Exploration of low-density polyethylene degrading Actinomycetes from dumping site

A Dissertation Report submitted

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By

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CERTIFICATE

This is to certify that this dissertation work entitled **"Exploration of low-density polyethylene degrading Actinomycetes from dumping site"** was successfully carriedout by **Sakariya Uma T.** towards the partial fulfilment of requirements for the degree of Master of Science in Microbiology of Atmiya University Rajkot. It is anauthentic record of her own work, carried out by him under the guidance of **Dr. Mousumi Das** during the academic year of 2022-23 The content of this report, infull or in parts, has not been submitted for the award of any other degree or certificate in this or any other University.

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D E C L A R A T I O N

I hereby declare that the work incorporated in the present dissertation report entitled **"Exploration of low-density polyethylene degrading Actinomycetes from dumping site"** is myown work and is original.

This work (in part or in full) has not been submitted to any University for the award of a anyDegree or a Diploma.

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Achievement of goal is not a one person's job. It is obtained by guidance and cooperation of others. Gratitude ought to be a way of life, something which we cannot give enough. It can mean a smile or a thank you gesture or appreciation.

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ABBREVIATIONS

LDPE	Low density polyethylene
MDPE	Medium density polyethylene
HDPE	High density polyethylene
PP	Polypropylene
PS	Polystyrene
PBT	Polybutylene terephthalate
PUR	polyurethane
PVC	Polyvinyl Chloride
POPs	Persistent organic pollutants
RMC	Rajkot Municipal Corporation
SCA	Starch casein agar
MSA	Mineral salt agar
$NaNO_3$	Sodium nitrate
MgSO4	Magnesium sulfate
KCl	Potassium chloride
$FeSO_4$	Ferrous sulfate
KH2PO4	Potassium Phosphate
K2HPO4	Dipotassium phosphate
MSM	Mineral salt medium

ABSTRACT

Low density polyethylene, a type of plastic, is frequently used as a material for packaging (LDPE). The ongoing accumulation of plastic in the environment harms the biosphere. It takes too long time for plastic to decompose naturally. The most environmentally acceptable solution to this persistent and expanding issue is the microbial degradation approach. The major objective of the current study is to identify and screen actinomycetes that degrade low density polyethylene. To eliminate plastic from the environment and stop the accumulation of plastic. A total 141 actinomycetes have been isolated from the soil at dumping site in Rajkot. 16 Actinomyces are obtained after these were screened by primary screening (solid medium and liquid medium) utilizing low density polyethylene powder. 8 of these isolates were assigned a high ability to degrade LDPE after further examination utilizing the clear zone method on these samples. The film degradation assay was used to determine the percentage of degradation, and the most promising isolate was located at dumping. Further SEM analysis confirmed the degradation of LDPE beads.

KEYWORDS: LDPE, Biodegradation, Actinomyces, SEM analysis of LDPE bead

1. INTRODUCTION

1.1 Type of Plastic

Plastics are used in the packing of products like food, medications, beauty products, cleansers, and chemical. The most popular polymers used for manufacturing include polyethylene (LDPE, MDPE, HDPE, LDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polybutylene terephthalate (PBT), and nylons. Around 30% of plastics are utilized for packaging purposes globally. (Saritha et. al., 2021)

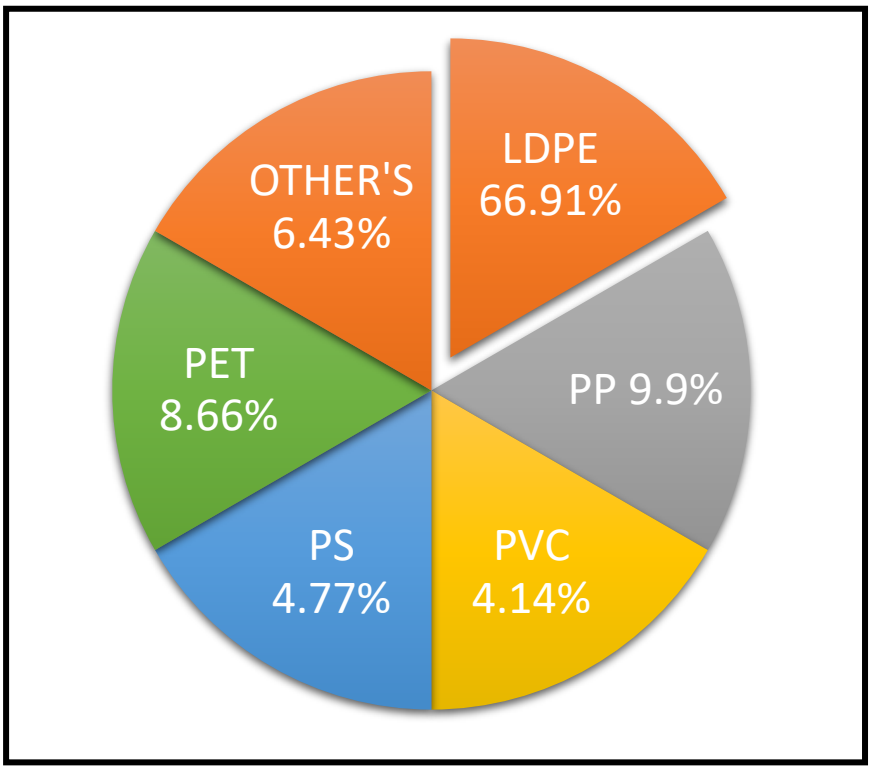


Fig. 1 composition of plastic waste in India

One of the main contributors to environmental pollution is low-density polyethylene. Low density polyethylene, a type of plastic, is frequently used as a material for packaging (LDPE). (Hussein, et. al., 2015) Long-chain ethylene monomers are used to create the polymer known as polyethylene. Low density polyethylene, which makes up around 60% of all plastic manufacture, is the non-biodegradable waste substance that is most common. Around 140 million tonnes of synthetic polymers are manufactured annually around the world, and the use

of polyethylene is growing at a rate of 12% per year (Shimao, et. al., 2001). Many micro-organisms accumulate PHA as intracellular energy and storage of carbon inclusions when the carbon is in excess to the other nutrients such as nitrogen, sulfur, phosphorus and oxygen (Madison and Huisman, 1999; Reddy et al., 2003).

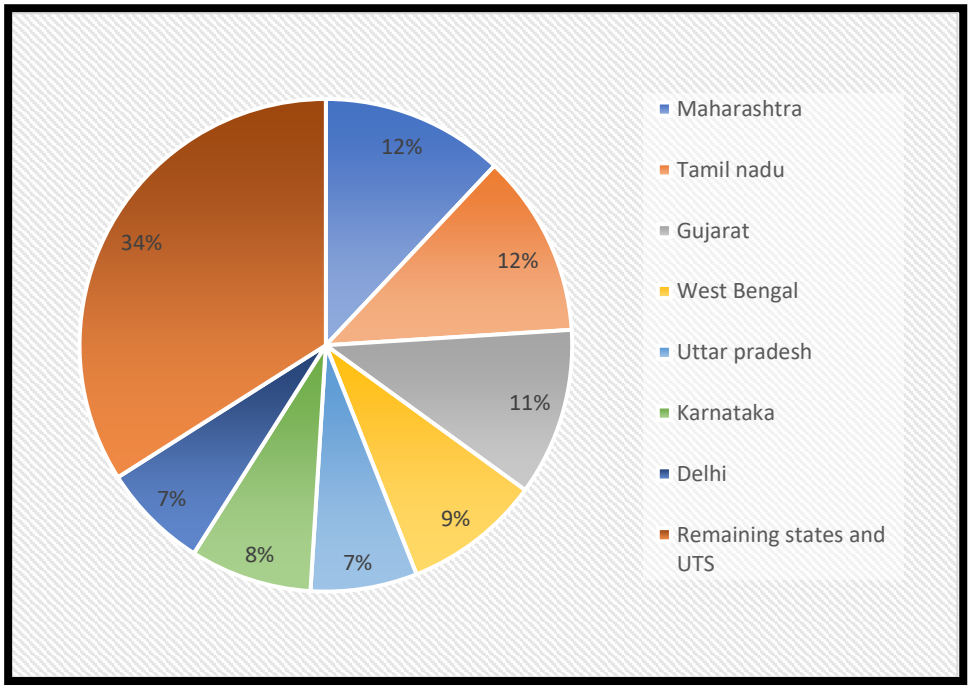


Fig. 2: States contributing to total plastic waste generation in India

1.2 Low density polyethylene

Low density polyethylene Degrading enzymes are produced by a number of bacteria, according to recent reports. The microbial species are connected to the deteriorating materials. In particular, actinomycetes produce hydrolytic enzymes that enable the breakdown of complex molecules into simpler ones, enabling them to thrive on various polymers. (Divyalakshmi et. al., 2017) Actinomycetes are second highest LDPE degrading. Low-density polyethylene (LDPE) is a type of thermoplastic polymer that is widely used for various applications. It is produced through the polymerization of ethylene monomers, which creates a long-chain polymer with a branched structure. The branched structure gives LDPE its unique properties, including flexibility, toughness, and transparency. LDPE is commonly used in packaging materials, such as plastic bags and films, due to its excellent flexibility and ability to conform to irregular shapes. (Ahmed et. al., 2017)

1.3 Bio-degradation

Degradation of plastic refers to any physical or chemical change in a polymer caused by environmental variables such as light, heat, moisture, chemical conditions, and biological activity. Biodegradable polymers are intended to decompose when discarded through the activity of living organisms. Microbial degradation of plastics is caused by enzymatic activities that result in polymer chain breaking into monomers. Microorganisms use polythene film as their sole source of carbon, resulting in partial plastic breakdown. Chemical, thermal, UV, and biodegradation processes can all be used to degrade PE. Hydro-biodegradation and oxo biodegradation are the two processes that help polyethylene to the hydro biodegradation reaction, which results in the destruction of plastics. Whereas oxy-biodegradation includes the reaction of plastic with oxygen to produce smaller molecules, which are then biodegraded by microorganisms and transformed into carbon dioxide, water, and biomass. (Bonhomme et. al., 2003).

1.4 Actinomyces

Actinomycetes are a phylum of gram-positive bacteria recognized within the domain bacteria (Gohel and Singh et. al., 2018). Actinobacteria are highly diverse group of prokaryotes dividing both characteristics of bacteria and fungi. They are like unicellular bacteria but do not have distinct cell wall and develop non-septate mycelium. The phylum actinobacteria constitutes one of the largest taxonomic units among the recognized major 18 family within the domain bacteria. The genera belong to the phylum actinobacteria show huge diversity in terms of morphology, physiology, and metabolic capabilities. They are widely distributed in terrestrial and marine environment like soil, alkaline dessert soil, lake, fresh water. (Sheikh et. al., 2018).

The purpose of this study was to isolate actinomycetes from dumped soil area and screening of the potential polyethylene degrading and identifying the high potential actinomycetes that degrade the low-density polyethylene.

