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## ORIGINAL ARTICLE

# Identification of Synthetic Plastic Degrading Bacteria From Soil Samples of East Gujarat

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**ABSTRACT**
*A polymer is a macromolecule composed of repeating structural units linked by covalent bonds. Plastic has replaced our habitual materials like wood, stone, horn, leather, paper, metal, glass, ceramics etc. Incineration, recycling and land filling are some of the common methods for handling plastic wastes. However, these methods are costly and often create new environmental and human health problems. So an attempt has been made to isolate the potent bacterium that degrades plastic from different soil environments. Results showed that there are 40 different bacterial isolated. From 40 bacterial isolates, 16 isolates were able to grow on MSM plus guaiacol plates indicating bacteria degrading synthetic plastic. Efficacy of the bacteria in degradation of plastics were analyzed in liquid (shaker) culture method and determined by (%w/w) decrement percentage. Bacterial isolates were identified using 16s-rRNA gene sequencing MEGA 10.0 software BLAST tool.*
***Key words:*** *Bacteria, biodegradation, environment, plastic.*

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## INTRODUCTION

The Greek word plastios, which means plastic - 'able to be molded into varied shapes' [1]. It is defined as the polymer which become mobile on heating and thus can be cast into varieties of moulds. The plastic is made up of silicon, oxygen, carbon, hydrogen, chloride and nitrogen. For extraction of the basic materials of plastics; coal, oil and natural gas are used [2]. Synthetic polymers are typically prepared from monomers derived from oil or gas by polymerization and by addition of various chemical additives; plastics are usually made from these. There are currently some 20 different groups of plastics, each with numerous grades and varieties [3]. The production of plastics has increased substantially over the last 60 years from around 0.5 million tonnes in 1950 to over 260 million tonnes today. In Europe alone the plastics industry has a turnover in excess of 300 million euros and employs 1.6 million people [4]. Almost all aspects of daily life involve plastics, in transport, telecommunications, clothing, footwear and as packaging materials that facilitate the transport of a wide range of food, drink and other goods [5].

Plastic are the product of the 20th century, they are largely synthetic materials made from crude oil, non-renewable and non-expensive resource. In their original forms, plastic were mimicking and replacing natural products such as lacquer, shellac, amber, horns, husks and tortoise shell [6]. Plastic is defined as the polymer which become mobile on heating and thus can be cast into moulds. Plastics are made up of linking of monomers together by chemical bonds. Polythene comprises of 64% of total plastic, which is a linear hydrocarbon polymer consisting of long chains of the ethylene monomers ($C_2H_4$). General formula of polyethylene is $C_nH_{2n}$, where 'n' is the number of carbon atoms [7]. Substantial quantities of plastic polymer have been accumulated in the natural environment and in landfills. Around 10% by weight of the municipal waste stream is plastic material [8]. Plastic debris causes aesthetic problems, and it also presents a hazard to maritime activities including fishing and tourism [9; 10]. Discarded fishing nets result in ghost fishing that may result in losses to commercial fisheries [9; 11]. Floating plastic debris can rapidly become

 

colonized by marine organisms and since it can persist at the sea surface for substantial periods, it may subsequently facilitate the transport of non-native or 'alien' species (8; 10; 12].

Synthetic plastics are extensively used in packaging of products like food, pharmaceuticals, cosmetics, detergents and chemicals. Approximately 30% of the plastics are used worldwide for packaging applications. This utilization is still expanding at a high rate of 12% per annum [13]. They have replaced paper and other cellulose-based products for packaging because of their better physical and chemical properties, such as their strength, lightness, resistance to water and most water-borne microorganisms. The most widely used plastics used in packaging are polyethylene (LDPE, MDPE, HDPE and LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), poly (ethylene terephthalate) (PET), poly (butylene terephthalate) (PBT), nylons. The widespread applications of plastics are not only due to their favorable mechanical and thermal properties but also mainly due to the stability and durability [14]. In fact, dioxins cause serious problems in the human endocrine hormone activity, thus becoming a major concern for the human health [15]. Dioxins also cause very serious soil pollution, causing a great concern for scientific community worldwide. Phthalates and Bisphenol A are closely related in thyroid causing dysfunction in humans. The burning of polyethylene, polyurethane, polyvinyl chloride and polystyrene produces toxic irritant products that lead to immune disorders and lung diseases, and are classified as possible human carcinogens [16]. The present study aimed to isolation and screening of synthetic plastic degrading bacteria from different soil environments after soil burial.

