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Research Article

Isolation, characterization and optimization of bacterial isolate SARR1 for biodegradation of pretreated low density polyethylene

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Abstract
Accumulation of low-density polyethylene (LDPE) has caused a threat to the environment because of its stable and inert nature as it cannot be degraded easily by microorganisms. Its lightweight, low cost, strength, durability, and its various other applications, have led to the wide usage of the polymer, which is exerting a negative effect on both marine and land biota. The development of an eco-friendly or a promising strategy is needed to reduce LDPE waste from both land and water. In the present study, observations have been made to isolate highly efficient LDPE degrading bacteria. The response surface methodology (RSM) was used to predict the best optimization of media for the degradation of LDPE by isolate SARR1. The isolate SARR1 was selected through primary screening by weight loss method and secondary screening using CO_2 evolution test, TTC and MATH Test. The isolate SARR1 showed 6.30 ± 0.25 g/L CO_2 evolution. The microbial adhesion hydrophobicity (MATH) was observed during log phase (100 to 56.89 ± 0.97 %) and stationary phase (100 to 82.92 ± 1.24 %). An isolate SARR1 converted the TTC into red coloured insoluble triphenyl formazan (TPF) after incubation of 7 days. The isolated bacteria SARR1 showed 38.3 ± 1.27 % biodegradation efficiency in the pretreated LDPE strips at 37 °C and pH 7.0 under optimized conditions within 30 days of incubation. This bioremediation and biodegradation approach is eco-friendly and safe for the environment. The results of treatment with isolate SARR1 had a potential hope to degrade LDPE at higher rate than natural degradation.

Keywords: Biodegradation, Bioremediation, Durability, Eco-friendly, LDPE, Bacteria

INTRODUCTION

Plastics are non-biodegradable and a potential xenobiotic compound that can cause impact globally (Ghosh *et al.,* 2019). The wide use of low-density polyethylene (LDPE) is increasing day by day because of its applications in pharmaceuticals industries, agriculture, households, and food packaging industries and due to its inert and durable nature (Erni-Cassola *et al.,* 2019). Out of total waste generated, LDPE contributes about 60-80 % of waste to the marine environment (Iniguez *et al.,* 2019). The remaining of the generated enters into the landfills and remains undegraded for a longer duration (Hahladakis and Iacovidou, 2019). The most significant ecological threat is its slow degradation rate or non-biodegradability in nature (Emadian *et al.,* 2017) as it is resistant to microbial degradation (Tokiwa *et al.,* 2009). Oxidative degradation of polyethylene releases harmful chemicals that have negative effects on the environment (Sojak *et al*., 2006). CPCB reports of the year 2017-18 estimates that India on an average generates 9.4 million tonnes of plastic wastes per annum which is approximately 26000 tonnes of plastic wastes per day and out of these 9.4 million tonnes of waste, only 5.6 million tonnes of plastic wastes are recycled per annum and the remaining 3.8 million tones left unchecked and littered. Degradation of plastic is difficult because of its stable nature and the basic sources responsible for the increase in its pollution are increasing population, changes in lifestyle, improper recycling, improper dis-

posal, and waste management systems (Balestri *et al.,* 2019). Improper handling of LDPE wastes is a threat to marine biota and the birds consuming it, intake of it blocks the intestines of fishes and decrease their gastric secretions, reduced food intake, affects the level of the hormones and increases reproductive failure chances and ultimately leads to their death (Azzarello and Van Vleet,1987). In recent years, a great emphasis is being led on screening potential microorganisms that can degrade LDPE and would help in the proper management of the littered waste (Skariyachan *et al.,* 2016, Idowu *et al.,* 2019). Studies have also shown LDPE as a possible substrate for heterotrophic microorganisms, which can breakdown these polymers under optimized conditions (Pathak and Kumar, 2017). These microbes release certain intracellular and extracellular enzymes which can break the bonds in LDPE. Both bacteria and fungi play a vital role in polyethylene degradation by natural metabolic mechanisms. The degradation of polyethylene begins with the attachment of microbes to the LDPE surface (Jamal *et al.,* 2018). In this study, an efficient bacterial strain SARR1 was isolated from the LDPE dumping site of Haryana, India, and its LDPE degradation potential was studied. The scope of the study is to isolate and identify potential microorganisms that can easily degrade LDPE, where biodegradation serves as a promising tool for elimination of the plastic and polyethylene wastes from the environment.

MATERIALS AND METHODS

LDPE and bacterial cultures

LDPE strips (40µ and size of 3×3 cm) were purchased from the local market of Rohtak, Haryana, India. The strips were sterilized using 75 % ethanol and then air-dried. The bacteria were isolated from the soil collected from the municipal wastes dumping sites of Ismailabad, Pehowa (Haryana) India. The sample was collected in a sterilized zip lock bags.

Media used

The mineral salt (MS) media (yeast extract 0.002 %, KCl 0.05 %, K_2HPO_4 0.12 %, $MgSO_4.7H_2O$ 0.05 %, $CaCl_2.2H_2O$ 0.01 %, $NaNO_3$ 0.3 %, KH_2PO_4 0.014 %, and $Fe(SO_4)_3$ 0.001 % amended with LDPE strips) was used for the cultivation of bacteria. To solidify the media, agar (2 %) was added and sterilized at 121 °C at 15psi for 15 min (Skariyachan *et al.,* 2016, Das and Kumar, 2015).

Isolation of potential bacterial isolates

The isolation of bacteria was done using the enrichment culture technique (Yang *et al.,* 2004). 10 g of soil sample was suspended in 90 mL of MS broth media amended with LDPE strips and incubated for 30 days at 37 °C under static conditions. After incubation 100 µL inoculums were spread on agar plates and further purified by streak plate method (Skariyachan *et al.,* 2016, Das and Kumar, 2015). The isolated culture would be submitted at National Centre for Cell Science (NCCS), Pune and is under process.

Screening of isolates

The primary and secondary screening methods were followed for the selection of promising bacterial isolates.

