INTERNATIONAL SEVEN JOURNAL OF MULTIDISCIPLINARY
ISSN: 2764-9547

Microbial consortia and polyethylene terephthalate degradation

Consorcios microbianos y degradación de polietilentereftalato

DOI: 10.56238/isevmjv2n5-010
Receipt of originals: 24/08/2023
Publication Acceptance: 09/15/2023

Leticia Guadalupe Navarro Moreno
Full-Time Research Professor, Tuxtepec Campus - Oaxaca, Mexico
E-mail: navarroleticia483@gmail.com

Andrea Rangel Cordero
Chemical Analyst at the Department of Microbiology of the Salvador Zubirán National Institute of Medical Sciences and Nutrition in Mexico City

Luis Felipe Collado
Luis Felipe Collado is an Intern of the Bachelor's Degree in Chemical Sciences at the University of Papaloapan, Tuxtepec Campus

Cirilo Nolasco Hipólito
Dr Cirilo Nolasco Hipólito is an expert in microbiology and works in the Bachelor's Degree in Food at the University of Papaloapan, Tuxtepec Campus

ABSTRACT
The world is currently facing a very serious pollution phenomenon, which is already part of our daily lives. Poorly discarded plastic becomes tons of waste that, when not properly disposed of, generate serious problems worldwide. An example of this is the damage to marine species. To reduce plastic pollution, especially from polyethylene terephthalate (PET) bottles, recycling techniques such as chemical degradation have been established. In an alkaline PET gradient, developed in the Biochemistry Laboratory of the University of Papaloapan, microbial growth with the appearance of mycelium was observed. The observed cluster was allowed to grow and some microbial strains were isolated and identified using microbiological and molecular biology methodologies. The results indicated the presence of several types of microorganisms, mostly fungi, that were part of the grouping formed in the alkaline residue. A series of studies have been initiated with these organisms with the aim of finding out how they develop the capacity to adapt to the degradation of PET as the only source of carbon. The importance of isolated microorganisms has opened up a wide range of questions related to biochemistry and microbial genetics; Evolution and biological phenomena related to environmental pollution.

Keywords: Polyethylene terephthalate (PET), Pollution, Microbiology, Microorganisms.

1 INTRODUCTION

We live surrounded by plastic, a material invented 150 years ago that was a revolution because it was resistant, light and cheap. For this and other reasons, polyethylene, polyester, polypropylene or polyvinyl chloride, among other plastics, are primarily present in our lives, day to day, more than we could imagine. These materials are used as building blocks for various everyday objects. In the same way, they are present in vehicles, in food processing and packaging, in mobile phones, in clothing, in the composition of many cosmetics and even in the utensils we use to eat (Libera, 2019). Due to the above, currently, throughout the world, there is a significant problem due to the contamination of water, air and soil, caused to a large extent by the large volumes of waste that are generated daily and that receive little or no adequate treatment. This situation has been aggravated by the fact that garbage, which is made up of waste of very varied composition, is generally collected and mixed during collection work, which makes it difficult to handle it in the end. There are numerous countries that have reported the above and have become aware of the problems that this phenomenon can generate, one of them is Peru, a country where they have studied water pollution as a result of pollution by various factors, including solid waste (Bendezu-Bendezu & Bendezu-Hernández, 2021).

Globally, it is known that 75% of plastics produced are wasted. This has been released by the report *Solving Plastic Pollution Through Accountability*. It has been estimated that, on average, 8 million tons of this waste reach the various seas of the world every year, bringing problems to more than 270 marine species, many of which end up entangled in this waste; in addition to the fact that more than 240 species have ingested these plastics through their food and water (WWF, 2019). The study group led by Rivera-Garibay indicated that the plastics industry is an example of overconsumption, due to the use of multiple packages, each one single-use, where producers hook consumers with the idea of recycling, but do not indicate the procedure to follow or a strategy for collecting the containers already used. This causes people to become responsible for the final destination of the waste. Eradicating the cultural pattern of "use and discard" is the main objective of Greenpeace's "Plastic-Free Oceans" campaign (Rivera-Garibay et al., 2020).

Another problem of pollution with plastic waste dumped into the sea is related to its fragmentation. When this happens, several phenomena can be generated, the first is its rupture and the second its accumulation on the seabed. In this way, they could be confused by fish, who will use them as food, thus including them in the food chain (Barboza et al., 2018). It is estimated that between 11.6 and 21.1 million tons of polyethylene, polystyrene and polypropylene microplastics are found in the Atlantic Ocean alone, in suspended water and in the upper 200 meters (Pabortsava & Lampitt, 2020).

The *Gyres Institute*, an advisory body to the United Nations Economic and Social Council, stated that there are areas of plastic accumulation in each of the five subtropical gyres located in the North and South Pacific, the North and South Atlantic, and the Indian Ocean (Eriksen et al., 2014). These areas are commonly referred to as "plastic islands," the largest and most well-known of which is the Great Pacific Garbage Patch. In 2013, *The Ocean Cleanup was founded*, whose main objective is to clean 50% of the Great Pacific Garbage Patch every 5 years based on ocean currents (Boyan Slat, 2013; The Ocean Cleanup, 2012). This system has only managed to capture 2 metric tons of discarded fishing nets, but no significant amounts of other plastic materials have been obtained as the plastic captured by this system is only retained for a few days. This is an example of the development of large-scale projects to try to solve this problem, although it includes some drawbacks such as the fact that this system can capture algae, jellyfish and fish eggs that are on the surface of the water, thus affecting the diet and populations of certain species.

In addition, the pollution of plastics in the environment has led to the production of microplastics as a side effect. Microplastics are defined as plastic fragments smaller than 5 mm. Nano plastics are even smaller, with a diameter of less than 0.05 mm. In addition to size, the European Chemicals Agency (ECHA) sets out some criteria for identifying microplastics. 1) Type (synthetic polymeric materials, chemically modified biopolymers), 2) state (solid, semi-solid) and 3) morphology (spheres, fibers, sheets). The sources of microplastics can be primary, where the microplastic is produced as is in the cosmetic industry (in the formulation of exfoliants, toothpaste, etc.) or as a raw material for the production of plastics (pellets). Secondary sources include their production as a result of the physical or chemical degradation of larger plastics or fibres, which reach the environment due to poor waste management. Plastics, in addition to the consequences on the environment, have an obvious direct effect on living beings, whether by ingestion, strangulation, entrapment or toxicity (Bollaín-Pastor & Vicente-Agulló, 2019).