## MATERIALS AND METHODS

### Sample collection

**Soil sample:** Soil sample were collected from different areas of Ahmedabad and Dahod, brought to the lab under preserved condition for further use. Area of Ahmedabad: Paldigam (a) Near Lake (b) Rhizopheric soil of Banyan tree; Area of Dahod: (1) Nursery(2) Dumping site (3) Muvalia (4) Nimlania

**Plastic sample:** Five different types of plastics were procured such as (1) Plastic water bottle (2) Polythene bag from vegetable vendor (3) Plastic Packing bag (4) Zip lock bag (5) 20µ polythene bag.

### Preparation of Burial Activity for Biodegradation:

The plastics samples were washed to remove the oils and spread out to air dry. After drying, it was cut into a piece of 10 cm x 2 cm for the laboratory study. Glass Bottle was used for submerged activity. A piece of plastics were added in the soil solution in different soil solution flask having 10 g of soil in 100 ml distilled water, Mineral salt Medium (MSM) containing (g/l) $K_2HPO_4$, 1g; $KH_2PO_4$, 0.2g; NaCl, 1g; $CaCl_2.2H_2O$, 0.002g; boric acid, 0.005g; $(NH_4)_2SO$, 1g; $MgSO_4.7H_2O$, 0.5g; $CuSO_4.5H_2O$, 0.001g; $ZnSO_4.7H_2O$, 0.001g; $MnSO_4.H_2O$, 0.001g and $FeSO_4.7H_2O$, 0.01g. were added to the system for enrichment devoid of any carbon source, kept at room temperature for 3 month under shaking condition.

### Primary screening of Bacteria:

Serial dilutions were prepared from incubated bottles until $10^{-7}$. The dilutions were spreaded on the Nutrient agar plates by spreading method. Then incubated at room temperature. 40 isolates of bacteria were isolated.

### Secondary screening of Bacteria:

The isolated bacteria were then inoculated on Mineral salt Medium (MSM) containing (g/l) $K_2HPO_4$, 1g; $KH_2PO_4$, 0.2g; NaCl, 1g; $CaCl_2.2H_2O$, 0.002g; boric acid, 0.005g; $(NH_4)_2SO$, 1g; $MgSO_4.7H_2O$, 0.5g; $CuSO_4.5H_2O$, 0.001g; $ZnSO_4.7H_2O$, 0.001g; $MnSO_4.H_2O$, 0.001g and $FeSO_4.7H_2O$, 0.01g. and Guaiacol media, devoid of any carbon source. Out of 40 isolates 16 isolates were able to grow on MSM plus Guaiacol plates.

### Identification of selected isolates

The 16 bacterial isolates were then identified macroscopically (colony morphology, surface pigment, shape, size, margin, surface), microscopically (Gram staining, shape, cell arrangement, granulation, presence of spore, motility) and biochemically on the basis of Bergey's Manual of Determinative Bacteriology.

### Microbial degradation of synthetic plastic under laboratory conditions

Selection was done on the basis of weight loss method. 100 ml MSM plus Plastic strip of 20 µg (2 x 5) cm; of weight 1 gm plus each bacterial isolates (VC2, VC4, VC6, VC8, VC10, VC12, VC14, VC16) was used. Under shaking condition for 3 months, then plastic was weighted to evaluate for any change in weight.

Percentage of decreasing of plastic film weight counted with formula:

$$\% \text{ decrease of plastic weight} = (R1 - R2) / R1$$

whereas: R1 = Initial Weight of Plastic Film;

R2 = Final Weight of Plastic Film

### Identification of bacterial strain

Three bacterial strains were then identified using 16s-rRNA gene sequencing MEGA 11 software BLAST and submitted to the NCBI GENE BANK.