Primary screening

The primary screening was done by using the weight loss method of LDPE (Bardají *et al.,* 2019). After the bacterial treatment, the strips were thoroughly washed with 2 % SDS (v/v) followed by distilled water, and dried overnight at room temperature. The degradation capability was calculated by the following formula (Sarker *et al.,* 2020).

$$\%\ Degradation\ of\ LDPE = \frac{I_w - F_w}{I_w} \times 100$$

.....Eq.1

Where,

I_w is the initial weight of the LDPE strip before bacterial treatment and F_w is the final weight of the LDPE strip after 30 days of bacterial treatment.

Secondary screening

After the primary screening, secondary screening was done to confirm the degradation of LDPE by the isolated bacteria using different types of polyethylene biodegradability tests, i.e. Sturm test (CO_2 evolution) (Esmaeili *et al.,* 2013), triphenyl tetrazolium chloride (TTC) test (Wolinska *et al.,* 2016, Kumari *et al.,* 2019), and microbial adhesion to hydrocarbons test (MATH) (Vague *et al.,* 2019).

Sturm test

This test was used to monitor the CO_2 evolution in the biometric flasks designed for aerobic biodegradation of LDPE. The amount of CO_2 evolved was determined by employing the titration method (Esmaeili *et al.,* 2013, Mohee and Unmar, 2007).

Triphenyl tetrazolium chloride (TTC) test

The bacterial isolate SARR1 showed highest CO_2 evolution in the Sturm test was selected for the TTC test. The 2, 3, 5-triphenyl tetrazolium chloride was used as a redox probe which indicates the capability and metabolic activity of the surface-adhered bacteria. 40 µL of 1.0 % TTC solution was added in autoclaved 20 mL MS media inoculated with isolate SARR1 and incubated for 7 days in dark conditions (Kumari *et al*., 2019).

Microbial adhesion to hydrocarbons test (MATH)

The microbial adhesion capability on LDPE strips sur-

face was analyzed by using this method (Gilan *et al.,* 2004, Sarkar *et al.,* 2020). The LDPE strip was inoculated with the isolate SARR1 in mineral salt broth media and incubated at 37 °C for 30 days under static conditions. 1.5 mL bacterial suspension was pooled at the different time intervals of growth phases. Different volumes of hexadecane (0.5 mL – 4 mL) were added to each pooled sample and vortexed for 2 min. and incubated at room temperature for 2 min. for the separation of the organic phase and aqueous phase. The optical density of the aqueous phase was measured using a UV visible spectrophotometer at 600 nm.

Morphological and biochemical characterization of LDPE degrading bacteria

The morphological, microscopic, and biochemical characterization of isolate SARR1 was done using the standard protocols of Bergey's manual (Whitman *et al.,* 1984).

Optimization of media with RSM

With the help of the response surface methodology, the growth conditions and % degradation of LDPE were optimized. Results were analyzed by using the software, Design-Expert version 13.0 (Stat-Ease Inc. Minneapolis, USA). The parameters optimized were pH, temperature, carbon source and nitrogen source (Table 1). The CCD (Central composite design) was employed and experimental design was obtained. The quadratic model was used to analyze the data. This particular model was best suited for the data with several constants in their model and also interprets interaction of the growth parameters with the variables.

LDPE pre-treatment using physical and chemical methods

Ultraviolet irradiation treatment to LDPE

The LDPE films were irradiated for 30 days under UV light in an ultraviolet chamber. The LDPE films were cut into strips (3×3 cm) for biodegradation study (Esmaeili *et al.,* 2013).

Heat treatment to LDPE

UV treated LDPE strips were placed at temperature 70 °C in a hot-air oven for a maximum of 30 days (Mourad, 2010)

Chemical treatment and biological treatment to LDPE strips

After both UV and heat treatments to the LDPE strips, these were further treated with concentrated nitric acid (99.9 %) for 10 days. The strips were removed, rinsed with sterile distilled water and then rinsed with 70 % ethanol for 30 min. The strips were oven dried overnight at 45 °C to 50 °C and measured their weight (Hasan *et al.,* 2007). After physical and chemical treatment to LDPE strips, biological treatment with isolate SARR1 was performed. The isolate SARR1 was inoculated in sterile MS broth media amended with pretreated LDPE strip and incubated for 30 days at 37 °C. After incubation, the biodegradation percentage was calculated using the weight-loss method.

Statistical analysis

The data was represented by a combination of average mean and standard deviation (mean ± SD). For the study of low-density polyethylene degradation, all the experiments were performed in triplicates. ANOVA (Analysis of Variance) was also carried out to determine the fit mechanism of the quadratic model.

RESULTS AND DISCUSSION

Isolation and primary screening of LDPE degrading bacteria

Five bacterial cultures were isolated from the soil samples of the waste disposal site. After the primary screening, based on the weight loss method of LDPE, the isolate SARR1 was observed as the most efficient strain for LDPE degradation (Fig. 1A). Bacterial cultures *Bacillus amyloliquefaciens* strain BSM-1 and strain BSM-2 have shown LDPE weight loss of 11.0 % and 16.0 %, respectively, within 60 days of incubation (Das and Kumar, 2015, Harshvardhan and Jha, 2013, Bhatia *et al.,* 2014). *Pseudomonas aeruginosa* strain SKN1 and strain SKN2 also showed LDPE weight loss of 10.32 % within 60 days of incubation (Nourollahi *et al*., 2019). In contrast to these studies, the bacterial isolate SARR1 was the most efficient strain that showed the biodegradation activity of 28.12 ± 1.09 % within 30 days of incubation only.

Secondary screening

Sturm Test (CO_2 evolution test)

The isolate SARR1 showed 6.30 ± 0.25 gL^{-1} CO_2 evolutions (Fig. 1B). The isolate SARR1 showed maximum CO_2 evolution and biodegradation activity. The fungal isolates *A. clavatus and Fusarium sp.* showed CO_2 evolution of 2.32 gL^{-1} and 1.85 gL^{-1}, respectively (Shah *et al.,* 2008, Gajendiran *et al.,* 2016).