It has been reported that microplastics are not only composed of structural polymers (macromolecules), but can be considered as a kind of complex cocktails of pollutants, because it has been proven that they can easily interact and sip toxic substances on their surface, and then release them (Campanale et al., 2020). These include chemical additives (phthalates), residual monomers, and substances such as flame retardants, biosides, and polyaromatic hydrocarbons.

Recent studies have detected the presence of microplastics in humans. This was based on a study of 47 human tissue samples (body, brain, lung, liver, adipose, spleen, and kidneys) by mass spectrometry, resulting in the presence of monomers of different synthetic polymers (ACS, 2020; Dalberg & WWF, 2019).

2 PROBLEM OF POLYETHYLENE TEREPHTHALATE (PET) CONTAMINATION IN MEXICO AND THE STATE OF OAXACA

In Mexico, the Statistical Yearbook of the Chemical Industry (ANIQ) reported the production, import, export, and apparent consumption of polyethylene terephthalate (PET) between 2010 and 2019 (ANIQ, 2021). Since 2010, production has remained steady at 1,000,000 tonnes, while imports increased by 91,000 tonnes in 2019. Exports increased by 135,000 tonnes and in relation to apparent consumption there was a decrease of 112,000 tonnes in 2019 compared to 2010. These data illustrate the role that this material has acquired in our lives and therefore the environmental problems that have been caused throughout its appearance in the Mexican market. Worldwide, Mexico is among the countries that produce the most PET waste, mainly due to the high consumption of soft drinks and bottled water. In 2017 Mexico ranked second in the world in the consumption of PET bottles, on average 200 PET bottles are generated per inhabitant in the country, today 300,000,000 tons of plastic are produced, of which only 3% is recycled (Cristán-Frías et al., 2003; El-Trochillero, 2005; Santillán, 2018).

Although Mexico is a country where activities such as PET collection and recycling are carried out, only 56% of the bottles generated are recycled in the entire country, with the exception of Mexico City where 90% is recycled (López-Casarín, 2019). This indicates that the rest of the PET is dispersed without having a post-consumer processing, and that in turn when it reaches the seas it could generate the formation of microplastics by mechanical fragmentation in the seas. As a result, fish stomachs have been reported in the regions of the Gulf of Baja California Sur, the Gulf of Mexico, Veracruz and in the Caribbean Sea region in the Port of Morelos (Reyes-Bonilla & Alvarez-Filip, 2019). They have also been reported in Playa Azul, Playa Capolita, and Zipolite in the states of Michoacán and Oaxaca (Beltrán-Villavicencio et al., 2016; Cruz-Salas et al., 2020). In the same way, scientists from the National Polytechnic Institute have carried out research studies related to microplastic pollution in Huatulco (Oaxaca), La Paz (Baja California), on the beaches of Cancun and Tulum and have begun studies in Chetumal (Quintana Roo), Acapulco (Guerrero) and Tecolutla (Veracruz). This has been done to obtain information on this environmental problem and thus determine the impact on people's health (IPN, 2020). The above indicates that in Mexico more plastic is produced than necessary and as a consequence tons of garbage are generated, which become a serious pollution problem because our country does not have a good waste management system, as reported by the Office of Scientific and Technological Information for the Congress of the Union (INCyTU, 2019a, 2019b).

In Oaxaca, the problem of plastic pollution is no less than in many states of the Mexican Republic. In this state, around 3,804.09 tons per day (ton/day) of municipal solid waste and special handling are generated, of which 1,062 tons/day are properly disposed of. This implies that more than 70% of the waste generated (2741.09 tons/day) is not subjected to adequate treatment or confinement. There are more than 209,926 open-air landfills located mainly in ravines, riverbanks, roadsides and vacant lots, which pollute surface and groundwater, as well as the soil and surrounding natural areas. This has had a major impact on human health and ecosystems. The state's secretary of the environment positioned PET waste as inorganic waste with commercial value.

Fig. 1 shows the percentages of the physical composition of the waste that has been collected in different regions of the state of Oaxaca (Hernández B et al., 2008; SEMARNAT, 2013). Waste generation in the Papaloapan region has represented the highest rate of solid inorganic waste generation of commercial value after the southern highland region, and fourth place in the generation of solid inorganic waste with no commercial value along with the northern highlands region. Of this waste generated in the Papaloapan region, only 30% of it has an adequate final disposal.

(Adapted from Hernandez B et al., 2008; SEMARNAT, 2013)

Figure 1. Percentage physical composition of waste generated in the eight regions of Oaxaca. ROV: Organic Waste with Commerical Value; ROSVC: Organic waste with commercial value; RO: Organic waste.