 

## RESULTS AND DISCUSSION

Primary and Secondary screening of Bacteria

To isolate the bacterial strains capable of degrading synthetic plastic, soil sample was collected from six different sites and five plastic samples were procured. A piece of plastics each were added in the soil solution in different soil solution flask having 10 g of soil in 100 ml distilled water, Mineral salt Medium (MSM) was added to the system for enrichment devoid of any carbon source. Serial dilutions were prepared from from incubated bottles as per requirements then spreaded on the Nutrient agar plates by spreading method. 40 isolates of bacteria were isolated. Isolated bacteria were then inoculated on Mineral salt Medium (MSM) and Guaiacol media, devoid of any carbon source. Out of 40 isolates 16 isolates were able to grow on MSM plus Guaiacol plates showing zone of clearance, used Guaiacol as a only carbon source.

### Identification of selected isolates

The 16 bacterial isolates were then identified macroscopically (colony morphology, surface pigment, shape, size, margin, surface) (Table 1), microscopically (Gram staining, shape, cell arrangement, granulation, presence of spore, motility) (Table 2) and biochemically (Table 3) on the basis of Bergey's Manual of Determinative Bacteriology.

**Table 1: Macroscopic study of isolated bacteria**

| Isolate no. | Shape | Size | Pigment | Margin | Texture | Elevation | Opacity | Special Characteristic |
|---|---|---|---|---|---|---|---|---|
| **VC1** | R | S | Cream White | Even | Smooth | Raised | Opaque | -- |
| **VC2** | R | B | Light Peach | Uneven | Smooth | Flat | Opaque | -- |
| **VC3** | R | M | Nil | Even | Smooth | Raised | Translucent | -- |
| **VC4** | R | S | White | Even | Smooth | Flat | Opaque | gives orange pigment at low temp. |
| **VC5** | R | M | White | Uneven | Rough | Flat | Opaque | -- |
| **VC6** | R | B | Off-White | Even | Smooth | Convex | Opaque | -- |
| **VC7** | R | S | White | Even | Smooth | Flat | Opaque | -- |
| **VC8** | R | M | White | Uneven | Smooth | Flat | Opaque | -- |
| **VC9** | R | M | White | Even | Smooth | Flat | Opaque | -- |
| **VC10** | R | S | Off-White | Uneven | Rough | Flat | Opaque | |
| **VC11** | R | M | Orange | Even | Smooth | Raised | Opaque | Mucoidal colony |
| **VC12** | R | L | Greenish blue | Uneven | Smooth | Convex | Translucent | -- |
| **VC13** | R | S | White | Even | Smooth | Flat | Opaque | -- |
| **VC14** | R | M | Nil | Even | Smooth | Flat | Opaque | -- |
| **VC15** | R | S | Nil | Even | Smooth | Raised | Translucent | -- |
| **VC16** | R | S | Pink | Even | Smooth | Raised | Translucent | Mucoidal colony |

**Table 2: Microscopic study of isolated bacteria**

| Isolate no. | Straight Rod | Cocci | Gram stain | Cell arrangement |
|---|---|---|---|---|
| **VC1** | + | - | - | Chain/ Single |
| **VC2** | + | - | + | Chain/ Single |
| **VC3** | + | - | - | Chain/ Cluster |
| **VC4** | - | + | + | Cluster |
| **VC5** | + | - | + | Chain |
| **VC6** | + | - | + | Single/ Chain |
| **VC7** | + | - | + | Single |
| **VC8** | + | - | + | Single/ Cluster |
| **VC9** | + | - | + | Chain/ Single |
| **VC10** | + | - | + | Single/ Cluster |
| **VC11** | + | - | + | Cluster |
| **VC12** | + | - | - | Single/ Chain |
| **VC13** | + | - | + | Chain/ Cluster |
| **VC14** | + | - | + | Single |
| **VC15** | + | - | + | Cluster |
| **VC16** | + | - | - | Cluster/Single |

 