AIM: To exploration of low-density polyethylene degrading actinomycetes from dumping site**.**

OBJECTIVE:

1. Isolation of Actinomycetes from Plastic dumping site.
2. Primary Screening of Actinomycetes for low density polyethylene degradation.
3. Secondary Screening of Actinomycetes for low density polyethylene degradation.
4. Degradation assay of low-density polyethylene (Beads) By isolated culture.
5. Molecular identification of low-density polyethylene degrading Actinomycetes.

2.REVIEW LITERATURE

2.1 Plastic degradation

A number of variables, such as the polymer's properties, the type of organism, and the pretreatment method, influence biodegradation. The mobility, crystallinity, molecular weight, functional groups, substituents, and plasticizers or additives that are added to the polymer, as well as the polymer's features like these, all play a big part in how quickly it degrades. [Artham and Doble et al., 2008]. Since synthetic polymers have only recently entered the natural world, evolution has not been able to create novel enzyme structures that can break them down (Mueller et al., 2006). As a result, plastics remain resistant to microbial attack. Recycling, burning, and landfilling are three methods used to dispose of plastic trash. Many communities are becoming more conscious of the effects of plastic pollution on the environment since discarded plastics persist in our ecosystem and negatively affect animals, the aesthetic attractiveness of cities, and the health of forests. By generating environmental pollution, improperly dumped plastic can seriously threaten human life. Moreover, the burning of polyvinylchloride (PVC) plastics results in the production of dioxins and furans, two persistent organic pollutants (POPs) [Jayasekara et al., 2005]. The study also determined that the ideal pH range for the degradation of polyester was 6-8 and that the optimal temperature ranges were between 40 and 60 C. Small strips of polyethylene were added to the medium containing casein broth and the degradation study was conducted by measuring the weight lost in the used polyethylene strips. Overall, these studies demonstrate the potential for various approaches to LDPE degradation, including thermal, photo, biodegradation, chemical, and enzymatic methods, to address the environmental concerns associated with this widely used plastic material. (Mueller et al., 2006) Biodegradation of Plastics: Challenges and Emerging Technologies" by (Barba-Ortega et al., 2021) - This review article discusses the challenges and potential solutions for the biodegradation of plastics, including enzymatic degradation, microbial degradation, and physical degradation. Plastic waste to energy: A review of incineration, pyrolysis and gasification by (Adediran et al., 2020) - This article reviews the use of incineration, pyrolysis, and gasification as methods for converting plastic waste into energy, highlighting their advantages and disadvantages.

"Accelerated degradation of polyethylene by UV/O3 treatment: A review" by Chen et al.

(2019) - This review article discusses the use of UV/O3 treatment to accelerate the degradation of polyethylene, including the mechanisms involved and the effect of various factors such as UV intensity and ozone concentration.

2.2 Low density polyethylene

Low density polyethylene (LDPE) is a widely used plastic material due to its excellent properties such as flexibility, transparency, and chemical resistance. However, its non-biodegradable nature and poor recyclability have led to serious environmental concerns, which have spurred research on the degradation and recycling of LDPE. In recent years, various approaches have been developed for LDPE degradation, including thermal, photo, and biodegradation, as well as chemical and enzymatic processes. Thermal degradation, which involves the use of heat to break down LDPE into smaller fragments, has been studied extensively. A recent study by (Jia et al., 2020) investigated the effects of temperature and pressure on the thermal degradation of LDPE, and found that higher temperatures and pressures led to increased degradation rates and lower molecular weights of the resulting fragments. Photodegradation, which involves the use of light to break down LDPE, has also been studied extensively. A study by (Khan et al., 2020) investigated the photodegradation of LDPE using a combination of sunlight and titanium dioxide nanoparticles, and found that the process led to a significant reduction in the molecular weight of the polymer. Biodegradation, which involves the use of microorganisms to break down LDPE, has also been explored as a potential solution for the disposal of LDPE waste. A study by (Reddy et al. 2020) investigated the biodegradation of LDPE by a bacterium called Pseudomonas putida, and found that the bacterium was able to degrade the polymer and produce biodegradable compounds. In addition to these methods, chemical and enzymatic degradation of LDPE have also been investigated. A study by (Ma et al. 2020) explored the use of enzymes to break down LDPE, and found that a specific enzyme called cutinase was able to degrade the polymer into smaller fragments. Chemical degradation, which involves the use of chemical agents to break down LDPE, has also been explored. A study by (Yamamoto et al. 2020) investigated the use of a chemical agent called 1,1,1-tris(hydroxymethyl) ethane to break down LDPE, and found that the process led to the formation of biodegradable compounds. Biodegradation, which involves the use of microorganisms to break down LDPE, has also been explored as a potential solution for the disposal of LDPE waste. A study by (Reddy et al. 2020) investigated the biodegradation of LDPE by a bacterium called

Pseudomonas putida, and found that the bacterium was able to degrade the polymer and produce biodegradable compounds.

One study conducted by (Sangale et al., 2017) isolated an LDPE-degrading actinomycete from soil samples collected from a landfill site. The actinomycete was identified as Streptomyces sp. and was found to degrade LDPE at a rate of 6.38% after 30 days of incubation. The study also showed that the actinomycete produced extracellular enzymes, such as lipases and esterase's, which are involved in the degradation of LDPE. Another study by (Krishnamoorthy et al., 2021) isolated an LDPE-degrading actinomycete from a plastic waste dumpsite in India. The actinomycete was identified as Streptomyces sp. and was found to degrade LDPE at a rate of 4.4% after 60 days of incubation. The study also showed that the actinomycete produced extracellular enzymes, such as cellulases and proteases, which are involved in the degradation of LDPE. In a study conducted by (Nanda et al., 2019), two LDPE-degrading actinomycetes were isolated from a plastic waste dumpsite in India. The actinomycetes were identified as Streptomyces sp. and Nocardiopsis sp., respectively. Both actinomycetes were found to degrade LDPE at a rate of 3.2% and 3.6%, respectively, after 60 days of incubation. The study also showed that the actinomycetes produced extracellular enzymes, such as lipases, esterases, and proteases, which are involved in the degradation of LDPE.

2.3 Actinomyces

A metagenomic investigation conducted by (Kumar et al., 2021) showed the density of Actinomycetes in the various soil and leachate samples taken from the waste disposal site known as Pirana, Ahmedabad, which contributed to the decomposition of plastic. *Actinomadura*, *Streptomyces*, and *Laceyella* are the isolates with the highest potential for degrading polyester, according to (Sriyapati et al., 2017) study. This could possibly be considered a particular type of bioplastic made from plants that degrades rapidly and safely in composting environments. Nonetheless, based on the process used to make it, bioplastic may have its own negative effects on the environment. For this worldwide problem to be solved, it is necessary to produce effective microorganisms and their products (Kathiresan et al., 2003). Based on morphological analysis, different types of actinomycetes were isolated from three different sites to explore the diversity of actinomycetes in the soils. This study explored (Waithaka et al., 2017). According to (Fotopoulou and Karapanagioti et al., 2017), degradation

is the breakdown of the polymer structure that results in a change in the material's physical and chemical properties as a result of a major change in its chemical structure.

Microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics (Gu et al., 2000a). The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the microorganisms that are responsible for the process of degradation differ from each other and they have their own optimal growth conditions in the soil. Plastics are potential substrates for heterotrophic microorganisms [Glass and Swift, 1989] There are at least two categories of enzymes that are actively involved in biological degradation of polymers: extracellular and intracellular depolymerases [Gu et al., 2000b]. Overall, these studies demonstrate that LDPE-degrading actinomycetes are a promising solution to the problem of plastic pollution. Further research is needed to identify and characterize more LDPE-degrading actinomycetes, as well as to optimize the conditions for their growth and degradation activity.

3.MATERIALS & METHODS

3.1 Samples collection

Soil sample were collected from plastic dumping site. 14 of soil samples were collected from 7 dumping site, 3 site located in RMC dumping, 2 site located in Saurashtra University and another site located in Sadhu-Vaswani Road. soil sample were collected at depth 3-5cm from different sites. The samples were collected in air tight bag.

3.1.1 Isolation of low-density polyethylene degrading micro-organisms:

Soil samples were collected from the dumping sites of selected districted of Rajkot (GUJARAT). 1gm of soil sample with 10 ml of sterile distilled water and thoroughly mixing to create soil suspension. 9 ml of sterile, distilled water were used to dilute 1 ml of soil suspension to a 10-fold dilution. For each soil sample, dilutions up to 10-8 were created independently. From 10-3 to 10-8 dilutions, a suspension (0.1 ml) was spread in Starch Casein Agar. After that, the plates were incubated for 7 days at 28 °C. (Fotopoulou and Karapanagioti et al., 2017)

Identification of the isolates were performed according to their morphological Characteristics, gram staining.

3.2 Primary Screening

3.2.1. In solid medium

Low density polyethylene powder was added to mineral salt agar [NANO3: 2 g; MgSO4: 0.5 g; KCl: 0.5g; FeSO4: 0.01 g; KH2PO4: 0.14 g; K2HPO4: 1.2 g; Yeast extract: 0.02 g; agar: 30 g] at a final concentration of 1% (W/V) respectively. Mixture was sonicated for 1 hour. Autoclaved it at 121°C, 15lbs pressure for 15 min. Sterilized media was cooled and poured into sterile Petri plates. After solidification, 0.1ml culture spread in mineral salt agar plate. After that, the plates were incubated for 1week at 28 °C. (Usha et al., 2011)

3.3.2 In liquid medium

In a sugar tube, 25 ml of liquid MSM were poured along with 1% LDPE powder as substrate. After being autoclave sterilized, sugar tubes were inoculation with loop-full culture and incubated for 7 days at 150 rpm and 28°C in a shaker incubator. A UV-visible spectrophotometer was used to measure the OD in order to estimate the bacterial growth of the isolates at 600 nm. (Hussein et al., 2015)

3.3 Secondary Screening

3.3.1 Clear zone method

At a final concentration of 1% (W/V), Polyethylene Glycol was added to mineral salt agar. At 121°C and 15 lbs. of pressure, it should be autoclaved. In sterilized petri plates, sterilized media was chilled before being added. In the middle of petri plates, streak cultures after solidification. for 2 weeks, incubated at 28 °C. After incubation, add 1% Coomassie brilliant blue dye for staining (20 min). Remove dye and add distain (25 min). Clear zone visualization. (Nademo et al. 2023)

3.4 Degradation Assay

Low-density polyethylene (beads) was added to the mineral salt broth at a final concentration of 1% (W/V). It should be autoclaved at 121 °C and 15 lbs. of pressure. Inoculated portent polythene-degrading microorganisms. The flasks were kept in the shaker incubator for a month at 28 °C and 120rpm. Low density polyethylene (beads) was cleaned in 70% ethanol over a period of time, allowed to air dry, and then weighed to verify the final weight. (Waithaka et al. 2017) The weight loss is measured using the formula:

$$\boldsymbol{Weight\ loss}\ (\%) = \frac{(\boldsymbol{Initial\ weight\ -\ Final\ weight})}{\boldsymbol{Initial\ weight}} \times \mathbf{100}$$

3.5 Biochemical characterization of Potent Isolates

3.5.1 Methyl Red test

First, prepare the MR broth tubes were autoclaved and sterilized then add the experimental bacterial culture using sterile techniques. Both tubes were incubated at 28ºC for 5-7 days. After appropriate incubation adds 2-3 drops of methyl red indicator to observe the colour changes. After the addition of the indicator remaining the red colour is a positive test and the colour changed to yellow is a negative test.