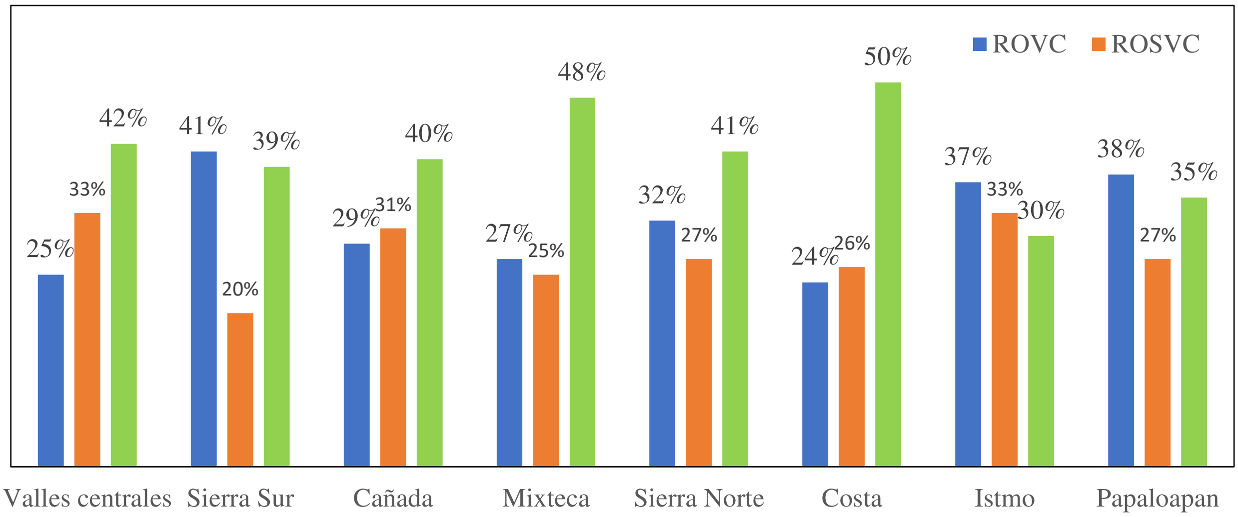


Table 1 shows the composition of the different types of waste with commercial value and the number of tons generated daily. It indicates that the amount of PET collected in the Papaloapan region represents the second waste generated in this territory. The waste that has been collected has come to represent 68% of the 1062 tons that are collected daily in the state of Oaxaca. In relation to PET, it has been observed that, as the years have passed, it has been proven that waste made up of soft drink bottles, chlorine bottles, detergents, oils (edible and car), drinkable yogurt, gelatins, cosmetics, among others, can be commonly observed in urban areas of Oaxaca. To illustrate this, Figure 2 shows photographs of the solid waste that can be found in some areas of the city of Tuxtepec, Oaxaca. Different plastics such as polyethylene bags, water bottles, soft drinks, processed food labels, cardboard, snack packaging, diapers, adhesives and clothing, as well as various organic waste, are distinguished.

Table 1:

Tons Per Day									
By-products	**Central Valleys**	**Sierra Sur**	**Glen**	**Mixtec**	**Sierra Norte**	**Coast**	**Isthmus**	**Papaloapan**	**Total**
Aluminium	6.81					0.39	2.26	0.17	9.63
Cardboard	11.56	6.94	2.68	13.07	1.35	9.12	6.68	0.72	52.14
Leather	9.95	1.31	0.60	0.36	1.39	0.05	2.26	1.52	17.44
Waxed cardboard packaging	57.09	3.98	1.64	1.36	0.98	5.06	9.93	5.42	85.45
Years	41.90	6.35	1.08	2.46	1.35	2.32	6.02	3.25	64.73
Ferrous material	10.48	2.22	0.39	0.23	0.66	0.08	2.55	…	16.59
Paper	21.75	11.76	5.50	7.44	4.38	10.79	21.87	14.35	97.54
Film Plastic	23.18	6.14	3.95	13.71	5.02	3.91	12.67	9.74	78.33
Rigid plastic	44.52	…	…	2.66	…	9.23	6.81	4.94	68.16
PET	**81.71**	**14.25**	**4.56**	**8.31**	**3.08**	**8.52**	**9.80**	**8.69**	**138.93**
Rag	18.28	2.02	1.68	2.58	2.25	1.07	5.00	2.57	35.45
Glass	17.91	8.39	2.87	11.03	2.36	8.39	9.20	7.10	67.25
Total	345.14	63.36	24.95	63.22	22.82	58.93	95.05	58.47	731.94

Adapted from Hernandez B et al., 2008; SEMARNAT, 2013

Figure 2. Garbage dumps scrambled with plastic in several places in San Juan Bautista Tuxtepec, Oaxaca

Source: Abad, 2021

3 RECYCLING AND DEGRADATION OF PET

Degradation is a generally irreversible process that causes a change (even if it is minimal) in the structure of a material and is characterized by the loss of its properties. This can occur through the action of various phenomena such as heat, humidity, solar radiation or enzymatically. The degradation of a synthetic material such as plastic is a complex process caused by the breaking of certain chemical bonds, causing both physical and chemical variations, which translate into characteristic changes that can be observed with the naked eye (Gutiérrez-Pescador, 2013; Madrigal-Cardiel et al., 2022).

In the case of the degradation of synthetic polymers such as PET, this consists of breaking the polymer's chains and forming shorter derivatives that can be broken down or disposed of more easily. When the fragments are degraded and used as a carbon source for energy production by microorganisms, the process is called biodegradation and the material is considered biodegradable (Yepes-Aguirre, 2014).

Chemical degradation is a process caused by the reactions that can be carried out between some chemical reagents and polymers, resulting in various original compounds or shorter chains of the same polymer as products (Posada-Bustamante, 2012). These reactions include: a) hydrolysis, which can be acidic, neutral or basic, generating terephthalic acid and ethylene glycol, b) glycolysis, where oligomers are generated, and c) methanolysis in which the products are ethylene glycol and dimethyl terephthalate.

Taking into account that PET has been reported as a non-biodegradable material, the fact of finding microorganisms capable of growing in the presence of alkaline degrades of this type of plastic may suggest that organisms could generate resistance mechanisms that allow them to adapt to the environment in which they grew. This has led to the identification and study of the different fungi and yeasts isolated from a microbial conglomerate grown in a chemical degraded PET.

4 METHODOLOGY

Alkaline degradation of polyethylene terephthalate (PET): Alkaline degradation was carried out generating potassium terephthalate as the main product with a pH of 12 ((Ramírez-Duran et al., 2006; Ramírez-Hernández et al., 2010; Ramírez-Hernández & Navarro-Moreno, 2010). This degradation was stored in a glass jar for several months, at the end of which the growth of some forms characteristic of fungal growth was observed.

Isolation of fungi grown in the chemical degradation of PET: A conglomerate sample of the PET degradate solution was taken and seeded in the medium of Dextrose Potato Agar (PDA). It was incubated at 37°C and its growth was observed every 24 hours, recording its growth characteristics. Subsequently, isolated colonies without contamination with other microorganisms were selected and seeded in separate media until axenic cultures were obtained.

