Isolation And Biochemical Characterization of Halophiles from Brine Pit and Examining Its Plastic Degrading Capasity

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***Abstract*—Plastics have caused a great deal of ecological problems that have attracted a lot of attention worldwide. Plastic products are useful and they bring convenience to our lives. However, as a persistent pollutant, they can remain in natural environment for hundreds of years or longer. Biodegradation is the process of degradation of large polymer molecules by groups of living organisms, some of which break down the polymer chain into oligomers and monomers. Others are able to use these products, converting them to simpler waste compounds, and still others are able to use the excreted wastes. Microbial degraders and their metabolic enzymes are among the environmental agents that participate in the degradation process, which results in a conversion of the carbon in the polymer chains into smaller biomolecules or into carbon dioxide and water. Halophiles are organisms represented by archaea, bacteria, and eukarya for which the main characteristic is their salinity requirement, halophilic "salt-loving". Halophilic microorganisms constitute the natural microbial communities of hypersaline ecosystems, which are widely distributed around the world. Halophiles are having the significant capacity to degrade the plastics.**

***Index Terms*—Halophiles, Plastic degradation, Environmental pollution.**

I. INTRODUCTION

Plastics have caused a great deal of ecological problems that have attracted a lot of attention worldwide. Plastic products are useful and they bring convenience to our lives. However, as a persistent pollutant, they can remain in natural environment for hundreds of years or longer (Magalhães, S et al., 2020). Biodegradation is the process of degradation of large polymer molecules by groups of living organisms, some of which break down the polymer chain into oligomers and monomers. Others are able to use these products, converting them to simpler waste compounds, and still others are able to use the excreted wastes. Microbial degraders and their metabolic enzymes are among the environmental agents that participate in the degradation process, which results in a conversion of the carbon in the polymer chains into smaller biomolecules or into carbon dioxide and water (Mir, S et al.,2017). In this way, they contribute to soil fertility, decrease plastic accumulation in the environment, and reduce the cost of waste management. Furthermore, biodegradable plastics could be useful for the production of valuable metabolites (monomers and oligomers) (Tokiwa et al., 2009). The biodegradation rate is strongly influenced by several polymer properties, such as the presence of branching and additional functional groups that promote higher hydrophilicity; the relative share of crystalline and amorphous regions; the presence of polar covalent bonds, such as ester or amide bonds, instead of carbon–carbon bonds; the molecular weight and length of the carbon chain; the size and form of the substrate (powder, fibers, pellets, films); environmental agents like UV, pH, temperature, and moisture; and the enzyme characteristics (Ahmed, T et al., 2017). Additionally, the rigidity could be increased by antioxidants and stabilizers added during plastic production. According to the rate of biodegradation, plastics are divided in two main groups: biodegradable, characterized by a higher rate process, and non-biodegradable, the biodegradation of which is slow or

mediated by a still-unknown process. Most currently used fossil-based plastics are non-biodegradable, e.g., PE, PP, PS, and PVC. As large molecules of polymers cannot enter directly into the microbial cell, microorganisms responsible for primary degradation carry out extracellular degradation, and the received intermediates are further degraded by secondary degraders. (Dussud et al., 2021).

II. METHODOLOGY

Collection of Samples

Sediment sample was collected in a sterile container from the brine pit located at Nadhi paalam area, Ramanathapuram District, Tamil Nadu, India. The collected samples were processed immediately.

Isolation of halophiles

Halophilic bacteria were isolated from the salt pans water sample using nutrient agar medium with 20% Salt (Yeast extract - 2g/L, Peptone - 5g/L, Beef extract - 1g/L, Sodium chloride – 25% , Agar - 20g/L with final pH (at 25°C) 7.2±0.2. Ten grams of sediment sample was suspended in 90 ml of sterile distilled water blank and shaken vigorously for 15 minutes. It was serially diluted up to 10^{-5}. Then 100 µl of sample was taken from the dilution 10^{-1} to 10^{-5}. The plates were incubated at 37 ± 2°C for 7 days. After incubation, pigmented colonies were selected and sub cultured.