Triphenyl tetrazolium chloride (TTC) test

The TTC was reduced to red-colored insoluble triphenylformazan by the isolate SARR1 after incubation of 7 days (Fig. 1C). The viability and metabolic activities of the surface attached bacteria were observed with the production of triphenylformazan, which facilitates the direct monitoring of active respiration of SARR1. In the same way, *Kocuria palustris* M16, *B. pumilus* M27, and *B. subtilis* H1584 also showed positive viability test with TTC (Harshvardhan and Jha, 2013). The bacterium *Bacillus sp.* strain AIIW2 reduces

TTC proved the LDPE degradation (Kumari *et al.,* 2019).

Microbial adhesion to hydrocarbons (MATH) test

MATH test resulted that the bacterial isolate SARR1 was hydrophobic at different concentrations of hexadecane (0 to 4 mL). The % O.D. was decreased from 100 to 56.89 ± 0.97 % during the log phase as the concentration of hexadecane increased (Fig. 1D). Under stationary phase, turbidity was decreased from 100 to 82.92 ± 1.24 %. *Rhodococcus ruber* strain C208, C332, B334, and E478 showed maximum hydrophobicity at a very low concentration of hexadecane, and *Rhodococcus ruber* strain C208 showed 20 % reduction in the turbidity (Gilan *et al.,* 2004). A significant increase (32.0 %) in the hydrophobicity of *K. palustris* and *B. subtilis* has earlier been reported (Harshvardhan and Jha, 2013, Mukherjee *et al.,* 2016.

Morphological, physiological, and biochemical

Table 1. Parameters for RSM optimization for the % Degradation.

Factor	Name	Units	Type	Sub Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	pH		Numeric	Continuous	3.00	11.00	-1 ↔ 3.00	+1 ↔ 11.00	7.00	3.14
B	Temperature	°C	Numeric	Continuous	-5.00	55.00	-1 ↔ 15.00	+1 ↔ 55.00	33.52	17.48
C	Carbon	g	Numeric	Continuous	0.0000	4.00	-1 ↔ 1.00	+1 ↔ 3.00	2.00	0.9608
D	Nitrogen	g	Numeric	Continuous	0.5000	2.50	-1 ↔ 1.00	+1 ↔ 2.00	1.50	0.4804

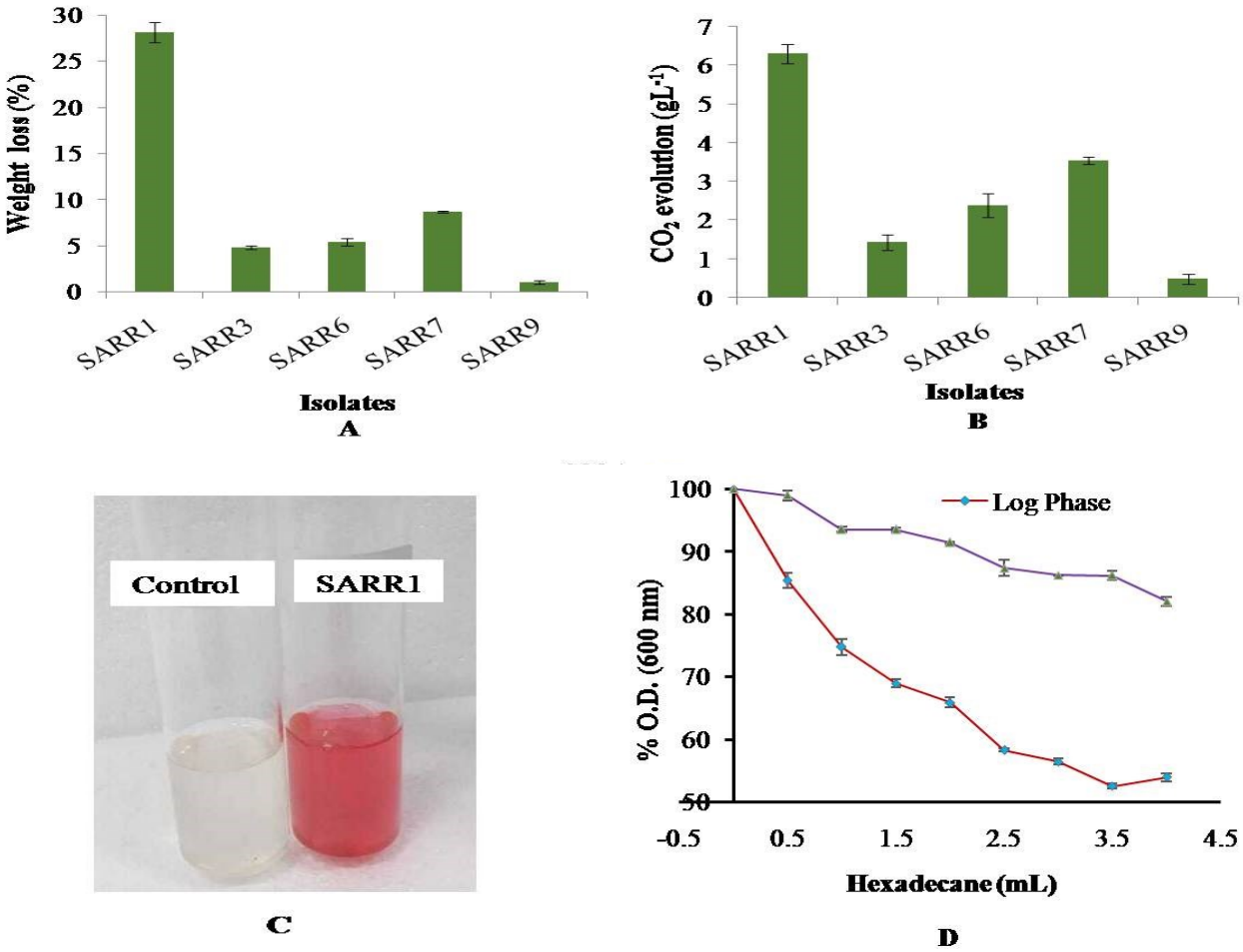

Fig.1. (*A) Primary screening of LDPE degrading bacterial isolates on the basis of weight loss method. Secondary screening of LDPE degrading bacterial isolates on the basis of (B) Sturm test (C) TTC test (D) MATH test.*

Table 2. Morphological, microscopic, physiological, and biochemical characterization of the isolate SARR1.