Identification of microorganisms: The first identification consisted of the analysis of the macroscopic (coloration, morphology, pigmentation, type of mycelium) and microscopic (type of spores, hyphae and coloration) characteristics of each fungus obtained. The second identification was carried out by seeding the isolated samples in Petri dishes in 4% SDA solid medium and letting them incubate at 30°C for a period of 48 h. When they grew, they were sampled and analyzed microscopically and the observations were compared with information contained in the specialized literature. The third identification was made by the Sanger method to determine the DNA sequence. Quantification was carried out on the NanoDrop equipment; (Gauthier, 2008; Sanger et al., 1977; Sikkema-Raddatz et al., 2013).

5 RESULTS

Figure 3 shows the microbial cluster identified in the alkaline medium containing potassium terephthalate (alkaline degradation product of polyethylene terephthalate; PET). The appearance of the conglomerate was cottony, with a dark color in the center and white on the edges. Microscopically, cottony-shaped structures typical of fungal growth were found. It was thought that it was possibly a contamination of the degradation, so a first microscopic observation was made in which typical structures of microscopic fungi (hyphae and spores) were observed. Because of this, the next step was to plant in media designed for mushroom cultivation. Several colonial types corresponding to different fungi were identified and isolated and later identified.

Figure 3. A conglomerate of microorganisms grown in a medium composed of an alkaline gradient of polyethylene terephthalate.

(Dr. Leticia Navarro's personal collection, 2019).

6 ISOLATION AND IDENTIFICATION OF FUNGI FROM THE MICROBIAL CONGLOMERATE

The fungi were isolated using microbiological seeding and reseeding techniques, as well as the analysis of their macroscopic and microscopic structures. The microorganisms were labeled as: a) brown fungus, b) red fungus (due to the pigment with which it stained the culture medium), c) white fungus, and d) black fungus. After microbiological analysis and using molecular biology techniques, it was possible to identify the genus and species of each of them. For the morphological identification of the fungi, they were seeded in SDA culture medium plus 4% glucose, which constitutes a more selective medium for the cultivation and isolation of yeasts and molds and that, due to its high concentration of dextrose and a low pH, favors growth, spore formation and pigment formation. in addition to inhibiting the growth of bacteria (Cercenado, 2006). The macroscopic and microscopic characteristics of the 4 isolates are presented along with their description. The book "Medical Important fungi: A guide to identification" 5th Edition was used. Davise H. Larone and the "Mycological Atlas" of the Clinical Microbiology Laboratory of the National Institute of Medical Sciences and Nutrition Salvador Zubirán for identification. Table 2 shows the names of the identified microorganisms. The description of each is shown below. All the photographs shown belong to the personal collection of Luis Felipe Collado.

Table 2. Microorganisms identified using microbiological and molecular biology techniques.

Nomenclature of microorganisms	Genus and species identified
White fungus	Acremonium sp
Coffee mushroom	Fusarium Proliferatum/Verticillioides
Red mushroom	Talaromyces Verruculosus
Black fungus	Aspergillus Niger

6.1 ACREMONIUM SP

Morphologically, faint white colonies with mycelium of cottony consistency without pigment generation were observed. Microscopically, conidia-like spores and nonseptate hyaline hyphae were present.

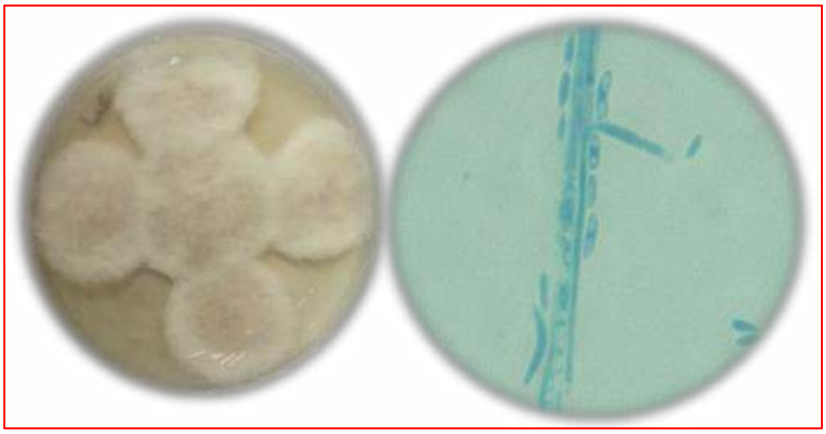
The literature mentions that fungi of the genus *Acremonium sp* are ascomycetes belonging to the group of hyalohyphomycetes that can be isolated from soil samples, plant remains, hay and some interior construction materials such as fiberglass or thermal materials used for heating. Two opportunistic species have been identified: A. *recifei and A*. alabamense, which can cause conditions such as mycetomas, keratitis and onychomycosis. This indicates their great ability to adapt to different environments (Rodríguez, 2016). Figure 2 shows the characteristics of *Acremonium* sp isolated from the cluster.

Figure 4. Macroscopic and microscopic characteristics of *Acremonium* isolated from a conglomerate grown in the presence of potassium terephthalate (PET degraded).

6.2 FUSARIUM PROLIFERATUM/VERTICILLIOIDES

As morphological characteristics, faint white colonies with a cottony consistency were observed, no pigment was produced in the culture medium. Over time, the colonies showed dark pink coloration and the surface became cottony, a pink to reddish pigment was observed in the culture medium. This characteristic was used as additional information for their identification. Microscopically, nonseptate macro-siphonated hyaline hyphae and abundant macroconidia were found.

Figure 5. Macroscopic and microscopic characteristics of Fusarium Proliferatum/Verticillioides isolated from a conglomerate grown in the presence of potassium terephthalate (PET degraded).



