiology: Mosby St Louis; 2007.
[25] Ruiz-Orejón LF, Sardá R, Ramis-Pujol J. Floating plastic debris in the Central and Western Mediterranean Sea. Marine Environmental Research 2016;120:136-144.
[26] Paul S, Sen B, Das S, Abbas SJ, Pradhan SN, Sen K, Ali SI. Incarnation of bioplastics: recuperation of plastic pollution. International Journal of Environmental Analytical Chemistry 2021:1-24.
[27] Kolvenbach BA, Helbling DE, Kohler H-PE, Corvini PF. Emerging chemicals and the evolution of biodegradation capacities and pathways in bacteria. Current opinion in biotechnology 2014;27:8-14.
[28] Lu Z, Guo W, Liu C. Isolation, identification, and characterization of novel Bacillus subtilis. Journal of Veterinary Medical Science 2018:16-0572.
[29] Ahmed T, Shahid M, Azeem F, Rasul I, Shah AA, Noman M, Hameed A, Manzoor N, Manzoor I, Muhammad S. Biodegradation of plastics: current scenario and future prospects for environmental safety. Environmental Science and Pollution Research 2018;25(8):7287-7298.
[30] Sarker RK, Chakraborty P, Paul P, Chatterjee A, Tribedi P. Degradation of low-density poly ethylene (LDPE) by Enterobacter cloacae AKS7: a potential step towards sustainable environmental remediation. Archives of Microbiology 2020;202:2117-2125.
[31] Dang TCH, Nguyen DT, Thai H, Nguyen TC, Tran TTH, Le VH, Tran XB, Pham TPT, Nguyen TG, Nguyen QT. Plastic degradation by thermophilic Bacillus sp. BCBT21 isolated from composting agricultural residual in Vietnam. Advances in Natural Sciences: Nanoscience and Nanotechnology 2018;9(1):015014.
[32] Vasile C. Degradation and decomposition. PLASTICS ENGINEERING-NEW YORK- 2000;59:413-476.
[33] Vasile C. Handbook of polyolefins: CRC press; 2000.
[34] Tribedi P, Gupta AD, Sil AK. Adaptation of Pseudomonas sp. AKS2 in biofilm on low-density polyethylene surface: an effective strategy for efficient survival and polymer degradation. Bioresources and Bioprocessing 2015;2(1):1-10.
[35] Kyaw BM, Champakalakshmi R, Sakharkar MK, Lim CS, Sakharkar KR. Biodegradation of Low Density Polythene (LDPE) by Pseudomonas Species. Indian J Microbiol 2012;52(3):411-9.