A	**Morphological and microscopic characterizations**	
1	Configuration	Round
2	Margin	Entire
3	Elevation	Convex
4	Surface	Smooth
5	Color	Cream
6	Cell shape	Rods
7	Gram's reaction	+
8	Endospore staining	+
9	Capsule staining	-
B	**Physiological characterization**	
I	**Growth at temperatures**	
1	4 °C	-
2	28 °C	(+)
3	37 °C	+
4	45 °C	(+)
5	50 °C	(+)
II	**Growth at pH**	
1	pH 3	-
2	pH 5	(+)
3	pH 7	+
4	pH 9	(+)
5	pH 11	-
C	**Biochemical characterization**	
1	Nitrate reduction test	-
2	Oxidase test	+
3	Catalase test	-
4	Citrate utilization	+
5	Urea hydrolysis	-
6	Indole test	-
7	Methyl red	-
8	Voges Proskauer's	-
9	Indole production	-
10	Urea hydrolysis	-

characterization of isolate SARR1

The colonies of isolate SARR1 were spherical, convex, and cream in colour. The isolate SARR1 was gram-positive, rod-shaped, and endospore-forming bacteria. The maximum growth of the isolate SARR1 was at 37 °C at pH 7. Biochemical characterization resulted in a positive oxidase and citrate utilization test while other tests were negative (Table 2). The bacteria can be identified using biochemical tests because each bacterium has slightly different metabolic properties (Eslami *et al.,* 2019).

Pre-treatment of LDPE using physical, chemical, and biological methods

The sequential pre-treatments of LDPE strips using physical and chemical methods were also monitored before biological LDPE degradation. After heat treatment, 0.35 ± 0.07 % weight loss of LDPE strips was recorded followed by 22.85 ± 2.28 % weight loss with isolate SARR1 under optimized conditions. Both the heat and UV treatment showed a 0.75 ± 0.21 % weight loss followed by 30.67 ± 1.15 % of LDPE weight loss with isolated SARR1. After heat and UV treatment, chemical treatment to the pretreated LDPE strips showed a weight loss of 1.09 ± 0.16 % and the isolate SARR1 further degraded the LDPE strip to 38.31 ± 1.27 % (Fig. 2). Similarly, *B. amyloliquefaciens* showed 0.5 – 1.3 % degradation on the pre-heat-treated LDPE and LLDPE strips **(**Novotný *et al*., 2018**).** Similarly, *B. borstelensis* showed 25 % more degradation after pre-treated ultraviolet irradiated LDPE (Hadad *et al.,* 2005).

Table 3. Experimental Design by RSM.

File Version	**13.0.3.0**		
Study Type	Response Surface	Subtype	Randomized
Design Type	Central Composite	Runs	27.00
Design model	Quadratic	Blocks	No blocks
Build Time (ms)	1.0000		

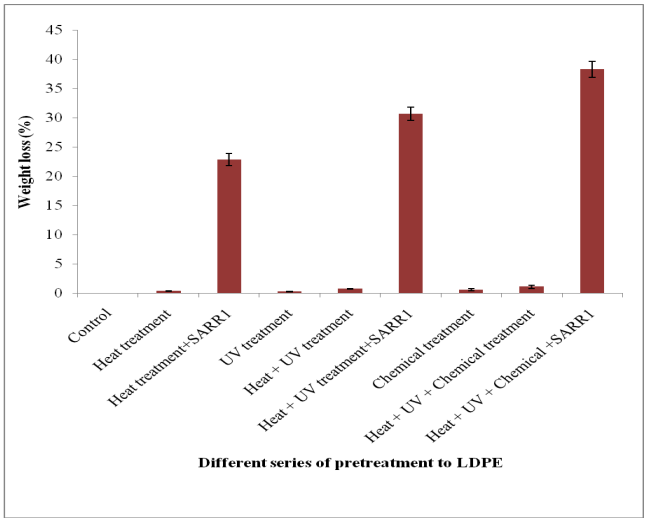

Fig. 2. *Effect of different pretreatment methods (UV treatment, heat treatment and chemical treatment) on the degradation of the LDPE and enhanced biodegradation of pretreated LDPE by an isolate SARR1.*

Response model of prediction

The 3D response surface plot was generated by using OVAT and the relationship between the variables and response was optimized through Three-dimensional (3D) response surface or contour plot analysis to check the effect of pH, temperature, carbon source and temperature on the degradation of LDPE by SARR1 (Table 1, 3; Fig. 3). The values of ANOVA (Table 4) were suggested that the model developed and represented be-