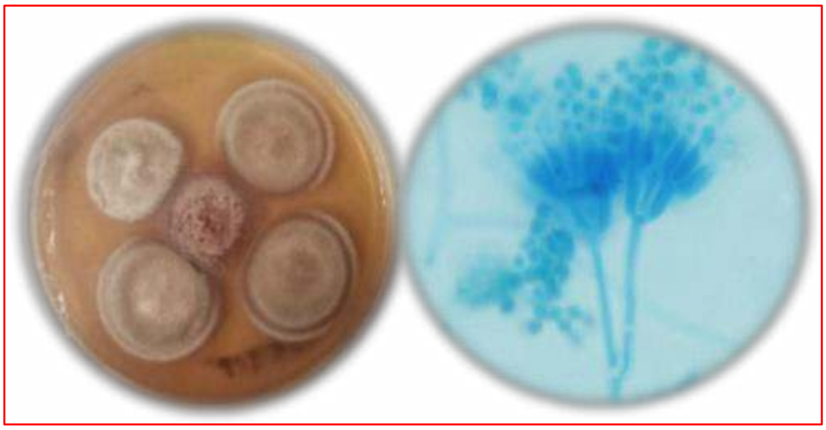
The literature indicates that this microorganism is an ascomycete that is part of the subdivision Deuteromycota, indicating that it does not have a sexual phase or, in some cases, is very rare. *Fusarium verticillioides* belongs to the latter category as it has a sexual phase called teleomorph, or perfect shape, which is very difficult to find in nature. Special conditions are required to observe it in vitro. It has been reported as the most damaging space to corn anywhere in the world. It is known to be the main pathogen of the crop and to cause substantial losses that vary from year to year. It is known as a necrotrophic pathogen because of its ability to cause the death of host tissue and then survives as a saprophyte in the stubble. It is a producer of mucotoxins such as fumosin, which is a neurotoxin, which harms animals (Torres-Toledano et al., 2016).

6.3 TALAROMYCES VERRUCULOSUS

Cream-colored colonies were obtained on the surface with red and green dots. On the reverse, a red pigment was observed in the culture medium. Microscopic analysis revealed septate branched hyaline hyphae, conidia, and chain spores. Due to the macroscopic and microscopic characteristics of the fungus, it was initially identified as *Penicillium sp.* However, as reported in the literature, and after making the identification by the Sanger sequencing technique, the organism was identified as *Talaromyces verruculosus*. Figure 4 shows the microorganism obtained.

INTERNATIONAL SEVEN JOURNAL
OF MULTIDISCIPLINARY
ISSN: 2764-9547

Figure 6. Macroscopic and microscopic characteristics of *Talaromyces verruculosus* isolated from a conglomerate grown in the presence of potassium terephthalate (PET degraded).

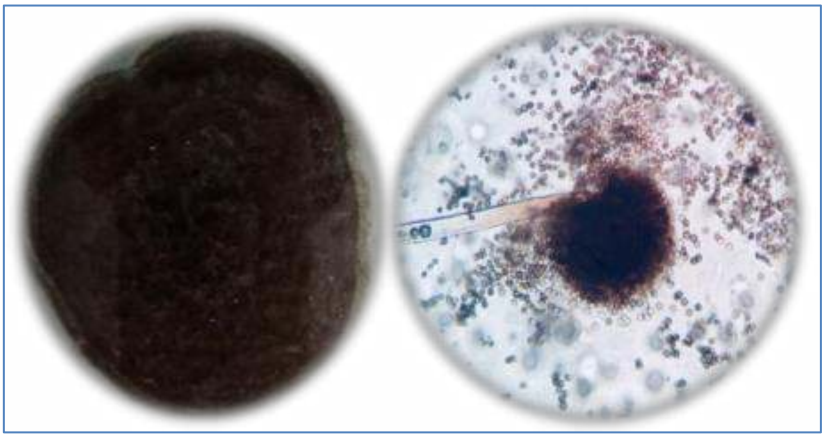


The genera Penicillium and Talatomyces have been reported to belong to filamentous fungi that have important environmental and biotechnological importance. They have low nutritional requirements and have a large and important enzyme content. These characteristics have allowed both spaces to be isolated from soils of tropical ecosystems, where they actively participate in biogeochemical cycles. Due to their close resemblance, the taxonomy of both genera is constantly revised using various identification techniques (Sousa-Ramos et al., 2018).

6.4 ASPERGILLUS NIGER

The morphological analysis showed colonies that initially presented yellow-white mycelium-like growth with a black border, after the growth time black spores were observed throughout the box. On the back of the petri dish, the colonies formed yellowish-green rings. The structure of the fungi showed septate hyaline hyphae, radial-headed conidiophores with branched and septate ends, conidia and chain spores. Using MALDI-TOF, the fungus was identified as *Aspergillus Niger*.

Figure 7. Macroscopic and microscopic characteristics of Aspergillus niger isolated from a conglomerate grown in the presence of potassium terephthalate (PET degraded).



This microorganism may or may not be pathogenic to humans. This depends on the species and the characteristics of each of them. This genus was described by P. A. Micheli in 1729 and is a ubiquitous hyaline filamentous fungus which can cause diseases of universal distribution. It is known that there are about 900 species of Aspergillus, which have been classified into 18 groups by Rapper and Fennell; of these, only 12 are related to some human diseases (Raper & Fennell, 1977). *Aspergillus niger* accounts for 2 to 3% of them. A report issued by Alcalá and his research group indicates that this fungus is a good example of what is called an "opportunistic pathogen" since it usually affects patients who are compromised in their state of health. Aspergillus owes its pathogenicity to the following characteristics: a) the small size of its conidia, which allows them to be aspirated and cause lung diseases; (b) its ability to grow at 37 °C, making it pathogenic to humans; c) its ability to adhere to epithelial and sometimes endothelial surfaces, as well as its great capacity to invade blood vessels, and d) its great capacity to produce extracellular toxic substances such as enzymes and cytokines that can harm mammals (Alcalá et al., 1997). other research has indicated that some strains of *Aspergillus niger* isolated from mining lands have high tolerance to concentrations of 1 to 5 mm of the metals mercury, lead, silver, zinc, chromium, cadmium and copper in artificial culture media (Villalba-Villalba et al., 2018).

It is now known that any compound can be toxic depending on its physicochemical and concentration characteristics, and that many organisms cannot live at extreme pH values. In the same way, the existence of extremophile organisms or organisms capable of adapting to adverse environmental conditions has been reported (Ramírez-Duran et al., 2006; Velásquez-Emiliani et al., 2018).

In this work, two important aspects that play a key role in our times have been linked: plastic pollution and the ability of microscopic fungi to grow in degraded materials such as polyethylene terephthalate. It was based on a microbial consortium formed by at least four fungi and one yeast (which was not shown in this work).

The literature shows that microbial consortia are common in nature and that they play important roles within nature. Consortia can be made up of microorganisms of the same species or different species such as bacteria and fungi, for example. One of the applications of microbial consortia is bioremediation.