3.5.2 Voges-Proskauer test

The experimental organism was inoculated into VP broth by loop inoculation using sterile techniques. The uninoculated tube was kept as a control. Both tubes were incubated at 28ºC for 5-7 days. After incubation adds alpha-naphthol and 40% KOH as an indicator. After adding the indicator, the crimson-red colour formation indicates the positive result and colour change is the negative result of the test.

3.5.3 Indole test

First, prepare the peptone water tubes were incubated with the bacterial culture broth culture using sterile needle techniques. The uninoculated broth was used as a control and both tubes were incubated at 28ºC for 5-7 days. After complete incubation, add 1 ml of Kovac's reagent in both tubes. After the addition of the reagent observed the cherry red colour on the top layer of the tube is a positive test and the absence of red coloration is indole negative.

3.5.4 Citrate utilization test

Simmons citrate agar slant was prepared and autoclaved for sterilization. Then slant was inoculated with the test organism by stab and streak inoculation. An uninoculated tube was kept as control. Both tubes were incubated at 28ºC for 5-7. After proper incubation observed the tubes for the growth and coloration of the medium. The colour of the medium if changed to blue indicates a positive result and green colour indicates a negative result.

3.5.5 Oxidase test

For the oxidase test, the test organisms were rubbed over the oxidase disc and saw the Colour changes of the disc. If the colour changes to purple that gives a positive result on the test.

3.5.6 Catalase test:

The catalase test was performed to detect the presence of catalase enzyme by inoculating a loopful of culture into slide containing 3% of hydrogen peroxide solution. Positive test was indicated by formation of effervescence or appearance of bubbles.

3.6 Molecular identification

3.6.1 16s rDNA sequencing

Molecular characterization of UA66 bacterial isolate was done with the help of Gene explore, Ahmedabad, Gujarat. Culture was sent to the GENE explore for 16S rRNA gene sequencing. For molecular identification DNA was isolated on 1% agarose gel, after that fragment of 16S rRNA gene was amplified by PCR. A single discrete PCR amplicon band was observed when resolved on Agarose and purified by column purification. 16S rRNA was used to carry out BLAST with NCBI GenBank database.

3.6.2 Scanning Electron Microscope

The surface morphology of LDPE bead was observed using Scanning Electron Microscopy (SEM). LDPE slabs placed on the sample holder and scanned at 5000× magnification. The control bead was also analyzed for comparison.

4.RESULTS

4.1 Isolation of actinomycetes form different plastic dumping site

The dumping sites are the most suitable regions for the collection as they are rich in plastic. The variation in population level of microorganisms associated with collected soil sample from 7 dumping site summarized in Table 1. And Fig. 3.

(A) (B)

(C)

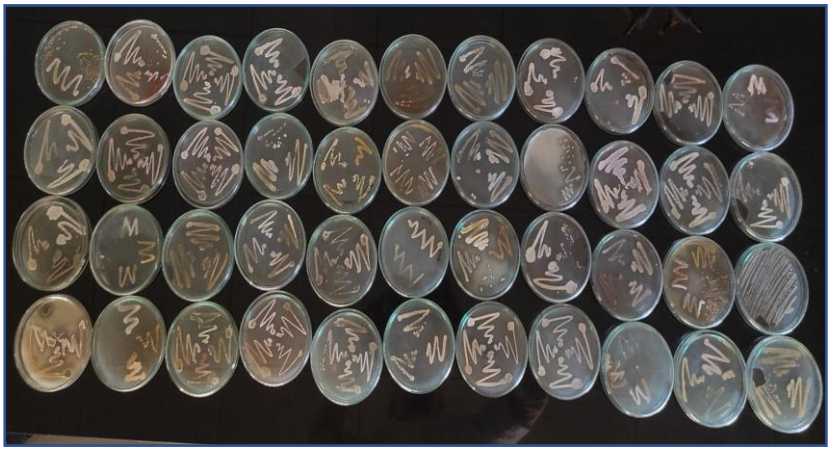
Fig. 3 dumping sites (A) RMC dumping site (B) Sadhu-Vaswani Road dumping site (C) soil sample

Table 1: Isolates from different dumping sites of Rajkot, Gujrat

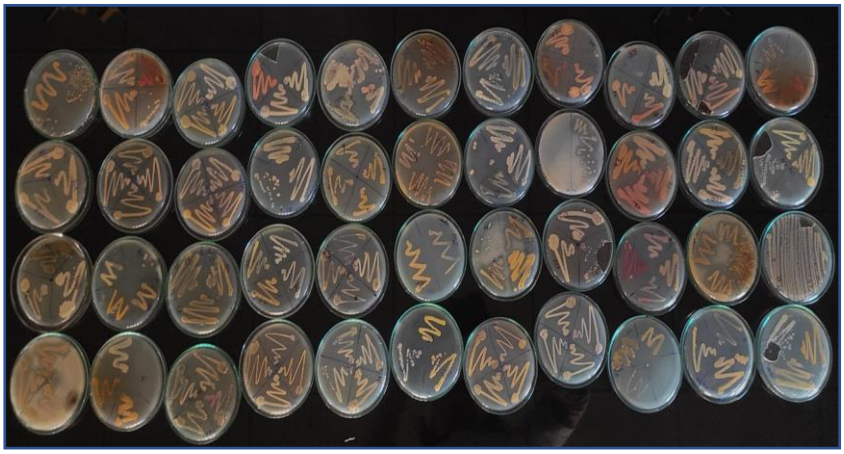
Site Location	No. of sample collection	Number of Isolate
RMC dumping site (Near Gate)	2	24
RMC dumping site (Near Plastic waste Department)	2	27
Raiya waste outside (Near RMC)	2	23
Saurastra University dumping site (Near Gate: 1)	2	18
Saurastra University dumping site	2	16
Sadhu Vaswani road dumping area (Behind the sadhu Vaswani school)	2	18
Sadhu Vaswani dumping site (Near Bhagat Singh Garden)	2	15

Table 1 reveals that Rajkot district has the highest number of microorganism isolates. 24 cultures isolates are RMC dumping site near Gate. 27 cultures isolates are RMC dumping site near plastic waste department. 23 cultures isolates are Raiya waste outside near RMC.18 cultures isolates are Saurastra University dumping site near gate 1. 16 cultures isolates are Saurastra University dumping site. 18 cultures isolates are Sadhu Vaswani riad dumping area behind the Sadhu Vaswani School. 15 cultures isolates are Sadhu Vaswani dumping site near Bhagat Singh Garden.

There are numerous reports on similar lines of work. As per the data available in the report of Waithaka et al., (2020). In this study Total 141 actinomycetes culture are isolated. (Fig. 4 follow for isolated culture) all total Actinobacterial isolates had shown round, milky white/ white/ black/ gray/ gray white/ yellow, entire and raised colonies.



(A)



(B)

Fig. 4 Isolation of actinomycetes culture on starch casein agar (A) Front side of isolates, (B) Back side of isolates. Total 141 cultures are isolated

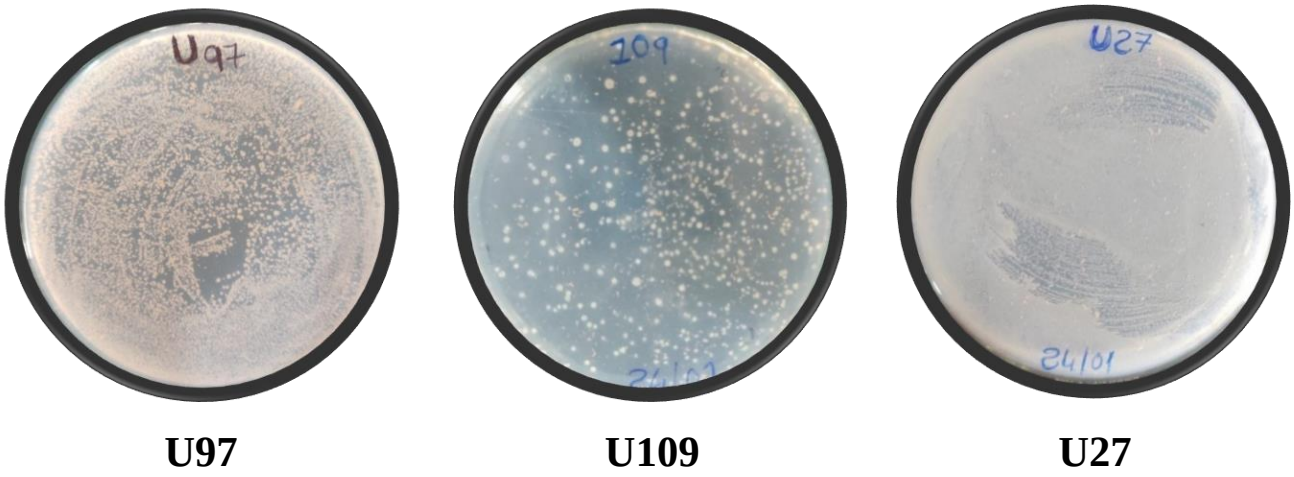
4.2 Primary Screening
4.2.1 solid medium

All 141 isolates were tested for their ability to degrade LDPE using solid MSM with 1% (w/v) LDPE powder. As indicated in **Table 2,** the efficiency to degrade has been recorded depending on the growth of culture colonies.