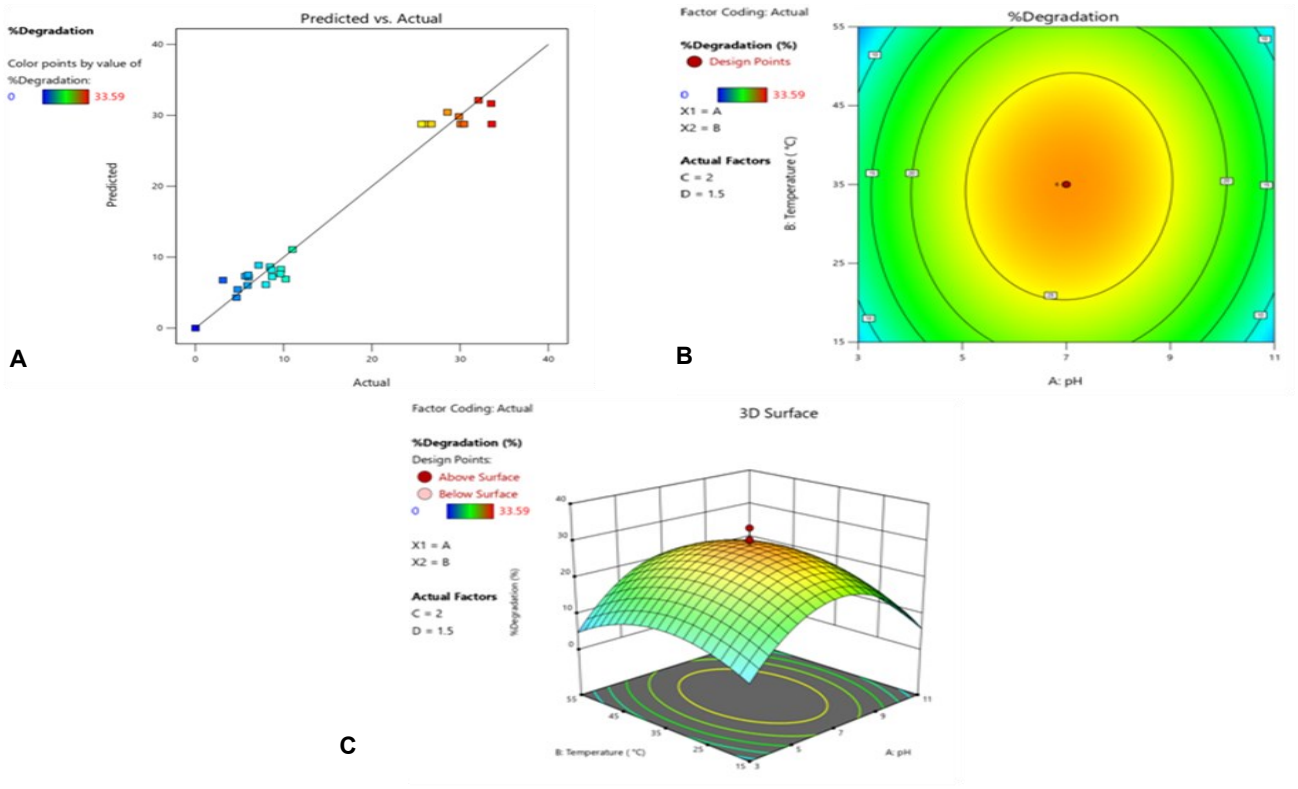

Fig. 3. *The prediction versus actual plot (A), 3D plot (B) and contour plot (C) showing % Degradation of LDPE.*

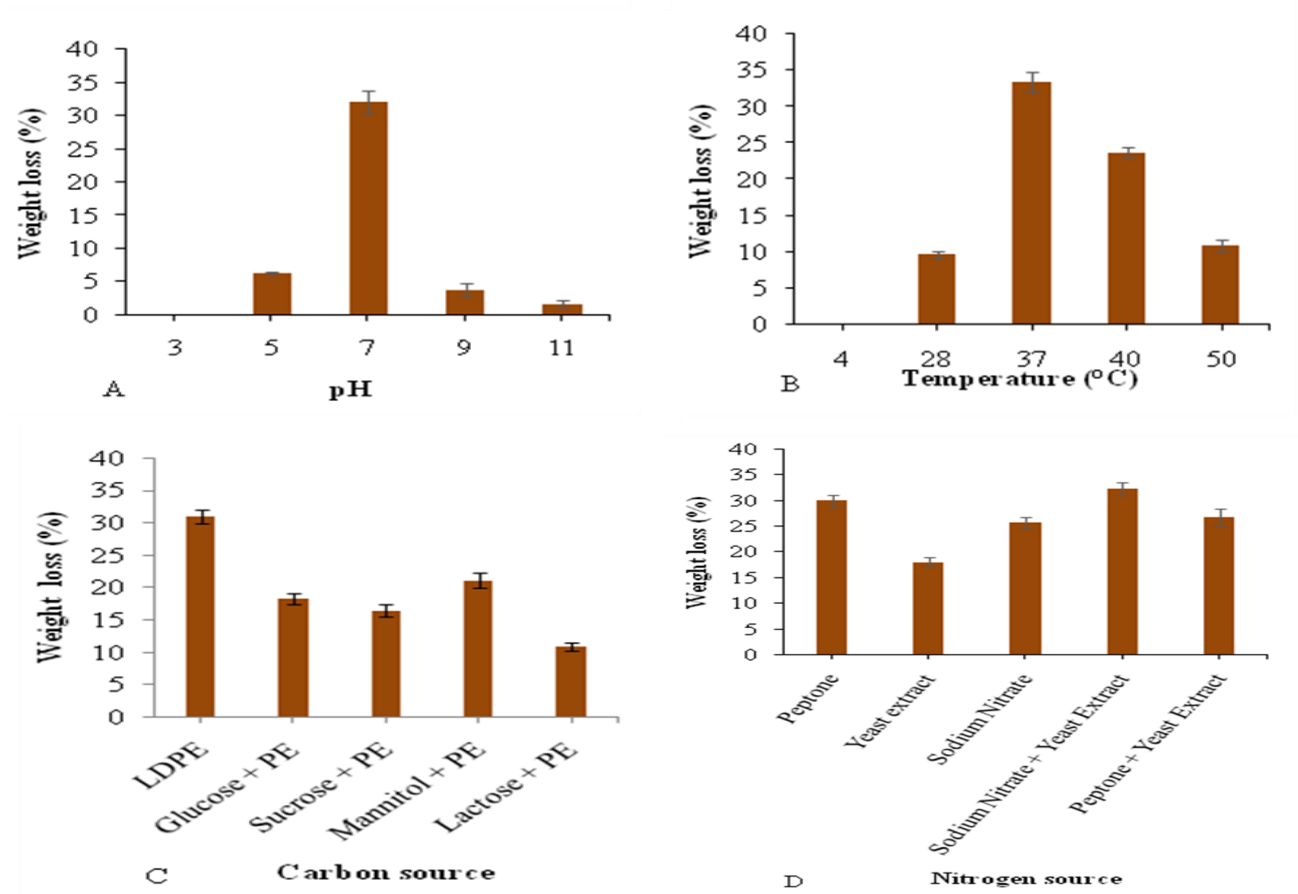

Fig. 4. *Optimization of different growth parameters for the biodegradation activity of isolate SARR1 (A) pH (B) Temperature (C) Carbon sources and (D) Nitrogen sources.*