In this regard, Tirado-Torres' group in 2015 mentioned that in the soil, microorganisms found in mixed populations can be identified by studying isolated and cultivated strains or consortia. Microbiological or molecular techniques can be used to identify them. They mention that, once the microorganisms have been isolated and identified, the technique used to adapt the soil biota to the pollutants is the enriched medium. For microorganisms isolated from soil samples, the methodology is to add a soil sample to an enriched medium containing the nutrients and growth factors necessary for the microorganisms. Concentrations and types of nutrients can vary in this technique and the pollutant to be degraded is added as a carbon source.

Within the oil industry, microbial consortia have also been used, which are formed depending on the characteristics of the microorganisms to be chosen. In relation to the above, the Toledo-León group mentions that microbial consortia have advantages to achieve effective bioremediation because they can often create networks with different levels of interaction, showing better adaptation, survival and permanence. These characteristics allow them to withstand fluctuating environments, making them robust to environmental changes (Toledo-León. Heidy et al., 2022).

In relation to the biodegradation of plastics, the RECOVER group has developed sustainable strategies to reduce plastic pollution, focusing on the research of biological organisms that allow the degradation and transformation of plastics. According to their research, the use of microbial consortia has been shown to improve the biodegradation of plastics such as linear low-density polyethylene (LLDPE). This has been achieved by using induced selection and the proliferation of plastic-degrading microorganisms in artificially contaminated microcosms, such as soil samples where plastic has been buried. The efficiency of degradation has been verified and work has been done on the identification of microorganisms and the study of their enzymatic profile. They have shown that plastic powder promotes microbial growth (Professional Waste, 2023).

A Mexican research group has reported *Penicillium pinophilum* and *Aspergillus niger* as possible low-density polyethylene-degrading microorganisms (Volke-Sepúlveda et al., 2002). It has also been reported that *Aspergillus nomius strain* JAPE1 and *Streptomyces sp*. strain AJ1 can degrade this type of plastic (Abraham et al., 2017). Priyanka and Archana established aerobic and anaerobic conditions to biodegrade plastics based on the collection of microorganisms from different sites (Priyanka & Archana, 2011). Once the degradation protocols have been established, this group of scientists have isolated and identified several organisms with potential for biodegradation. Similar experiments have been carried out and a wide variety of bacteria have been found that could be used as potential biodegradable polyethylene agents (Nowak et al., 2011).

In relation to PET, there are no reports of microorganisms that can degrade it in its commercial form, due to the chemical nature of this polymer or because microbial species have not developed the enzymatic machinery to be able to break the polymer matrix of these materials.

In this work, microbial consortia were not formed artificially, but naturally, since the microorganisms that were isolated were obtained from a residue of the degradation of a type of plastic reported as non-biodegradable, using a chemical method developed based on the alkaline hydrolysis of PET (Ramírez-Duran et al., 2006; Ramírez-Hernández et al., 2010; Ramírez-Hernández & Navarro-Moreno, 2010). The degradation products of the plastic were ethylene glycol and potassium polythylemerephthalate with a final pH of 12. The gradient was placed in a glass container and closed, not airtight. Over time, the growth of mycelial forms was detected, which were identified as a microbial consortium formed by fungi and yeasts. Four fungi and two yeast strains were isolated and identified. The different studies carried out with these fungi have shown that two of them cannot grow in culture media if they are not seeded together with the other microorganisms that make up the microbial consortium in addition to the fact that, when they are seeded in culture media enriched with varying concentrations of the degradation products of PET at different pH values, fungi have different growth and some are less tolerant than others. This demonstrates the possible symbiosis that should have been established as a requirement for the survival of species in the original conditions of polymer degradation.

The degradation products may have served as a source of carbon. The results indicate the importance of the study of biodegradation, but rather the importance of the relationships that microorganisms can establish between them in order to use different sources of carbon generated in polluted environments. Tolerance and resistance processes are crucial to be able to establish models of biodegradation of plastics.

7 CONCLUSIONS

Environmental pollution is a topic that encompasses many sub-topics of great interest. One of them is the study of microorganisms as possible biodegrading agents. This is because many microbes possess incredibly fascinating characteristics of growth and adaptation to extreme environments constituted by temperatures, pH, or concentrations of salts and variable toxic agents. Their study is becoming increasingly important as environmental pollution is increasing day by day.

The basis of the present research was the knowledge that microorganisms have special adaptation mechanisms that help them develop and grow, in this case, using the degradation products of polyethylene terephthalate (PET): potassium terephthalate or ethylene glycol. The study of the identified strains of *Talaromyces, Fusarium, Acremonium* and *Aspergillus* will be able to provide knowledge related to biology, biochemistry and genetics that allow them to adapt to an environment such as the one in which they developed. Understanding the mechanisms that help these fungi adapt could be important to establish, in the future, possible strategies for the biodegradation of various plastic compounds that are found to pollute the environment.

REFERENCES

Abad, C. (2021). Que asco Tuxtepec servicio de recoleccion de basura. *El Piñero*. https://www.elpinero.mx/que-asco-tuxtepec-amanecio-sin-servicio-de-recoleccion-de-basura/

Abraham, J., Ghosh, E., Mukherjee, P., & Gajendiran, A. (2017). Microbial degradation of low density polyethylene. *Environmental Progress & Sustainable Energy*, *36*(1), 147–154. https://doi.org/10.1002/ep.12467

ACS. (2020, October 21). *Methods for microplastics, nanoplastics and plastic monomer detection and reporting in human tissues*. American Chemical Society. https://www.acs.org/content/acs/en/pressroom/newsreleases/2020/august/micro-and-nanoplastics-detectable-in-human-tissues.html

Alcalá, L., Muñoz, P., Peláez, T., & Bouza, E. (1997). Aspergillus y aspergilosis. *Control Calidad SEIMC*. https://www.seimc.org/contenidos/ccs/revisionestematicas/micologia/asperguillus.pdf

ANIQ. (2021). *Volumen de Producción y Comercio Exterior*. Polietilen Tereftalato (PET). https://aniq.org.mx/Anuario/2022/Capitulo10/polietilen-tereftalato.html