**Table 3: Biochemical test of isolated Bacteria**

| Test / Isolate No. | Motility | Urea Utilization | Nitrate reduction | Lipid | Starch | Indole | Citrate | MR Test | VP Test | TSI | Catalase |
|---|---|---|---|---|---|---|---|---|---|---|---|
| VC1 | - | - | - | - | - | - | - | - | - | + | + |
| VC2 | + | + | - | - | + | - | + | - | + | - | + |
| VC3 | + | + | + | - | + | + | + | - | - | + | + |
| VC4 | + | + | - | - | - | - | - | - | - | - | + |
| VC5 | - | - | + | - | + | - | - | - | - | - | + |
| VC6 | + | + | + | v | + | - | + | - | - | + | + |
| VC7 | - | - | - | - | - | - | - | - | - | - | - |
| VC8 | + | - | - | + | + | - | + | - | + | | + |
| VC9 | + | - | | - | - | - | + | - | - | + | + |
| VC10 | + | - | + | - | - | - | v | - | + | - | + |
| VC11 | - | v | + | - | - | - | + | - | + | - | + |
| VC12 | + | + | + | - | + | + | + | - | + | - | + |
| VC13 | - | + | + | - | - | + | + | + | + | + | + |
| VC14 | - | - | + | - | - | - | - | - | + | - | + |
| VC15 | + | + | + | - | - | - | + | + | + | + | + |
| VC16 | + | - | - | - | + | + | - | + | + | - | + |

**Table 4: carbohydrate test of isolated Bacteria**

| Test / Isolate No. | GLUCOSE | FRUCTOSE | MALTOSE | LACTOSE | SUCROSE | MALTOSE | MANNITOL | MANNOSE | ADONITOL | ARABITOL | CELLOBIOSE | SORBITOL | TAGATOSE | TREHALOSE | RIBOSE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VC1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| VC2 | + | - | - | - | - | - | + | + | - | - | - | - | - | - | - |
| VC3 | + | - | - | - | - | - | + | + | + | - | - | - | - | - | - |
| VC4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| VC5 | + | + | + | + | + | + | - | - | - | - | + | + | - | - | + |
| VC6 | + | + | + | - | v | - | - | - | - | - | v | - | - | + | + |
| VC7 | + | + | + | + | + | + | - | - | + | + | - | - | v | + | + |
| VC8 | - | - | - | - | - | - | + | + | - | - | + | - | - | + | - |
| VC9 | - | - | + | - | - | + | - | - | - | - | + | + | + | + | + |
| VC10 | + | + | + | v | + | + | + | + | - | - | + | + | v | + | + |
| VC11 | - | + | + | - | - | - | + | + | - | - | + | - | - | + | - |
| VC12 | + | - | - | - | - | - | + | + | - | - | - | - | - | - | - |
| VC13 | + | - | + | - | - | + | + | + | + | + | + | - | - | - | - |
| VC14 | + | v | + | + | v | - | - | - | - | + | + | + | v | + | - |
| VC15 | - | + | - | - | - | - | + | + | + | + | + | + | + | + | + |
| VC16 | + | - | - | - | - | + | + | + | + | + | + | v | - | + | - |

**Microbial degradation of synthetic plastic under laboratory conditions**

The screened isolates were inoculated into 100 ml of MSM broth in a conical flask along with pre-weighed, sterilized 20 µg of size 2 x 5 cm weighed, washed with sterile distilled water and air dried. Initial weights

 

for all the pieces were checked. The flasks were incubated in a shaker for 3 month. After incubation the plastic pieces were removed, washed with sterile distilled water, then sprayed with alcohol, air dried and weighed. The final weight of the polyethylene strips were noted and percentage degradation was calculated (Table 5).