Table 4. Analysis of variance (ANOVA) for quadric model by CCD of RSM for % Degradation of LDPE optimizing growth by SARR1.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3361.26	14	240.09	28.38	< 0.0001	significant
A-pH	2.65	1	2.65	0.3130	0.5861	
B-Temperature	0.5006	1	0.5006	0.0592	0.8119	
C-Carbon	7.92	1	7.92	0.9365	0.3523	
D-Nitrogen	2.23	1	2.23	0.2631	0.6173	
AB	13.38	1	13.38	1.58	0.2325	
AC	0.6440	1	0.6440	0.0761	0.7873	
AD	1.14	1	1.14	0.1347	0.7200	
BC	5.70	1	5.70	0.6737	0.4278	
BD	5.53	1	5.53	0.6541	0.4344	
CD	0.0564	1	0.0564	0.0067	0.9363	
A^2	1090.93	1	1090.93	128.93	< 0.0001	
B^2	598.45	1	598.45	70.73	< 0.0001	
C^2	7.37	1	7.37	0.8711	0.3691	
D^2	7.81	1	7.81	0.9229	0.3557	
Residual	101.53	12	8.46			
Lack of Fit	52.67	7	7.52	0.7698	0.6374	not significant
Pure Error	48.87	5	9.77			
Cor Total	3462.79	26				

tween the OVAT and response with good accuracy and reliability. The F-value of Model 52.67 indicates that the model is significant. P-values obtained were less than 0.0500 implies model terms (A^2, B^2) are significant. The non-significant lack of fit is suitable to fit the model. The estimation of coefficient represents the expected change in response per unit change in factor value when all remaining factors are held constant. As a rough rule indication, VIFs less than 10 are significantly tolerable (Table 5). The recent study evaluated the PLA food packaging degradation by *Bacillus sp.* SNRUSA4 by using RSM approach and obtained the Box-Behnken design for the optimization studies (Sawiphak and Wongjiratthiti, 2021).

Optimization studies of isolate SARR1

Effect of different pH on degradation efficiency of isolate SARR1

The effect of different pH values (3.0, 5.0, 7.0, 9.0, and 11.0) on the degradation efficiency of the isolate SARR1 was also studied. The pH 7.0 was found the optimum pH for maximum degradation of LDPE and degraded 32.17 ± 1.69 % of LDPE (Fig. 4A). Similarly, *B. amyloliquefaciens* (Das and Kumar, 2015) and *Thiobacillus sp.* and *Clostridium sp.* (Islami *et al.,* 2019) showed maximum degradation of LDPE at pH 7.0.

Effect of different temperatures on degradation efficiency of isolate

The maximum LDPE degradation (33.52 ± 1.39 %) was observed at 37 ºC by the bacterial isolate SARR1 within 30 days of incubation (Fig. 4B). The bacterium *B. aryabhattai* also showed 3.85 ± 0.50 % degradation at temperature 37 °C within 30 days of incubation (Hou *et al.,* 2019). Similarly, 9 % of degradation has been shown by *Enterobacter cloacae* AKS7 after 45 days of incubation at 30 °C (Sarker *et al.,* 2020).

Effect of different carbon sources on degradation efficiency of isolate

The maximum LDPE degradation (30.90 ± 1.04 %) was achieved by the isolate SARR1 was with polyethylene as a sole source of carbon while media amended with glucose + LDPE showed 18.31 ± 0.90 % and lactose + LDPE showed 10.92 ± 0.64 % degradation activity under optimized pH 7.0 and temperature (37 °C) conditions (Fig. 4C). Similarly, a combination of glucose + LDPE in the media showed degradation of 7.13 ± 0.05

Table 5. Response surface quadric model using ANOVA and its coeffiecient values.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	28.76	1	1.19	26.18	31.35	
A-pH	0.4069	1	0.7272	-1.18	1.99	1.0000
B-Temperature	-0.1769	1	0.7272	-1.76	1.41	1.24
C-Carbon	-0.5746	1	0.5938	-1.87	0.7191	1.0000
D-Nitrogen	0.3046	1	0.5938	-0.9891	1.60	1.0000
AB	0.9144	1	0.7272	-0.6701	2.50	1.0000
AC	-0.2006	1	0.7272	-1.79	1.38	1.0000
AD	0.2669	1	0.7272	-1.32	1.85	1.0000
BC	-0.5969	1	0.7272	-2.18	0.9876	1.0000
BD	0.5881	1	0.7272	-0.9963	2.17	1.0000
CD	-0.0594	1	0.7272	-1.64	1.53	1.0000
A^2	-15.26	1	1.34	-18.19	-12.33	1.39
B^2	-7.28	1	0.8655	-9.17	-5.39	1.52
C^2	0.5542	1	0.5938	-0.7395	1.85	1.11
D^2	0.5704	1	0.5938	-0.7233	1.86	1.11

% by *P. fluorescens* (Mukherjee *et al.,* 2018). The isolates *Staphylococcus* sp. MMP10 and *Bacillus* sp. MGP1 also showed degradation activity of 2.08 % and 2.92 %, respectively, with LDPE as a sole carbon source (Kunlere *et al.,* 2019).

Effect of different nitrogen sources on biodegradation efficiency of isolate

Under optimized pH, temperature, and carbon sources, the isolate SARR1 showed maximum LDPE biodegradation of 32.32 ± 1.16 % in the presence of sodium nitrate and yeast extract. It was decreased (30.03 ± 1.13 %) in the presence of peptone compared to sodium nitrate + yeast extract (Fig. 4D). Similarly, *P. aeruginosa* strain SKN1 (Nourollahi *et al.,* 2019) and *L. fusiformis* (Mukherjee *et al.,* 2016) showed degradation of 10.32 ± 0.129 % and 7.006 ± 0.05 %, respectively in a medium amended with peptone. Whereas 6.975 ± 0.05 % LDPE degradation was achieved NH_4NO_3 as a nitrogen source in the media (Mukherjee *et al.,* 2016). However, *B. borstelensis* degraded LDPE 11.0 ± 0.1 % in the presence of KNO_3 as a nitrogen source (Hadad *et al.,* 2005).