Table 2: Growth ability of isolates in solid medium

No.	Isolates	Growth ability of isolates		
		Maximum	Moderate	Minimum
1	UA1		++	
2	UA3	+++		
3	UA4	+++		
4	UA6			+
5	UA7	+++		
6	UA8	+++		
7	UA10		++	
8	UA11		++	
9	UA12			+
10	UA14			+
11	UA16	+++		
12	UA17			+
13	UA18	+++		
14	UA19	+++		
15	UA20	+++		
16	UA21	+++		
17	UA22			+
18	UA23	+++		
19	UA24	+++		
20	UA27			+
21	UA29		++	
22	UA30	+++		
23	UA31			+
24	UA32	+++		
25	UA33		++	
26	UA35		++	

27	UA37			+
28	UA40		++	
29	UA46		++	
30	UA49			+
31	UA50		++	
32	UA54	+++		
33	UA55			+
34	UA58		++	
35	UA60	+++		
36	UA61	+++		
37	UA65			+
38	UA66	+++		
39	UA67		++	
40	UA68		++	
41	UA71		++	
42	UA75		++	
43	UA79	+++		
44	UA83		++	
45	UA86		++	
46	UA90			+
47	UA92		++	
48	UA96	+++		
49	UA97	+++		
50	UA98	+++		
51	UA107			+
52	UA109		++	
53	UA130		++	
54	UA137		++	



U97 **U109** **U27**

U3 **U4** **U21**

U54 **U58** **U107**

Fig. 5: Primary screening of LDPE degrading isolates, using mineral salt agar with 1% low density polyethylene powder, at 28 °C for 7 days

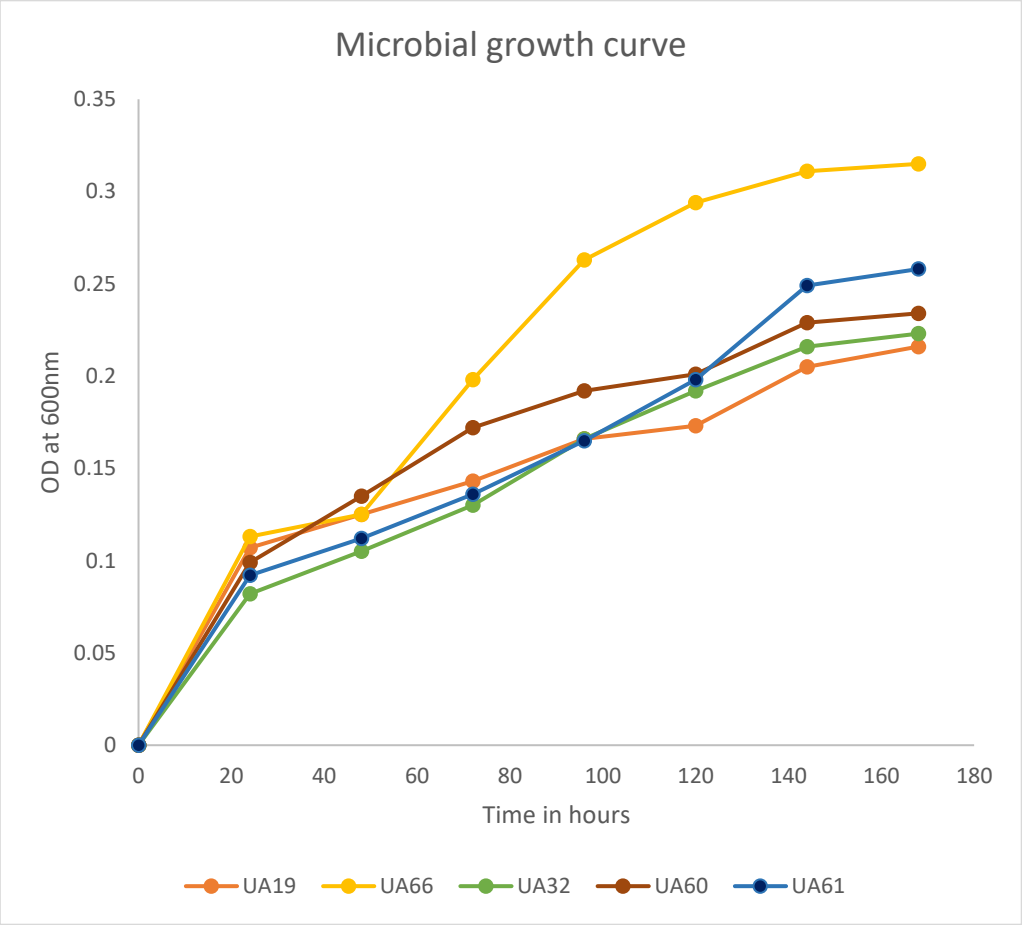
4.2.2 LIQUID MEDIUM

The ability of 16 isolates from 54 LDPE degrading bacteria to grow in liquid MSM with 1% LDPE powder was tested, and the growth density at 600 nm over the course of seven days of incubation was calculated. Table (3). It is necessary for the growth of microbes and fungus to have a variety of nutritional components, including carbon, nitrogen, phosphorus, and other mineral sources.

Table 3: Primary screening of selected bacterial isolates using optical density as a bacterial growth in liquid mineral salt medium, 150 rpm at 28°C for 7 days.

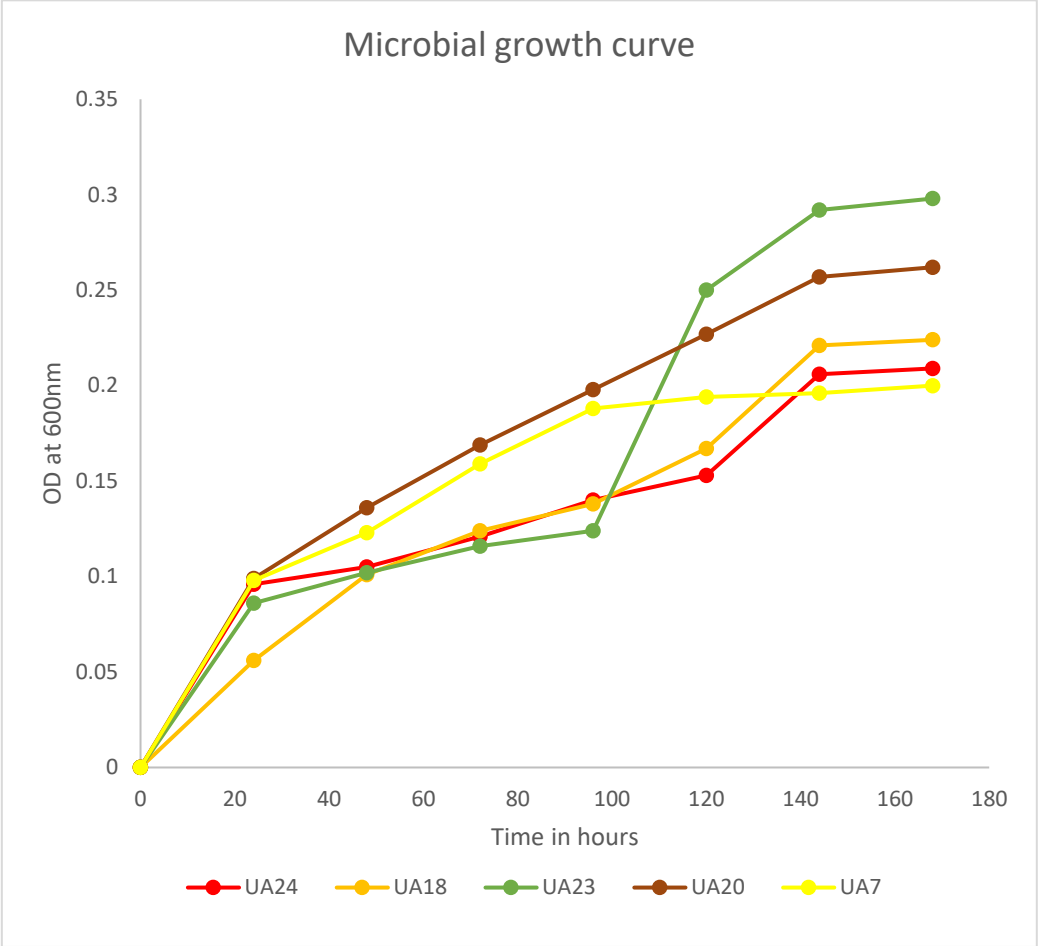
No.	Isolate	OD of growth
1	UA1	0.193
2	UA3	0.142
3	UA4	0.110
4	UA6	0.148
5	UA7	0.257
6	UA8	0.200
7	UA10	0.167
8	UA11	0.172
9	UA12	0.124
10	UA14	0.188
11	UA16	0.203
12	UA17	0.134
13	UA18	0.232
14	UA19	0.298
15	UA20	0.201
16	UA21	0.155
17	UA22	0.126
18	UA23	0.201
19	UA24	0.209
20	UA27	0.081
21	UA29	0.166
22	UA30	0.209
23	UA31	0.131
24	UA32	0.223
25	UA33	0.144
26	UA35	0.142
27	UA37	0.142
28	UA40	0.173

29	UA46	0.148
30	UA49	0.099
31	UA50	0.155
32	UA54	0.147
33	UA55	0.142
34	UA58	0.174
35	UA60	0.234
36	UA61	0.234
37	UA65	0.136
38	UA66	0.315
39	UA67	0.157
40	UA68	0.165
41	UA71	0.138
42	UA75	0.153
43	UA79	0.243
44	UA83	0.156
45	UA86	0.165
46	UA90	0.098
47	UA92	0.139
48	UA96	0.243
49	UA97	0.246
50	UA98	0.258
51	UA107	0.122
52	UA109	0.125
53	UA130	0.153
54	UA137	0.178



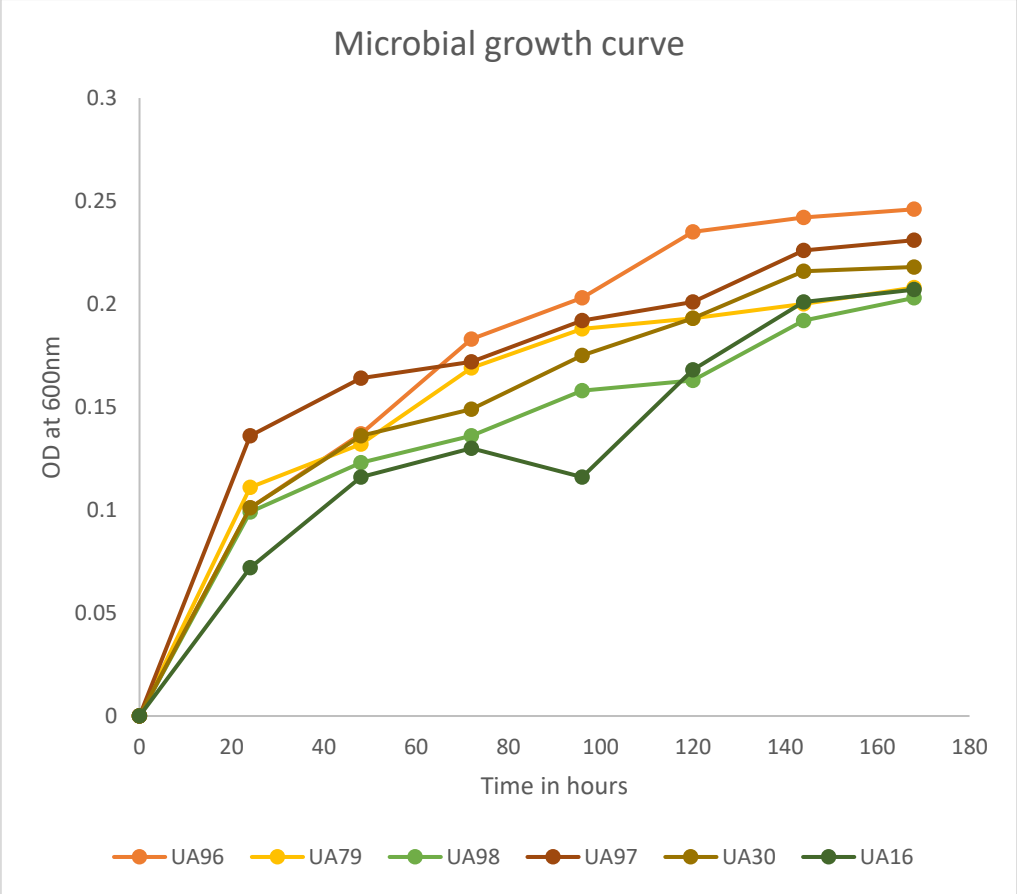
<u>Fig.6</u> Growth of Actinomycetes in M9 medium, incubated at 28°C, OD at 600nm for 7 days: [A] OD at 600nm UA19, UA66, UA32, UA60, UA61 culture growth in M9 medium.