**Table 5: Microbial degradation of synthetic plastic under laboratory conditions**

| **Isolate no.** | Initial weight (gm) | Final weight (gm) | Weight loss (Difference) in % |
|---|---|---|---|
| **VC2** | 1 | 0.98 | 0.02 |
| **VC4** | 1 | 0.97 | 0.03 |
| **VC6** | 1 | 0.95 | 0.05 |
| **VC8** | 1 | 0.95 | 0.05 |
| **VC10** | 1 | 0.98 | 0.02 |
| **VC12** | 1 | 0.96 | 0.04 |
| **VC14** | 1 | 0.99 | 0.01 |
| **VC16** | 1 | 0.97 | 0.03 |

**Identification of bacterial strain**

The top three isolates with maximum weight loss – VC6, VC8 and VC12 were selected. The gene sequence of the isolated strains VC6, VC8 and VC12 were analyzed with its closely related sequences using NCBI BLAST tool and identified as *Bacillus cereus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Phylogenetic tree exhibiting evolutionary relationship of the isolate is shown in Figure 1; Figure 2; Figure 3.

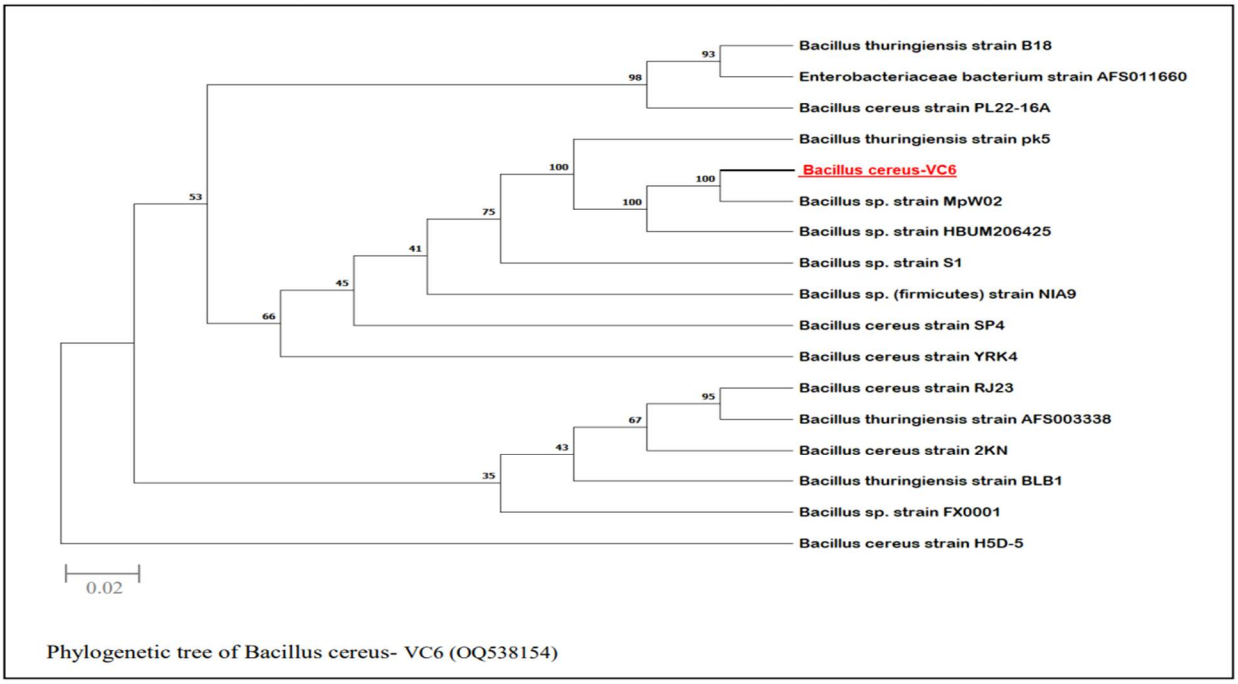


**Figure 1: Phylogenetic tree of Bacillus cereus- VC6 (OQ538154)**

 