Conclusion

In the present scenario of globalization, more attention is needed for safe disposal of LDPE before its commercialization. In this study, an effective and eco-friendly degradation of LDPE using isolate SARR1 for 30 days developed substantial microbial degradation of LDPE. Moreover, the growth of SARR1 in the presence and absence of LDPE not only represented the adherence towards the surface but also showed the capability to utilize the LDPE strips within 30 days of incubation. The isolate represents cell-surface hydrophobicity that causes enhancement in the degradation of LDPE. The RSM analysis was used to optimize the media and degradation efficiency of LDPE. The prediction versus actual plot indicated the degradation rate of SARR1 was accurate. Based on the above studies, the isolate SARR1was selected for further research work. Furthermore, the isolate SARR1 can be used to study the cellular mechanisms for the utilization of complex carbon sources (LDPE). To study these mechanisms, genome sequencing with *In Silico* approach can be a useful tool for identifying the enzymes and their coding genes. To make effective commercialization of the isolate SARR1, the LDPE degrading microbial enzymes needs to be extracted and further characterized.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Azzarello, M. & Van Vleet, E. (1987). Marine birds and plastic pollution. *Mar. Ecol. Prog. Ser.,* 37, 295-303. https://doi.org/10.3354/meps037295.
2. Balestri, E., Menicagli, V., Ligorini, V., Fulignati, S., Raspolli Galletti, A.M. & Lardicci, C. (2019). Phytotoxicity assessment of conventional and biodegradable plastic bags using seed germination test. *Ecol. Indic.,* 102, 569-580. https://doi.org/10.1016/j.ecolind.2019.03.005.
3. Bardají, D. K. R., Furlan, J. P. R. & Stehling, E. G. (2019). Isolation of a polyethylene degrading *Paenibacillus* sp. from a landfill in Brazil. *Arch. Microbiol.,* 201, 699-704. https://doi.org/10.1007/s00203-019-01637-9.
4. Bhatia, M., Girdhar, A., Tiwari, A. & Nayarisseri, A. (2014). Nayarisseri, Implications of a novel *Pseudomonas* species on low density polyethylene biodegradation: an in vitro to in silico approach. *Springerplus.,* 3. https://doi.org/10.1186/2193-1801-3-497.
5. Das, M. P. & Kumar, S. (2015). An approach to low-density polyethylene biodegradation by *Bacillus amyloliquefaciens*. *3 Biotech.,* 5, 81-86. https://doi.org/10.1007/s13205-014-0205-1.
6. Emadian, S.M., Onay, T.T. & Demirel, B. (2017) . Biodegradation of bioplastics in natural environments. *Waste Manag.,* 59 ,526-536. https://doi.org/10.1016/j.wasman.2016.10.006.
7. Erni-Cassola, G., Wright, R. J., Gibson, M. I. & Christie-Oleza, J. A. (2019). Distribution of plastic polymer types in the marine environment; A meta-analysis. *J. Hazard. Mater.,* 369, 691-698. https://doi.org/10.1016/j.jhazmat.2019.02.067.
8. Eslami, H., Shariatifar, A., Rafiee, E., Shiranian, M., Salehi, F., Hosseini, S.S., Eslami, G., Ghanbari, R. & Ebrahimi, A.A. (2019). Decolorization and biodegradation of reactive Red 198 Azo dye by a new *Enterococcus faecalis–Klebsiella variicola* bacterial consortium isolated from textile wastewater sludge. *World J. Microbiol. Biotechnol.,* 35.
9. Esmaeili, A., Pourbabaee, A. A., Alikhani, H. A., Shabani, F. & Esmaeili, E. (2013). Biodegradation of Low-Density Polyethylene (LDPE) by mixed culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* in Soil. *PLoS One.,* 8. https://doi.org/10.1371/journal.pone.0071720.
10. Gajendiran, A., Krishnamoorthy, S. & Abraham, J. (2016). Microbial degradation of low-density polyethylene (LDPE) by *Aspergillus clavatus* strain JASK1 isolated from landfill soil. *3 Biotech.,* 6,1-6. https://doi.org/10.1007/s13205-016-0394-x.
11. Ghosh, S., Qureshi, A. & Purohit, H. J. (2019). Microbial degradation of plastics: Biofilms and degradation pathways. *Contam. Agric. Environ. Heal. Risks Remediat.,* 184-199. https://doi.org/10.26832/aesa-2019-cae-0153-014.
12. Gilan, I., Hadar, Y. & Sivan, A. (2004). Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcus* ruber. *Appl. Microbiol. Biotechnol.,* 65,97-104. https://doi.org/10.1007/s00253-004-1584-8.
13. Hadad, D., Geresh, S. & Sivan, A. (2005). Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *J. Appl. Microbiol.,* 98,1093-1100. https://doi.org/10.1111/j.1365-2672.2005.02553.x.
14. Hahladakis, J. N. & Iacovidou, E. (2019). An overview of the challenges and trade-offs in closing the loop of post-consumer plastic waste (PCPW): Focus on recycling. *J. Hazard. Mater.,* 380. https://doi.org/10.1016/j.jhazmat.2019.120887.
15. Harshvardhan, K. & Jha, B. (2013). Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India. *Mar. Pollut. Bull.,* 77,100-106. https://doi.org/10.1016/j.marpolbul.2013.10.025.
16. Hasan, F., Shah, A. A., Hameed, A. & Ahmed, S. (2007). Synergistic effect of photo- and chemical treatment on the rate of biodegradation of low density polyethylene by *Fusarium* sp. AF4. *J. Appl. Polym. Sci.,* 105,1466-1470. https://doi.org/10.1002/app.26328.
17. Hou, L., Xi, J., Chen, X., Li, X., Ma, W., Lu, J., Xu, J. & Lin, Y.B. (2019). Biodegradability and ecological impacts of polyethylene-based mulching film at agricultural environment. *J. Hazard. Mater.,* 378. https://doi.org/10.1016/j.jhazmat.2019.120774.
18. Idowu, I. A., Atherton, W., Hashim, K., Kot, P., Alkhaddar, R., Alo, B. I. & Shaw, A. (2019). An analyses of the status of landfill classification systems in developing countries: Sub Saharan Africa landfill experiences. *Waste Manag.,* 87,761-771. https://doi.org/10.1016/j.wasman.2019.03
19. Islami, A. N., Tazkiaturrizki, T. & Rinanti, A. (2019). The effect of pH-temperature on plastic allowance for Low-Density Polyethylene (LDPE) by *Thiobacillus* sp. and *Clostridium* sp. *J. Phys. Conf. Ser.,* https://doi.org/10.1088/1742-6596/1402/3/033003.
20. Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., Hussain, T., Ali, M., Rafiq, M. & Kamil, M.A. (2018). Bacterial biofilm and associated infections. *J. Chinese Med. Assoc.,* 81,7-11. https://doi.org/10.1016/j.jcma.2017.07.012.
21. Kumari, A., Chaudhary, D. R. & Jha, B. (2019). Destabilization of polyethylene and polyvinylchloride structure by marine bacterial strain. *Environ. Sci. Pollut. Res.* 26,1507-1516. https://doi.org/10.1007/s11356-018-3465-1.
22. Kunlere, I. O., Fagade, O. E. & Nwadike, B. I. (2019). Biodegradation of low density polyethylene (LDPE) by certain indigenous bacteria and fungi. *Int. J. Environ. Stud.,* 76, 428-440. https://doi.org/10.1080/00207233.2019.1579586.
23. Mohee, R. & Unmar, G. (2007). Determining biodegradability of plastic materials under controlled and natural composting environments. *Waste Manag.,* 27,1486-1493. https://doi.org/10.1016/j.wasman.2006.07.023.
24. Mourad, A. H. I. (2010). Thermo-mechanical characteristics of thermally aged polyethylene/ polypropylene blends. *Mater. Des.,* 31,918-929. https://doi.org/10.1016/j.matdes.2009.07.031.
25. Mukherjee, S., Chowdhuri, U. R. & Kundu, P. P. (2016). Bio-degradation of polyethylene waste by simultaneous use of two bacteria: *Bacillus licheniformis* for production of bio-surfactant and *Lysinibacillus fusiformis* for bio-degradation. *RSC Adv.*,6,2982-2992. https://doi.org/10.1039/c5ra25128a.
26. Mukherjee, S., Roy Chaudhuri, U. & Kundu, P. P. (2018). Biodegradation of polyethylene via complete solubilization