The ability of UA19, UA66, UA32, UA60, UA61 isolates grow in liquid MSM with 1% LDPE powder was tested, and the growth density at 600 nm over the course of seven days of incubation. The isolates UA19, UA66, UA32, UA60, UA61 caused optical density ranging 0.298, 0.315, 0.223, 0.234, 0.234 respectively. (Fig. 6)



<u>Fig. 7</u> Growth of Actinomycetes in M9 medium, incubated at 28°C, OD at 600nm for 7 days: OD at 600nm UA24, UA18, UA23, UA20, UA7 culture growth in M9 medium.

The ability of UA24, UA18, UA23, UA20, UA7 isolates grow in liquid MSM with 1% LDPE powder was tested, and the growth density at 600 nm over the course of seven days of incubation. The isolates UA24, UA18, UA23, UA20, UA7 caused optical density ranging 0.209, 0.232, 0.201, 0.201, 0.257 respectively. (Fig. 7)



<u>Fig :8</u> Growth of Actinomycetes in M9 medium, incubated at 28°C, OD at 600nm for 7 days OD at 600nm UA96, UA79, UA98, UA97, UA30, UA16 culture growth in M9 medium.

The ability of UA96, UA79, UA98, UA97, UA30, UA16 isolates grow in liquid MSM with 1% LDPE powder was tested, and the growth density at 600 nm over the course of seven days of incubation. The isolates UA96, UA79, UA98, UA97, UA30, UA16 caused optical density ranging 0.243, 0.243, 0.258, 0.246, 0.209, 0.203 respectively. (Fig 8)

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4.3 Secondary screening

4.3.1 Clear zone method

Coomassie Brilliant blue dye reacts with polymer. And give blue colour. Where the clear zone occurs, the polymer degrades by organism.

Table 4: Secondary screening of isolates by Clear zone method

No.	Isolates	LDPE biodegradation by (clear zone method)
1	UA60	2.8cm
2	UA24	2.25cm
3	UA79	3.90cm
4	UA98	3.2cm
5	**UA66**	**4.42cm**
6	UA7	3.2cm
7	UA20	2.64cm
8	UA18	2.30cm

In this process the zone of clearance was observed by adding 1 % concentrations of PEG followed by staining with Coomassie blue. Out of 16 only 8 isolates give a clear zone on the mineral salt agar plate. UA66 isolate culture gives a 4.42-cm clear zone. table (4). Follow fig. 9.

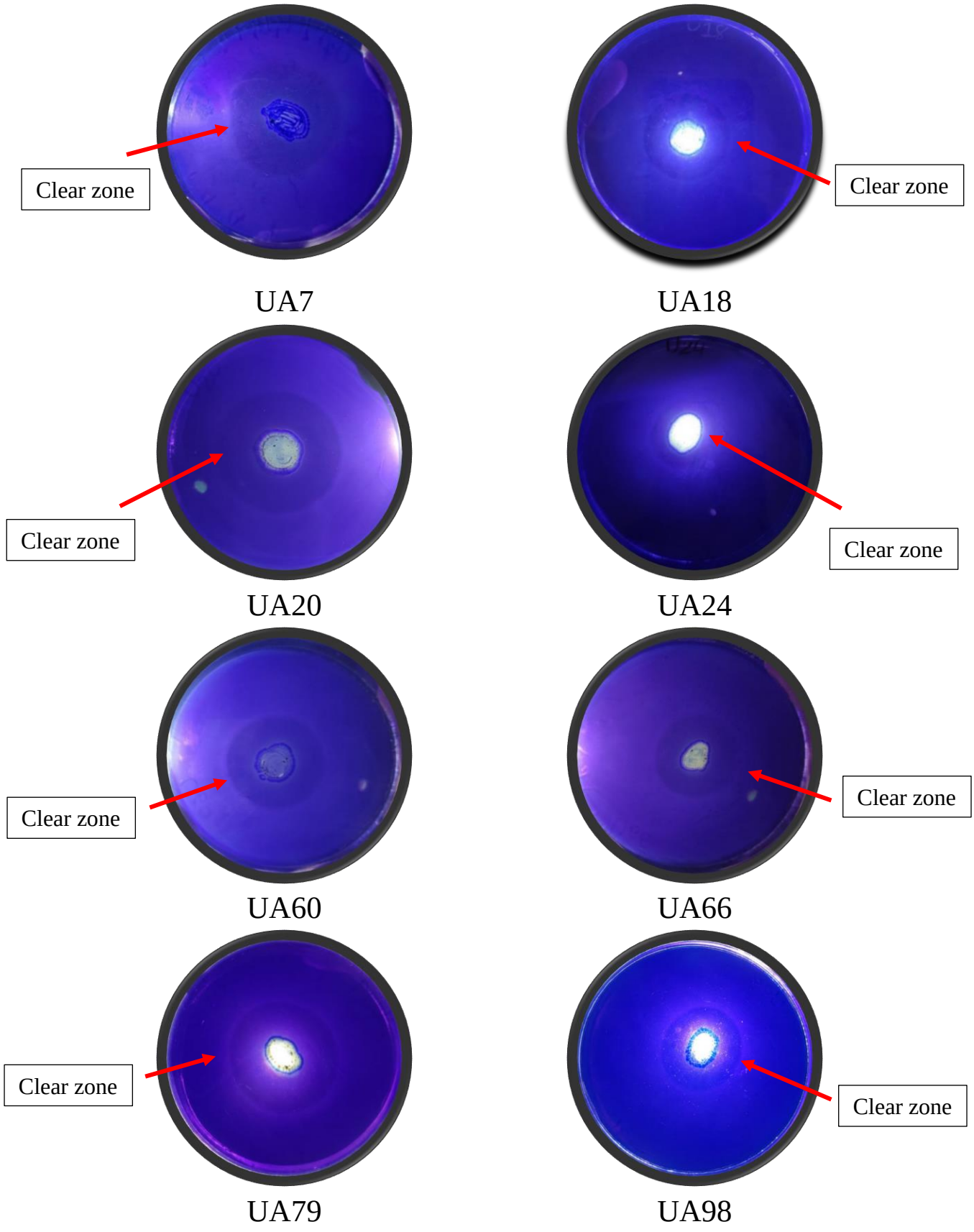


Fig 9: Secondary screening of isolates by Clear zone method

4.4 Degradation assay

Pre-weighed LDPE films were subjected to degradation with the above-mentioned eight isolates. Out of the eight isolates, UA66 showed maximum degradation in liquid M9 medium which confirmed higher plastic degrading capacity. (fig.11)

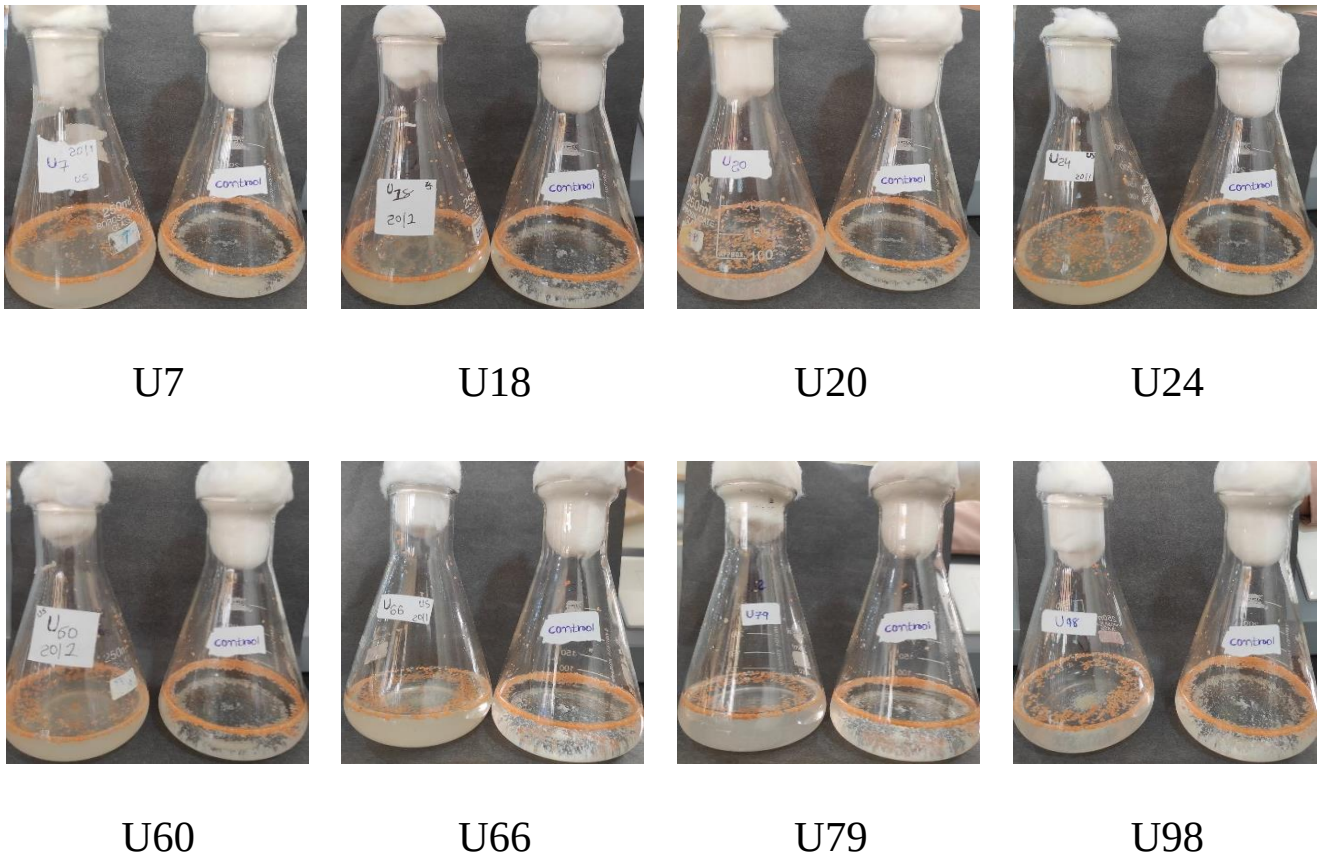


Fig.10: Degradation of low-density polyethylene incubated with actinomyces in shaker cultures under laboratory condition

In (Fig.10) Shows, Turbidity and Colour change in Mineral salt medium. Test and control both are shown in fig. According to Dry weight loss in the degraded low-density polyethylene, the source supported not only the organism's survival but also its growth.