Barboza, L. G. A., Dick Vethaak, A., Lavorante, B. R. B. O., Lundebye, A.-K., & Guilhermino, L. (2018). Marine microplastic debris: An emerging issue for food security, food safety and human health. *Marine Pollution Bulletin*, *133*, 336–348. https://doi.org/10.1016/j.marpolbul.2018.05.047

Beltrán-Villavicencio, M., Vazquez, A., Carlos, J., & Zeferino, A. (2016). *Anaerobic digestion of used disposable diapers View project*. https://www.researchgate.net/publication/329554229Separaciónycaracterizacióndemicro-plásticosenplayasmexicanas

Bendezu-Bendezu, M. A., & Bendezu-Hernández, C. V. (2021). Efecto de los parámetros fisicoquímicos y biológicos sobre la calidad del agua del río pisco. *South Florida Journal of Development*, *2*(4), 5606–5614. https://doi.org/10.46932/sfjdv2n4-049

Bollaín-Pastor, C., & Vicente-Agulló, D. (2019). Presencia de Microplásticos en Aguas y us Potencial Impacto en as Salud Pública. In *Rev Esp Salud Pública* (Vol. 93). https://scielosp.org/pdf/resp/2019.v93/e201908064/es

Boyan Slat. (2013, February). *The ocean clean up*. The Ocean Clean Up. https://theoceancleanup.com/boyan-slat/

Campanale, Massarelli, Savino, Locaputo, & Uricchio. (2020). A Detailed Review Study on Potential Effects of Microplastics and Additives of Concern on Human Health. *International Journal of Environmental Research and Public Health*, *17*(4), 1212. https://doi.org/10.3390/ijerph17041212

Cristán-Frías, A., Ize, I., & Gavilán, A. (2003). La situación de los envases de plástico en México. *Gaceta Ecológica*, *69*, 67–82. http://www.redalyc.org/articulo.oa?id=53906905

Cruz-Salas, A. A., Alvarez-Zeferino, J. C., Martínez-Salvador, C., Enríquez-Rosado, M. del R., Gutiérrez-Ortiz, M. del R., Vázquez-Morillas, A., & Ojeda-Benitez, S. (2020). Cuantificación y caracterización de microplásticos y residuos sólidos urbanos en playa Zipolite, Oaxaca. *Ciencia y Mar*, *71*(3–21), 1–21.

Dalberg, & WWF. (2019). *Naturaleza sin Plástico: Evaluación de la ingestión humana de plásticos presentes en la naturaleza*. http://awsassets.panda.org/downloads/analisis_de_estudio_de_ingestion.pdf

El-Trochillero. (2005). El día Mundial del Medio Ambiente, #SinContaminaciónPorPlástico. *Https://Eltrochilero.Com/Plastico-Recicla-Mexico/*.

Eriksen, M., Lebreton, L. C. M., Carson, H. S., Thiel, M., Moore, C. J., Borerro, J. C., Galgani, F., Ryan, P. G., & Reisser, J. (2014). Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS ONE*, *9*(12), e111913. https://doi.org/10.1371/journal.pone.0111913

Gauthier, M. G. (2008). *Simulation of polymer translocation through small channels: A molecular dynamics study and a new Monte Carlo approach* [Ph. D.]. University of Ottawa (Canada).

Gutiérrez-Pescador, J. G. (2013). *Biodegradación de polietileno de baja densidad por consorcios microbianos. Tesis de grado* [B. Sc.]. Universidad Nacional Autónoma de México.

Hernández B, C. P., Katsurada H, M., Álvarez M, L., García-Torres, E., & Sosa M, P. (2008). *Programa para la Prevención y Gestión Integral de Residuos Sólidos Gestión Integral de Residuos Urbanos y de manejo Especial del Estado de Oaxaca. Entrega final*. https://www.gob.mx/cms/uploads/attachment/file/187442/Oaxaca.pdf

INCyTU. (2019a). *Plásticos en los océanos*. https://foroconsultivo.org.mx/INCyTU/documentos/Completa/INCYTU_19-034.pdf

INCyTU. (2019b, November). Plásticos en los océanosIlustración: Eduardo Balderas. *NOTA-INCyTU*, *34*. https://foroconsultivo.org.mx/INCyTU/documentos/Completa/INCYTU_19-034.pdf

IPN. (2020). Realiza IPN estudios sobre contaminación por microplásticos en playas Mexicanas. In *Instituto Politécnico Nacional*. https://www.ipn.mx/assets/files/ccs/docs/comunicados/2020/03/c-041.pdf

Libera. (2019). *Impacto del abandono del plástico en la naturaleza*. https://proyectolibera.org/storage/otros/Impacto-de-los-plásticos-abandonados_LIBERA-def-1.pdf

López-Casarín, J. (2019, October 30). Reciclaje en México. *El Economista*. https://www.eleconomista.com.mx/opinion/Reciclaje-en-Mexico-20191030-0001.html

Madrigal-Cardiel, M. Á., Velázquez-Roque Silvano, Maldonado-Paleo Jesús, Zurita-Luna, U., & Hernández-Damián, J. (2022). Diseño y simulación de un molino compacto para polietileno tereftalato. *South Florida Journal of Development*, *3*(1), 232–244. https://doi.org/10.46932/sfjdv3n1-018

Nowak, B., Pająk, J., Drozd-Bratkowicz, M., & Rymarz, G. (2011). Microorganisms participating in the biodegradation of modified polyethylene films in different soils under laboratory conditions. *International Biodeterioration & Biodegradation*, *65*(6), 757–767. https://doi.org/10.1016/j.ibiod.2011.04.007

Pabortsava, K., & Lampitt, R. S. (2020). High concentrations of plastic hidden beneath the surface of the Atlantic Ocean. *Nature Communications*, *11*(1), 4073. https://doi.org/10.1038/s41467-020-17932-9

Posada-Bustamante, B. (2012). La degradación de los plásticos. *Revista Universidad EAFIT*, 67–86. https://publicaciones.eafit.edu.co/index.p

Priyanka, N., & Archana, T. (2011). Biodegradability of Polythene and Plastic By The Help of Microorganism: A Way for Brighter Future. *Journal of Environmental & Analytical Toxicology*, *01*(02). https://doi.org/10.4172/2161-0525.1000111