by the action of *Pseudomonas fluorescens*, biosurfactant produced by *Bacillus licheniformis* and anionic surfactant. *J. Chem. Technol. Biotechnol.,* 93,1300-1311. https://doi.org/10.1002/jctb.5489.

27. Nourollahi, A., Sedighi-Khavidak, S., Mokhtari, M., Eslami, G. & Shiranian, M. (2019). Isolation and identification of low-density polyethylene (LDPE) biodegrading bacteria from waste landfill in Yazd. *Int. J. Environ. Stud.,* 76,236-250. https://doi.org/10.1080/00207233.2018.1551986.
28. Novotný,Č. Malachová, K., Adamus, G., Kwiecień, M., Lotti, N., Soccio, M., Verney, V. & Fava, F. (2018). Deterioration of irradiation/high-temperature pretreated, linear low-density polyethylene (LLDPE) by *Bacillus amyloliquefaciens. Int. Biodeterior. Biodegrad.,* 132,259–267. https://doi.org/10.1016/j.ibiod.2018.04.014.
29. Pathak, V. M. & Kumar, N. (2017). Review on the current status of polymer degradation: a microbial approach. *Bioresour. Bioprocess.,* 4. https://doi.org/10.1186/s40643-017-0145-9.
30. Sarker, R. K., Chakraborty, P., Paul, P., Chatterjee, A. & Tribedi, P. (2020). Degradation of low-density poly ethylene (LDPE) by *Enterobacter cloacae* AKS7: a potential step towards sustainable environmental remediation. *Arch. Microbiol.,* 202,2117-2125. https://doi.org/10.1007/s00203-020-01926-8.
31. Sawiphak, S. & Wongjiratthiti, A. (2021). Optimisation of Culture Conditions for PLA food-packaging Degradation by *Bacillus* sp. SNRUSA4. *Pertanika J. Sci. Technol.,* 29 (1).
32. Shah, A. A., Hasan, F., Hameed, A. & Ahmed, S. (2008). Biological degradation of plastics: A comprehensive review. *Biotechnol. Adv.,* 26,246-265. https://doi.org/10.1016/j.biotechadv.2007.12.005.
33. Skariyachan, S., Manjunatha, V., Sultana, S., Jois, C., Bai, V. &Vasist, K. S. (2016). Novel bacterial consortia isolated from plastic garbage processing areas demonstrated enhanced degradation for low density polyethylene. *Environ. Sci. Pollut. Res.,* 23,18307-18319. https://doi.org/10.1007/s11356-016-7000-y.
34. Sojak, L., Kubinec, R., Jurdakova, H., Hájeková, E. & Bajus, M. (2006). High resolution gas chromatographic-mass spectrometric analysis of polyethylene and polypropylene thermal cracking products. *J. Anal. Appl. Pyrolysis.,* 78,387-399. https://doi.org/10.1016/j.jaap.2006.0 9.012.
35. Tokiwa, Y., Calabia, B. P., Ugwu, C. U. & Aiba, S. (2009). Biodegradability of plastics. *Int. J. Mol. Sci.,* 10,3722-3742. https://doi.org/10.3390/ijms10093722.
36. Vague, M., Chan, G., Roberts, C., Swartz, N. A. & Mellies, J. L. (2019). *Pseudomonas* isolates degrade and form biofilms on polyethylene terephthalate (PET) plastic. *BioRxiv.,* 647321. https://doi.org/10.1101/647321.
37. Whitman, W. B., Goodfellow, M., Kämpfer, P., Busse H.-J., Trujillo M.E., Ludwig W., Suzuki, K. & Parte A., (1984). Bergey's Manual of Systematic Bacteriology, Volume 5:The Actinobacteria. *New York Springer,* ©2012. 1640f. http://books.google.com/books?id=66UMS7A2KisC&pgis=1%0Ahttp://www.springer.com/life+sciences/book/978-0-387-95043-3.
38. Wolinska, A., Zapasek, M. & Stepniewska, Z. (2016). The optimal TTC dose and its chemical reduction level during soil dehydrogenase activity assay. *Acta Agrophys*., 23.
39. Yang, H.S., Yoon, J.S. & Kim, M.N. (2004). Effects of storage of a mature compost on its potential for biodegradation of plastics. *Polym. Degrad. Stab.,* 84,411-417. https://doi.org/10.1016/j.polymdegradstab.2004.01.014.