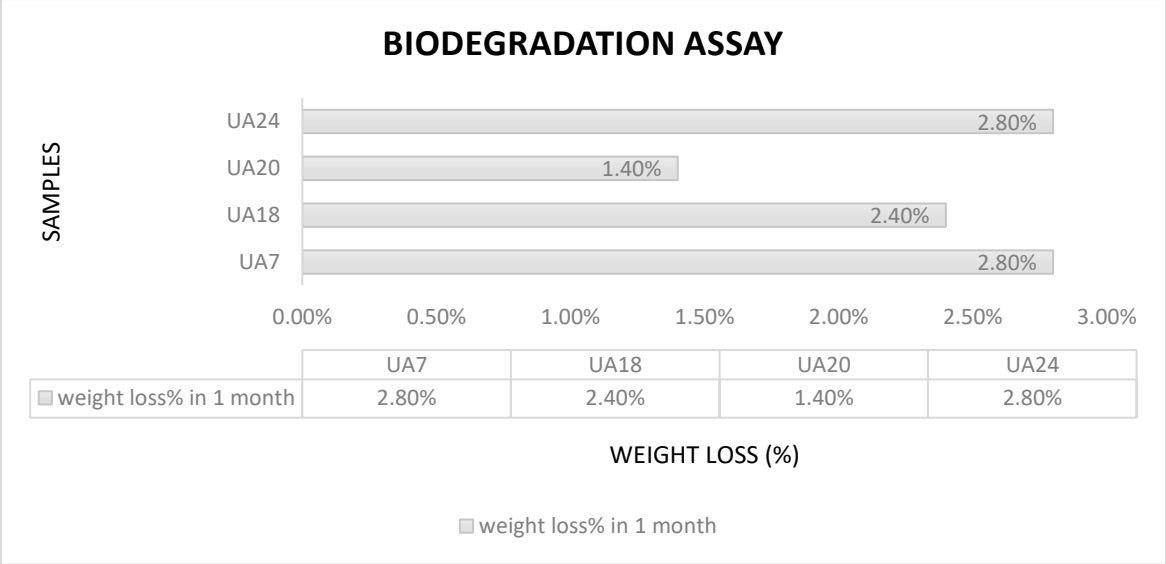


Fig.11 Biodegradation assay: UA7, UA18, UA20, UA24 isolates give a weight loss % in 1 Month

Weight loss was measured in 1 month, the bacterial isolates UA7, UA18, UA20, UA24 caused biodegradation ranging from 2.80%, 2.40%, 1.40%, 2.80% respectively. All isolates have a degradation capacity which is confirmed by biodegradation assay test.

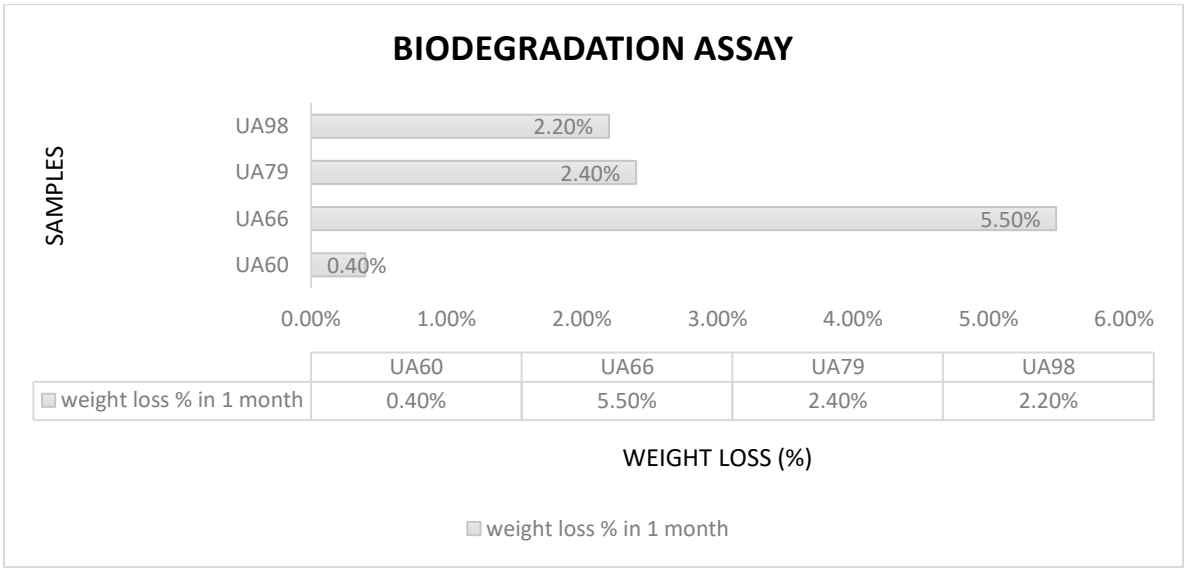


Fig.12 Biodegradation assay: UA60, UA66, UA79, UA98 isolates give a weight loss % in 1 Month

Weight loss was measured in 1 month, the bacterial isolates UA60, UA66, UA79, UA98 caused biodegradation ranging from 0.40%, 5.50%, 2.40%, 2.20% respectively. UA66 culture displayed high degradability measuring 5.5%.

4.5 Morphological and biochemical Characterization polyethylene (LDPE) degrading isolates

Table 5, showing the following colony characteristic features ranging from small to medium in size, powdery dry, rough, black to white colonies exhibiting typical actinobacterial colony characteristics

Table 5: Properties of Actinomyces

Colony characteristics	Colony name							
	UA7	**UA18**	**UA20**	**UA24**	**UA60**	**UA66**	**UA79**	**UA98**
Size	Small	Small	Medium	Medium	Medium	Small	Medium	Medium
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colour	White	White	White	White	White	Black	White	Black
Reverse Pigment	Yellow	Yellow	Yellow	Yellow	Yellow	Yellowish Black	Yellow	Black
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Texture	Powdery	Powdery	Powdery	Powdery	Powdery	Powdery	Powdery	Powdery
Elevation	Raised	Flat	Raised	Raised	Raised	Raised	Flat	Raised
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Gram Staining	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

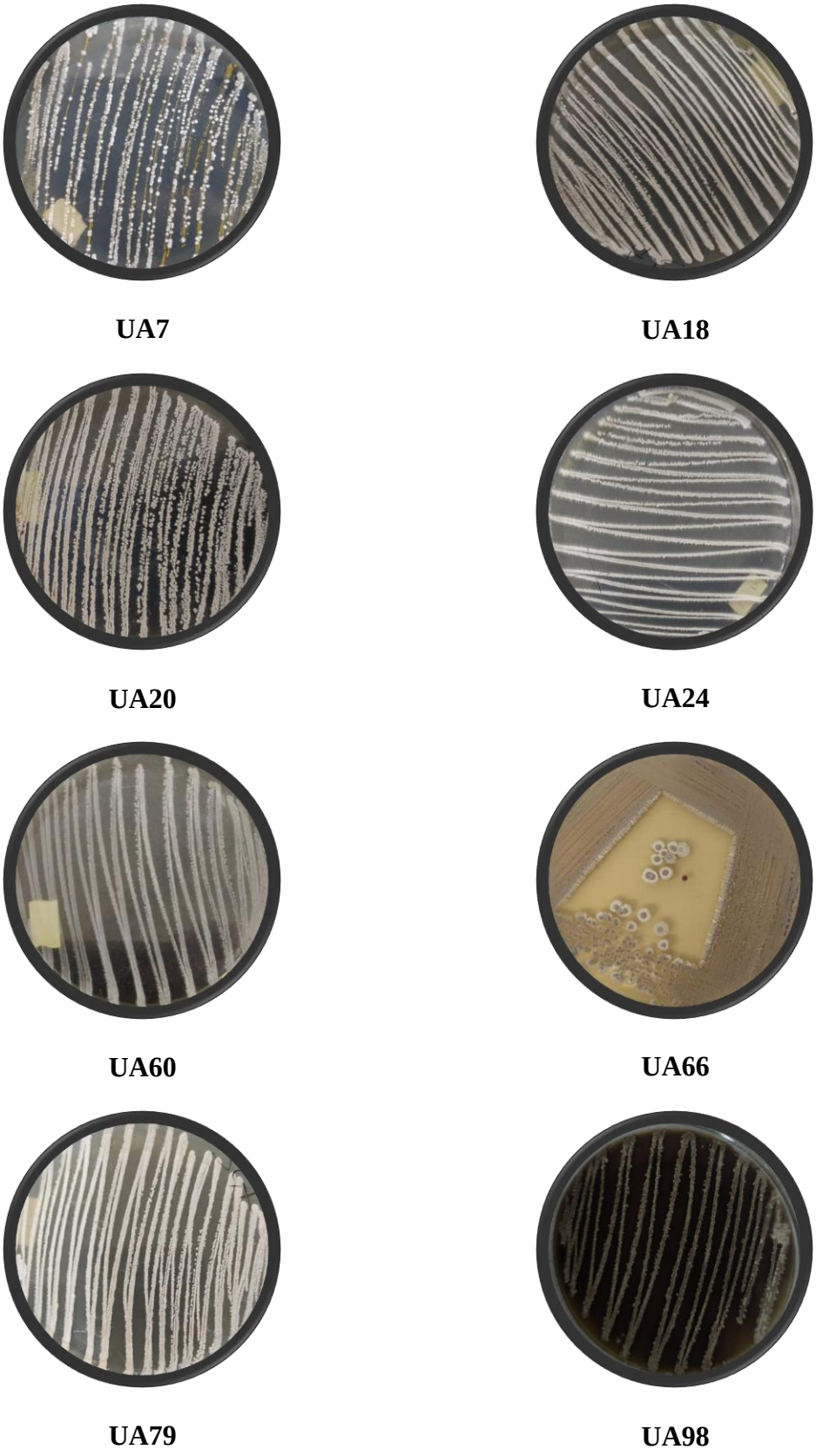


Fig. 13: Potent culture on Starch casein agar

Fig.13 shows the Potent Actinomycetes strain UA7, UA18, UA20, UA24, UA60, UA66, UA79, UA98 on Starch casein agar medium. UA7, UA18, UA20, UA24, UA60 and UA79 have white powder colony and yellow reverse pigment, UA66 gave black colour colony and yellowish black reverse pigment, UA98 gave black powdery colony with black reverse pigment.