Phenotypic characteristics of halophiles

The isolates were examined for colony and cell morphology. Colony morphology was focused on pigmentation, surface and shape. Further, the isolates were stained by differential staining, The biochemical tests like indole, Methyl red, Voges-Proskauer, Citrate utilization, Urease, Catalase, Oxidase, Starch and Caesin utilization were performed

III. PLASTIC DEGRADATION BY HALOPHILES

Plastic degradation by halophiles, begin by preparing a Nutrient agar medium with 20% NaCl in sterile Petri dishes, which will support the growth of halophilic bacteria. Inoculate the mediumwith a halophilicbacterial strains (*HA1, HA2, HA3 & HA4)* and sterilize 1.5 cm diameter plastic pieces (from by cleaning them with ethanol, then allow them to dry. Incubate at the optimal temperature (usually 30-37°C) for 3 to 7 days until bacterial growth is visible. Incubated plates are regularly check for any physical changes in the plastic such as cracking, discoloration, or biofilm formation, which can indicate bacterial activity and plastic degradation. The weigh of plastic samples before and after the incubation period to assess the extent of degradation by monitoring any changes in their weight. Additionally, bacterial growthobserve around the plastic, which suggests microbial colonization and potential plastic degradation. A control setup should be prepared by placing plastic in the same conditions but without the halophilic bacteria, to compare degradation with the experimental group. Regular observations should be made, and any physical or microbial changes should be documented over time.

IV. RESULT AND DISCUSSION

Collection of Sample

Halophiles are organisms that can tolerate and survive under moderate to high saline conditions. The microorganisms survive under those condition produce several coloured pigments, which in turn has several biotechnological potentials. In this study, totally 2 sediments samples were collected from different parts of the brine pit located at Nadhi paalam area, Ramanathapuram District, Tamil Nadu, India.

Fig. 1: Collection of sediment samples from brine pit, Ramanathapuram District, Tamil Nadu, India

Isolation of halophiles

Halophilic bacteria were isolated using halophilic agar medium prepared with distilled water. The plates were incubated and observed after seven days, it showed morphologically different colonies. The serially diluted sample yielded approximately 50 × 106 CFU/g. The morphology of the isolated colonies

was distinctive. Most of them were white, yellow, creamy and pale yellow in color.

Fig. 2: Pure cultures of pigment producing halophilic bacteria

The shape of the colonies was circular, irregular and the surface of the colonies was smooth, mucoid in nature. Four different yellow-colored colonies (HA 1, HA 2, HA 3, HA 4) were taken for further studies.

Phenotypic characteristics of halophiles

The sediment samples of salt pan showed circular colonies. Most of them were smooth, shiny, mucoid. The gram staining results showed many gram-negative rods.

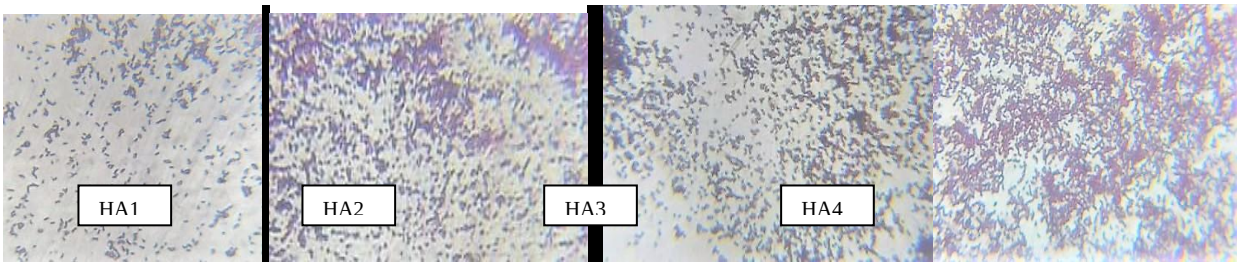

Fig. 3: Gram staining

Table 1: Morphology of isolated halophiles

Isolates	Colony color	Form	Surface	Gram stainig
H 1	Yellow	Circular	Slimy, Mucoid	Negative rods
H 2	Pale yellow	Circular	Slimy	Negative rods
H 3	Creamy yellow	Circular	Slimy	Negative rods
H 4	White	Circular	Mucoid	Negative rods

Biochemical characterization

The biochemical results showed in Table 2. The colony morphology, gram staining and biochemical results of the isolated halophilic bacteria were compared with bacterial identification key, Bergey's manual.