Ramírez-Duran, N., Serrano, R. J. A., & Sandoval-Trujillo, H. (2006). Microorganismos extremófilos. Actinomicetos halófilos en México. *Revista Mexicana de Ciencias Farmacéuticas*, *37*(3), 56–71. http://www.redalyc.org/articulo.oa?id=57937307

Ramírez-Hernández, A., & Navarro-Moreno, L. G. (2010). El polietileno, síntesis y degradación. *AlephZero-Comprendamos*, *57*, 1–10. http://www.comprendamos.org/alephzero/57/polietileno.html

Ramírez-Hernández, A., Navarro-Moreno, L. G., & Conde-Acevedo, J. (2010). Degradación química del poli(etilen tereftalato) Síntesis y degradación. *Revista Colombiana de Química*, *39*(3), 321–331. https://www.redalyc.org/pdf/3090/309026685002.pdf

Raper, K. B., & Fennell, D. I. (1977). *The Genus Aspergillus*. Krieger Publishing Company.

Residuos Profesionales. (2023, June). *Avances significativos en la degradación de plásticos mediante consorcios microbianos*. Residuos Profesionales. https://www.interempresas.net/Plastico/Articulos/483179-Avances-significativos-degradacion-plasticos-mediante-seleccion-inducida-microcosmos.html

Reyes-Bonilla, H., & Alvarez-Filip, L. (2019). *Estudio sobre el impacto de la contaminación por microplásticos en peces de México Population genomics of Octopus hubbsorum in northwestern Mexico View project Marine Protected Areas View project*. https://doi.org/10.13140/RG.2.2.31686.63049

Rivera-Garibay, O. O., Álvarez-Filip, L., Rivas, M., Garelli-Ríos, O., Pérez-Cervantes, E., & Estrada-Saldívar, N. (2020). *Impacto de la contaminación por plástico en áreas naturales protegidas mexicanas*. https://www.greenpeace.org/static/planet4-mexico-stateless/2020/09/12e508ed-estudio_impacto_contaminacion-plastico.pdf

Rodríguez, B. (2016, June 22). *Acremonium sp.* Atlas de Identificación Micológica. https://atlasdemicologia.wordpress.com/author/atlasdemicologia/

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences*, *74*(12), 5463–5467. https://doi.org/10.1073/pnas.74.12.5463

Santillán, M. L. (2018). Una vida de plástico. *Ciencia UNAM*, 1–5. https://ciencia.unam.mx/leer/766/una-vida-de-plastico

SEMARNAT. (2013). Resumen Ejecutivo del Programa Estatal para la Prevención y Gestión Integral de los Residuos Sólidos Urbanos y de Manejo Especial en el Estado de Oaxaca. https://www.oaxaca.gob.mx/semaedeso/wp-content/uploads/sites/59/2022/08/Resumen-Ejecutivo-PEPGIRSUME.pdf

Sikkema-Raddatz, B., Johansson, L. F., de Boer, E. N., Almomani, R., Boven, L. G., van den Berg, M. P., van Spaendonck-Zwarts, K. Y., van Tintelen, J. P., Sijmons, R. H., Jongbloed, J. D. H., & Sinke, R. J. (2013). Targeted Next-Generation Sequencing can Replace Sanger Sequencing in Clinical Diagnostics. *Human Mutation*, *34*(7), 1035–1042. https://doi.org/10.1002/humu.22332

Sousa-Ramos, S. M., Cruz, R., Barbosa, R. do N., Machado, A. R., Costa, A. F. da, Motta, C. M. de S., & de-Oliveira, N. T. (2018). Penicillium and Talaromyces Communities of Sugarcane Soils (Saccharum officinarum L.): Ecological and Phylogenetic Aspects. *Journal of Agricultural Science*, *10*(4), 335. https://doi.org/10.5539/jas.v10n4p335

The Ocean Cleanup. (2012). *The largest cleanup in history* . Cleaning up the Garbage Patches. https://theoceancleanup.com/

Toledo-León. Heidy, Barrios-San Martín, H., Plá-Pérez, A., Romero-Silva, R., & Acosta-Díaz, S. (2022). Diseño y caracterización de un consorcio bacteriano para la degradación de ripios de perforación base diésel. *Revista CENIC Ciencias Químicas*, *53*(2), 102–112. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S2221-24422022000200102&lng=es&tlng=es.

Torres-Toledano, M., Granados-García, V., Rafael López-Ocaña, L., & Torres Toledano, M. (2016). *Aportaciones originales Carga de la enfermedad renal crónica en México*. Intersistemas, S.A. de C.V. https://www.anmm.org.mx/publicaciones/ultimas_publicaciones/ENF-RENAL.pdf

Velásquez-Emiliani, A. V., Quintero-De La Hoz, M., Jiménez-Vergara, E. Y., Blandón-García, L. M., & Gómez-León, J. (2018). Microorganismos marinos extremófilos con potencial en bioprospección. *Revista de La Facultad de Ciencias*, *7*(2), 9–43. https://doi.org/10.15446/rev.fac.cienc.v7n2.67360

Villalba-Villalba, A. G., Cruz-Campas, M. E., & Azuara-Gómez, G. V. (2018). Aspergillus niger Tiegh., isolated in Sonora, Mexico: metal tolerance evaluation. *Revista Chapingo Serie Ciencias Forestales y Del Ambiente*, *24*(2), 131–146. https://doi.org/10.5154/r.rchscfa.2017.03.023

Volke-Sepúlveda, T., Saucedo-Castañeda, G., Gutiérrez-Rojas, M., Manzur, A., & Favela-Torres, E. (2002). Thermally treated low density polyethylene biodegradation by Penicillium pinophilum and Aspergillus niger. *Journal of Applied Polymer Science*, *83*(2), 305–314. https://doi.org/10.1002/app.2245

WWF. (2019). *Solving PlaStic Pollution through accountability*. www.panda.org

Yepes-Aguirre, L. M. (2014). *Degradación de polietileno de baja densidad utilizando hongos. revisión sistemática de la literatura*. https://repository.javeriana.edu.co/bitstream/handle/10554/16184/YepesAguirreLauraMaria2014.pdf?sequence=1&isAllowed=y