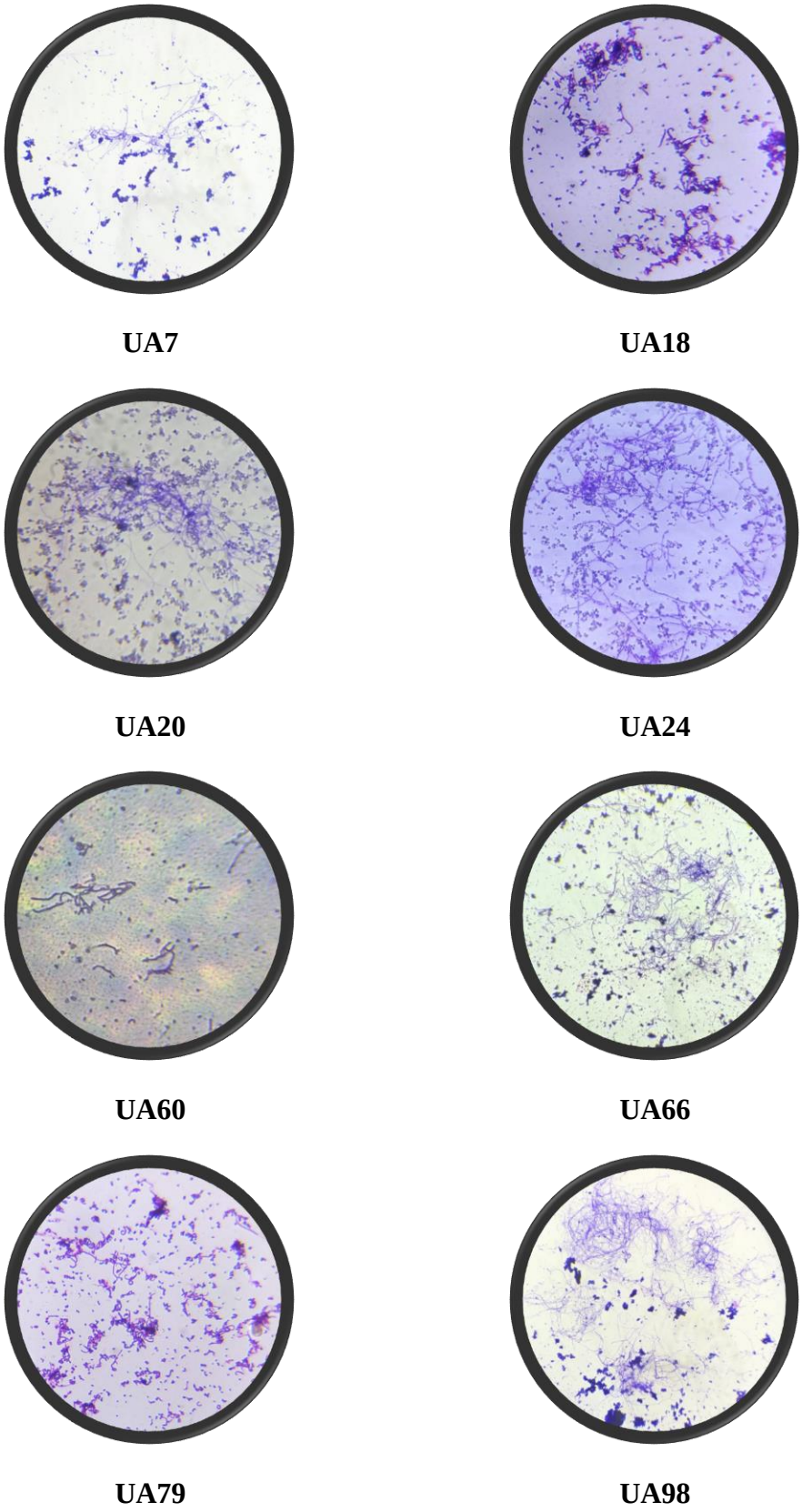


Fig. 14: Gram staining of potent

Contains gram staining of actinomycetes strain UA7, UA18, UA20, UA24, UA60, UA66, UA79, UA98 under oil immersion lanes, Gram staining showing long, filamentous like structure, branched gram-positive bacteria.

Table: 6 Biochemical characterizations of Potent Isolate

No.	Isolate	Biochemical test					
		MR	VP	Citrate	Indole	Oxidase	Catalase
1	UA7	-	+	-	-	+	-
2	UA18	-	+	+	-	+	+
3	UA20	-	-	-	-	-	+
4	UA24	+	-	+	-	-	-
5	UA60	-	-	+	-	+	-
6	UA66	-	-	+	-	+	+
7	UA79	-	-	+	-	-	-
8	UA98	+	-	-	-	+	-

Table 6 shows the biochemical test of isolated Actinomycetes strain UA7, UA18, UA20, UA24, UA60, UA66, UA79, UA98. Biochemical test like Methyl Red, Voges-Proskauer test, Indole test, Citrate test, Oxidase and catalase test.

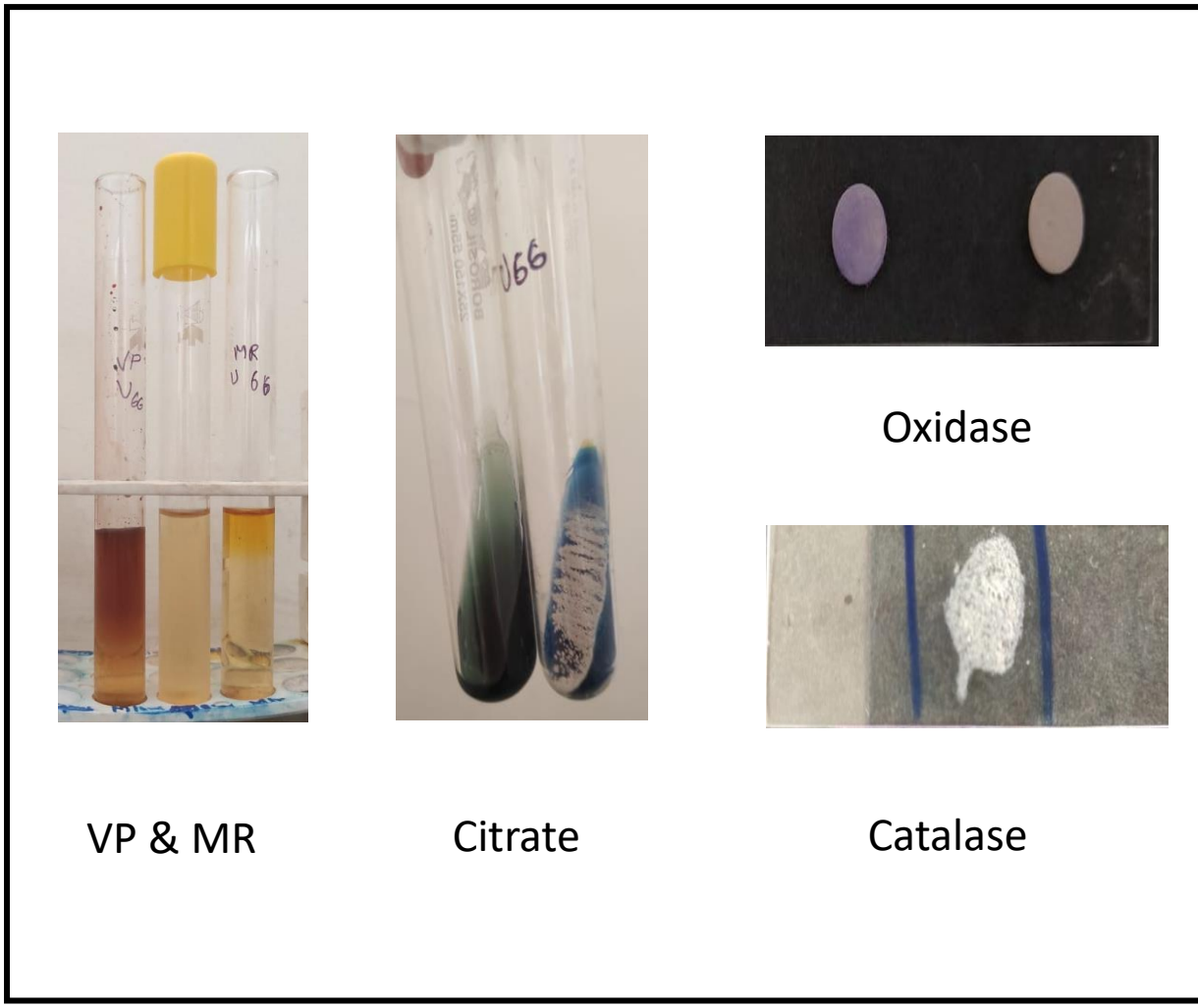


Fig. 15: Biochemical test of UA66

Fig.14 shows the image of biochemical test of UA66 isolate culture in this MR test give yellow colour. It indicates negative result, VP also negative, Indole negative, Citrate slant change colour green to blue it indicate citrate positive, oxidase disk change in blue colour it indicates positive result, in catalase test organism form bubble after adding hydrogen peroxide solution it indicates positive test.

4.6 Molecular identification

4.6.1 16s rDNA sequencing

The sample labelled **UA66** is closely related to *Streptomyces sp*. Based on nucleotide homology analysis. According to 16s rDNA sequencing UA66 is 99.95% similar to ***Streptomyces viridosporus***. The sequence data were submitted to the gene bank (NCBI) and can be accessed through the **Accession number OQ660494** for strain UMA66. phylogenetic tree of UA66 is shown in fig. 16

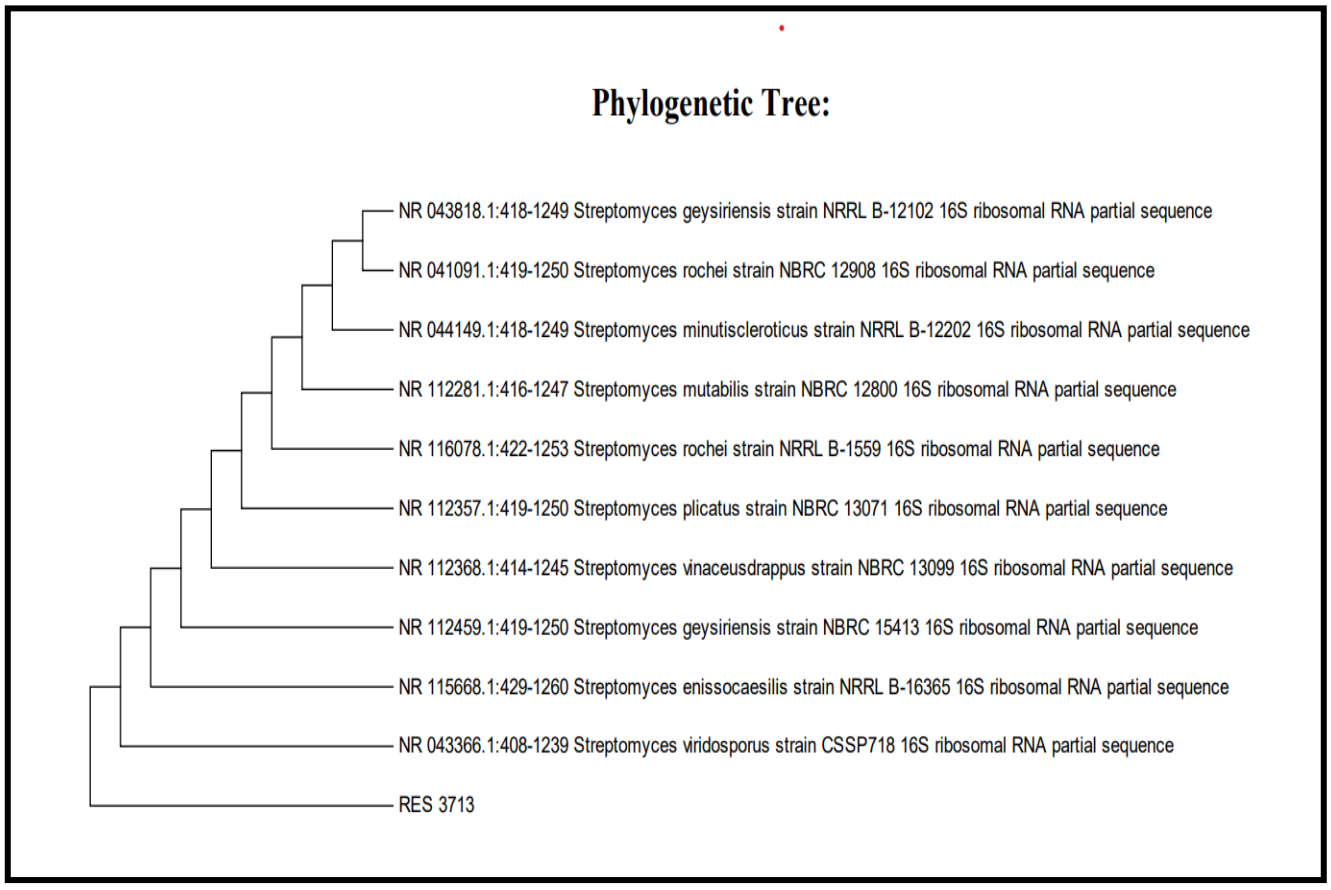
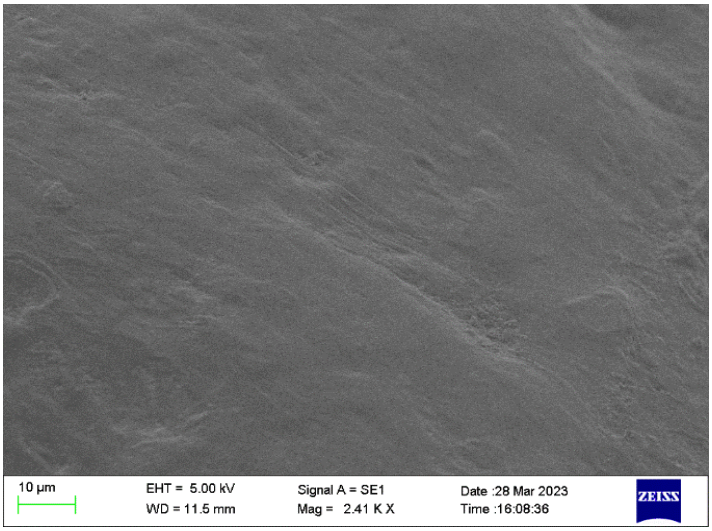