Table: 2 Biochemical results of pigmented halophilic bacteria

Isolates	Indole	MR	VP	Citrate	Urease	Amylase	Catalase	Oxidase	Protease
HA 1	-	+	-	+	+	+	+	-	+
HA 2	-	+	-	+	+	+	+	-	+
HA 3	-	+	-	+	+	+	+	-	+
HA 4	-	+	-	+	+	+	+	-	+

V. PLASTIC DEGRADTION

Observations showed robust growth around and on the plastic surface. Over time, the surface of the plastic bag showed visible signs of rupture and degradation. The plastic material exhibited surface cracks and damage, suggesting the halophiles' potential role in plastic degradation. After a period of incubation (7 days at 37ºC), significant microbial growth was observed around the plastic surface. Compared to other halophiles HA1 showed the significant result in plastic degradation. This degradation suggests that the halophiles are capable of breaking down the polymer chains of the plastic material, possibly through enzymatic action that weakens the integrity of the plastic.

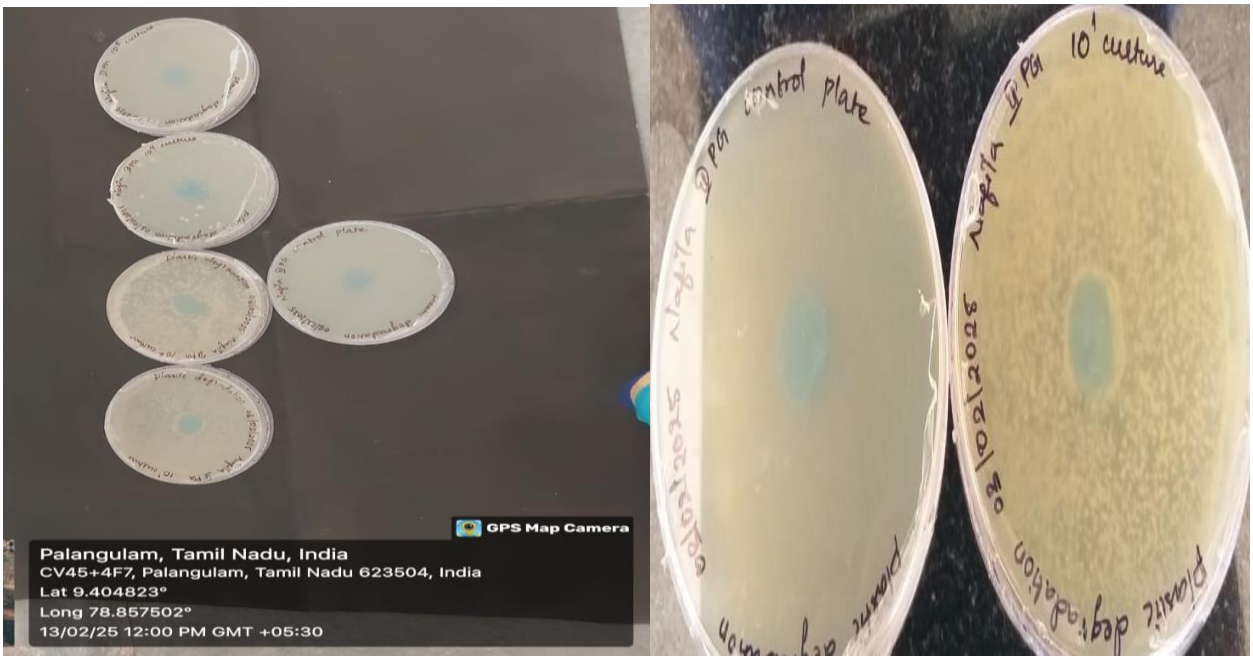

Fig. 4: Plastic degradation by Halphiles

VI. DISCUSSION

Halophiles (“salt-loving”) constitute the natural microbial communities of hypersaline ecosystems, which are widely distributed around the world which are classified in three different categories: slight (1–3%); moderate (3–15%); and extreme (15–30%). The halophiles are also gaining environmental importance because of their greater use in several bioremediation processes (Manjula et al., 2018). Plastics have caused a great deal of ecological problems that have attracted a lot of attention worldwide. Plastic products are useful and they bring convenience to our lives. However, as a persistent pollutant, they can remain in natural environment for hundreds of years or longer. By using Halophiles to degrade the plastic is the very efficient and cost-effective method.

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VIII. ETHICAL STATEMENT

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Conflict of interest: Authors declared no conflict of interest
Ethical Approval: There is no ethical issue to do this Project.

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