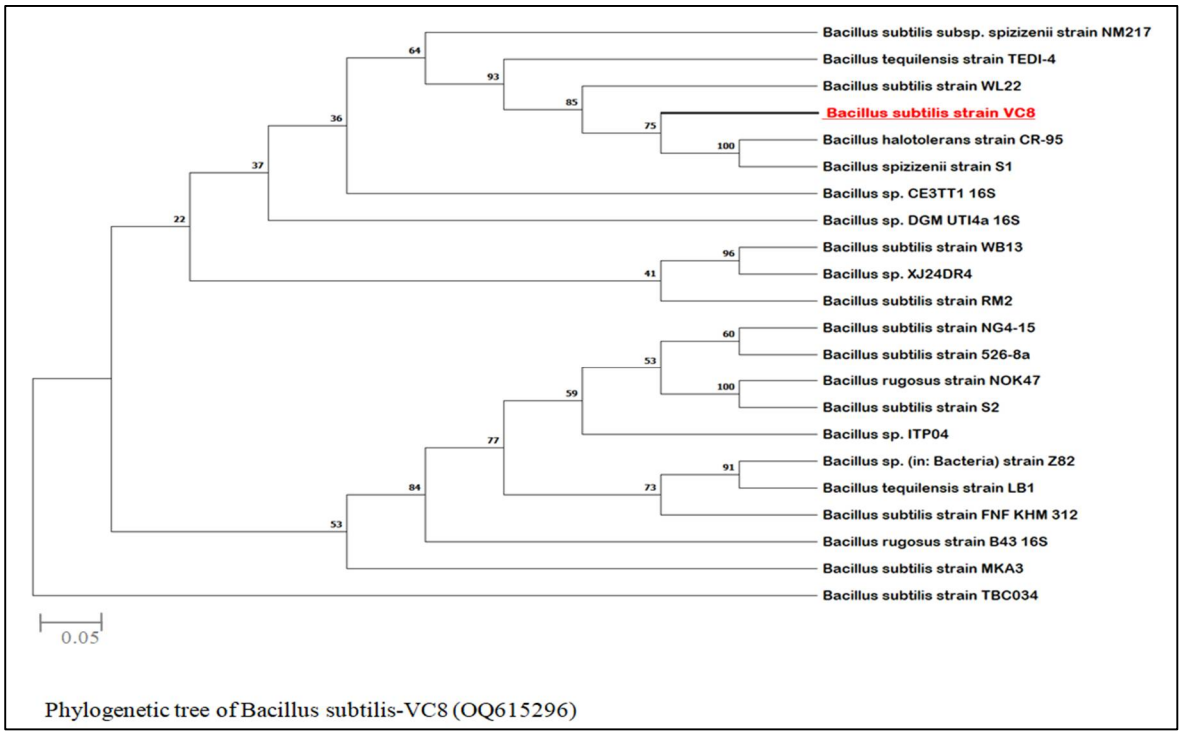


**Figure 2: Phylogenetic tree of Bacillus subtilis-VC8 (OQ615296)**

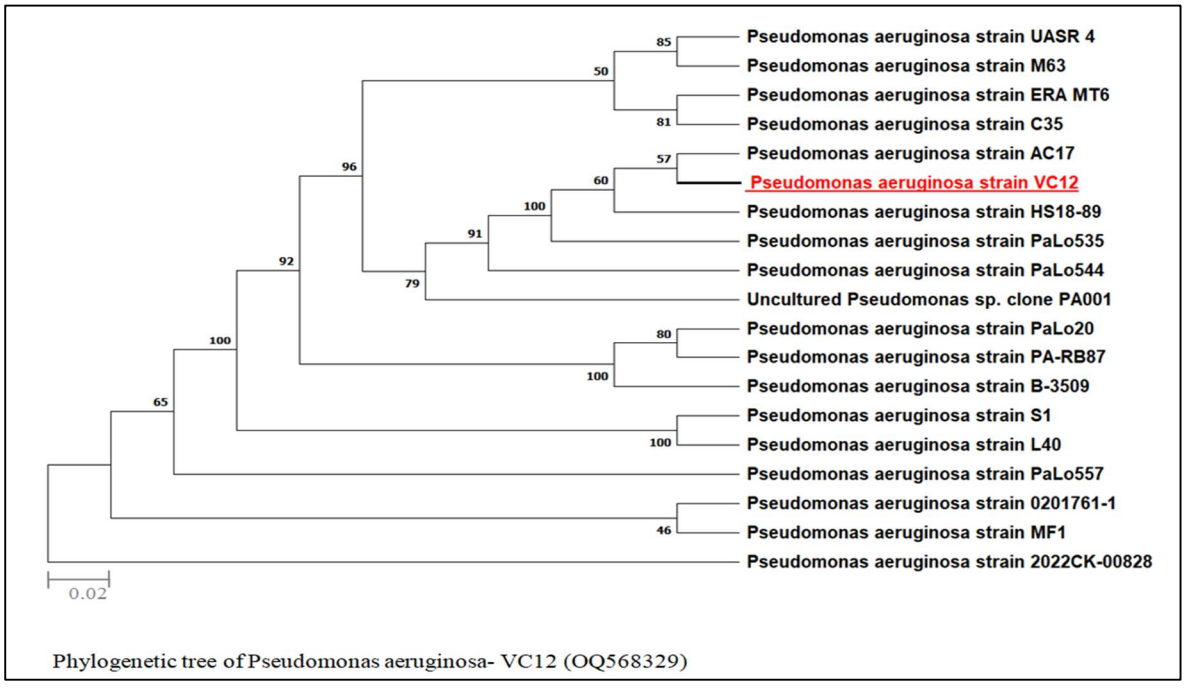


**Figure 3: Phylogenetic tree of Pseudomonas aeruginosa- VC12 (OQ568329)**

Hadad et al., (2005) reported the *Desulfotomaculum nigrificans* degrade 10.2%, 13.2% and 16.2 % of polythene bag at 10, 20 and 30 days incubation respectively. At the same time *P. alcaligenes* degraded 10.5%, 14.7% and 16.2 % of polythene bag at 10, 20 and 30 days incubation respectively. An increase in incubation period there is a dramatic increase in weight loss of polythene bag. Among the two isolates tested, *P. alcaligenes* was found to be more effective in degradation of polythene bag at 30 days [17]. Previously, Norman et al., (2002); Tadros et al., (1999); have reported on the biodegradability potential of *P. fluorescens* and *P. aeruginosa* on synthetic plastics [18; 19]. After pretreatment with nitric acid, *P. aeruginosa* was able to degrade 0.25 gram of LDPE by 50.5% in 2 months [20]. However, no chemical pretreatment was needed for *Pseudomonas* spp. AKS2 to degrade LDPE films, albeit only 5% of the total

 

mass of 300 mg was degraded within 45 days [21]. Also without any pretreatment, an uncharacterized *Pseudomonas* spp. was found to degrade 28.6% of low MW PE (MW 1700 Da) in a sterilized compost condition after 40 days [22].

According to previous study *Bacillus cereus* was able to degrade polyethylene, LDPE and polythene carry bags with the efficiency of 7.2%, 17% and 12.5% respectively [23]. This condition caused by different activity of depolymerase enzyme for each bacteria isolates . In Screening procedure, Augusta et al. (1993); reported