Fig. 16: Molecular phylogenetic analysis

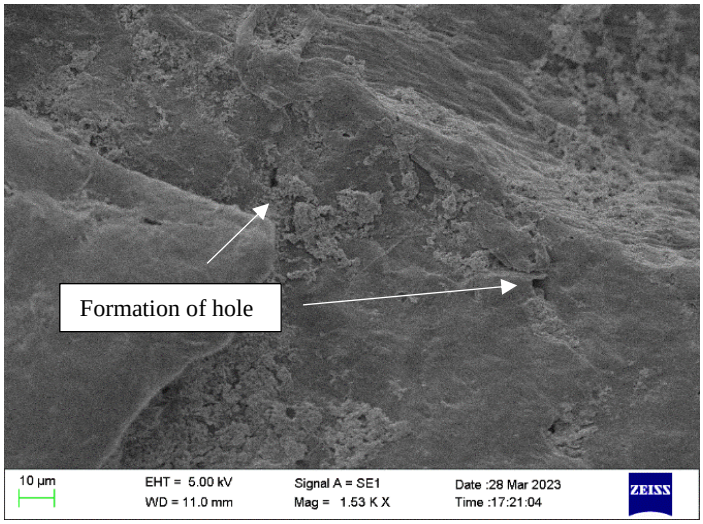
The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

4.6.2 Scanning Electron Microscope

The SEM analysis can reveal the changes in the morphology and surface structure of the plastic beads as they degrade. In the early stages of degradation, the surface of the beads may become rougher and more porous as the polymer chains begin to break down. As degradation progresses, the surface may become more cracked, fragmented, Formation of holes of LDPE structure confirmed degradation capacity of *Streptomyces spp*. (fig. 17)



A



B

Fig. 17: Scanning electron microscopy (A) control bead test (B) bead treated with UA66 culture

5.DISCUSSION

This study has covered the major concerns about the Low-density polyethylene, area examined has been the biodegradation of low-density polyethylene by the liquid culture method. It is clear that most recalcitrant polymers can be degraded to some extent in the appropriate environment at the right concentration. The present study deals with the isolation, identification and degradative ability of low-density polyethylene degrading Actinomyces from soil. Different types of changes are produced by the Actinomyces during morphological and biochemical analysis. In the present study pieces of low-density polyethylene were inoculated in the liquid culture medium containing actinomyces isolates and kept for 1 month to observe the percentage of weight loss by actinomyces. The result shows the degradative ability of the microorganisms after one month of incubation. The 5.5 percentage of weight loss due to degradation was found by *Streptomyces spp*.

Although the creation of the biofilm initially results in an increase in weight (0.02%) of the polyethylene, later measurements show that the polyethylene is being used as a carbon source leading to a sharp decrease in the weight of the LDPE strip. When weight was measured every two to six months, the bacterial isolates Pseudomonas sp., A. niger, A. flavus, and Streptomyces caused biodegradation ranging from 4.34% to 24.22%, 10.78% to 26.17%, 5.69% to 16.45%, and 12.42% to 46.7%, respectively. Streptomyces also displayed high degradability, measuring 12.42% to 46.7%. [Usha et al., (2011)]. [Singh et al., (2012)] carried out degradation of LDPE using Aspergillus fumigatus According to their work, Aspergillus fumigatus was able to degrade 4.65% of polyethylene. Aspergillus glaucus was the most active species overall, outpacing Aspergillus niger in its ability to break down 7.26% of plastics in a month. According to [Singh et al. (2012)], Penicillium sp. was more active in lowering LDPE (up to 6.58%) than A. fumigatus (which decreased weight by 4.65%). According to [Kathiresan & Bingham (2001)], the biodegradation rate of polythene by bacteria ranged from 2.19 to 20.54% and from 0.56 to 8.16% for plastics. According to (Raaman et al., 2012) The degradation rate of LDPE strip by aspergillus niger ranged from 5.8%.

The study conducted by (Zahra et al., 2010) aimed to investigate the potential biodegradation of low-density polyethylene (LDPE) by fungi isolated from solid waste. The

researchers collected various fungi strains from landfill waste and tested their ability to degrade LDPE in a solid waste medium. The researchers found that some of the isolated fungi strains showed significant biodegradation of LDPE after 30 days of incubation. The degradation was confirmed through visual observation, weight loss, and Fourier-transform infrared spectroscopy (FTIR) analysis, which showed changes in the chemical structure of the LDPE polymer. The study also investigated the effect of environmental factors such as temperature and pH on the biodegradation process. The results showed that the fungi strains were able to degrade LDPE at a wide range of temperatures and pH levels, suggesting that they could potentially be used in various environmental conditions. Overall, the study suggests that fungi isolated from solid waste have the potential to be used for the biodegradation of LDPE, which could have significant environmental benefits by reducing plastic waste accumulation. However, further research is needed to optimize the biodegradation process and determine the full potential of these fungi strains. The study also highlights the importance of proper waste management and the potential for using waste as a source of biodegrading organisms.

The study found that several bacterial strains were able to degrade LDPE effectively under laboratory conditions. The researchers confirmed the degradation of LDPE through weight loss, scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR) analysis. The SEM images showed surface changes in the LDPE film, and the FTIR analysis showed changes in the chemical structure of the polymer. The researchers also investigated the effect of environmental factors such as temperature, pH, and nutrient concentration on the biodegradation process. The results showed that the LDPE-degrading bacteria were able to function at a wide range of temperatures and pH levels, suggesting that they could be useful in various environmental conditions. Overall, the study suggests that bacteria isolated from soil contaminated with plastic waste have the potential to be used for the biodegradation of LDPE. However, further research is needed to optimize the biodegradation process and determine the full potential of these bacterial strains. The study also highlights the importance of proper waste management and the potential for using waste-contaminated soil as a source of biodegrading organism

6. CONCLUSION

In the current study, the experiment was conducted to detect the ability of actinomycetes as a plastic degradation and according to the results, it can be concluded that the plastic dumping site is the hub of the potent actinomycetes degrading low-density polyethylene. The primary and secondary screening proved that some actinomycetes can take up LDPE as a carbon source and further the clear zone assay proves that actinomycetes can degrade LDPE. SEM analysis confirmed the degradation of LDPE beads. Actinomycetes weight loss of 5.5%. It can be concluded that UA66 is a potent LDPE degrader. According to the 16sDNA sequencing, UA66 is closely related to Streptomyces sp.

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ACHIEVEMENT

No.	Achievement	Organization	Date
1	International online course on "MICROSCOPY- A N OVERVIEW"	Sacred heart college (autonomous) Tirupattur, Tamil-nadu	3/2/2023-9/2/2023
2	Attend national conference "EMERGING PARADIGM AGRICULTURAL MICROBIOLOGY"	Atmiya University, Rajkot, Gujarat.	11/2/2023
3	Poster presented in National conference "MICROBIOMES TO MECROMOLECULES: CONNECTING THE DOTES"	Gujarat university, Ahmadabad, Gujarat.	22/2/2023-23/2/2023
4	Sequence submission in NCBI	National Center for Biotechnology Information	27/3/2023

Poster presented in National conference

"MICROBIOMES TO MECROMOLECULES: CONNECTING THE DOTES"

Gujarat university,

Ahmadabad,

Gujarat

NATIONAL CONFERENCE

MICROBIOMES TO MACROMOLECULES: CONNECTING THE DOTS

February 22 & 23, 2023

CERTIFICATE

This is to certify that

Dr./Mr./Ms. Sakariya Uma

has actively participated in National Conference on "MICROBIOMES TO MACROMOLECULES: CONNECTING THE DOTS" organized by Department of Microbiology & Biotechnology, University School of Sciences, Gujarat University, Ahmedabad – 380 009 on 22nd & 23rd February 2023.

He / She presented paper Oral / Poster / only Attended under P.G. / Ph.D. / Faculty category.

Prof. (Dr.) Meenu Saraf
Convenor
Director, School of Sciences
Professor and Head,
Department of Microbiology and Biotechnology
Gujarat University

Dr. Rakeshkumar R. Panchal
Organizing Secretory
Associate Professor
Department of Microbiology and Biotechnology
Gujarat University

Prof. (Dr.) H. A. Pandya
Patron
Hon. Vice-chancellor
Gujarat University

Department of Science & Technology
Government of Gujarat

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TRANSCRIPTING BRIGHTER BIO FUTURE

Sequence submission in NCBI

Streptomyces sp. strain UMA66 16S ribosomal RNA gene, partial sequence

GenBank: OQ660494.1

FASTA Graphics

Go to:

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  AUTHORS   Sakariya,U.T., Yagnik,U.B., Bhuva,S.A., Bhatt,M.K. and Das,M.B.
  TITLE     Direct Submission
  JOURNAL   Submitted (21-MAR-2023) Department of Microbiology, Atmiya
            University, Kalawad Road, Rajkot, Gujarat 360005, India
COMMENT     Sequences were screened for chimeras by the submitter using
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