that the zone of clearance around the colony is due to extracellular hydrolyzing enzymes secreted by the target organism into suspended polyesters agar medium [24]. On the contrary, from the study of Vatsel and Anbuselvi (2014) different strains was recovered and were identified as *E.coli*, *Staphylococcus*, *Pseudomonas*, *Klebsiella* and *Bacillus*. The isolated microorganisms from polyethylene dumped areas can be interacted with polythene and undergo changes in mechanical properties of tensile strength, optical changes of cracking, erosion and decolorization. It is clear that natural polymers can be degraded to some extent by microbes. The biodegradation of plastics by isolated bacteria showed clear zone. It shows the initiation of biodegradation. Maximum degradation was found to be by *Staphylococcus* species and the minimum degradation was found to be by pseudomonas species. *Staphylococcus* showed 52% degradation and pseudomonas showed 11% degradation by weight loss [25].

## CONCLUSION

Bacteria isolated from soil have the ability to degrade plastic. The present study implicates the ability to degrade environmental-friendly plastic and utilizing it as sole carbon source is different in different species. The gene sequence of the isolated strains VC6, VC8 and VC12 were analyzed and identified as *Bacillus cereus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* with percentage degradation (w/w) as 0.05%, 0.05% and 0.04%, in 3 months respectively. It can be concluded that the soil contains potential candidates for bioremediation of plastic waste and it will reduce solid waste which causes environmental issues.

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