Isolation of plastic waste decomposing bacteria from the soils of different areas of Anbar province

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Abstract :

The current study focused on the possibility of isolating and diagnosing plastic-decomposing bacteria from soil samples in different areas of Anbar province, the pureAkar PVC medium (polyvinyl chloride) and the pure PS polyester acar medium were used to isolate the plastic-decomposing bacteria, as 10 bacterial isolates capable of growing on the pure PVC (polyvinyl chloride) medium and the pure PS polyester acar medium were obtained to isolate well characterized 3 Of which with its ability to grow efficiently on both mediums, and the method of measuring the diameter of the colony or the transparent area around the colony was used to measure the growth efficiency on both mediums and choose the most efficient isolation of which is the isolation that was given the local symbol C1 isolated from the soil of plastic landfills in the Sufi area of Ramadi, after which the isolation was diagnosed by morphological and biochemical tests that appeared to belong to the bacteria *Kytococcucsedentarius* and the diagnosis was confirmed by the phyticdevice Vitek 2 compact system used three types of plastic waste (nylon bags, water bottles, water cups) The results showed that nylon bags were the best carbon source used by bacteria as the only source of carbon and energy at a temperature of 30 ° C pH 7.

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different end products Most plastics are derived from non-renewable raw materials (such as natural gas, oil or coal) and biorenewable materials such as vegetable oils, carbohydrates and vegetable proteins Starch, lignin, celluloseand other workerscan be used in plastic production (de Vargas Mores *et.al.,* 2018).

The uncontrolled use of plastics in packaging, transportation, industry and various uses in rural and urban areas over the past few decades has led to an increase in serious problems associated with the disposal of plastic waste and pollution, the effective decomposition of plastic bags takes about 1000 years and

Introduction:

Plastic is defined as a polymer that becomes mobile when heated, so it can be poured into molds Plastic consists of carbon, hydrogen, silicon, oxygen, chloride and nitrogenn (Kale et.al., 2015), plastic is a lightweight, strong, durable and low-cost industrial or semi-synthetic ring vehicle (Van Eygen *et.al.,* 2017)

Plastic is used to produce a very wide range of products and has quickly moved to all aspects of human life Plastics cover a wide range of synthetic polymeric materials (such as polypropylene, polyethylene, polyvinyl chloride, polystyrene, nylon and polycarbonate) produced a wide range of

Although many studies have shed light on the potential role of microorganisms in the analysis of marine plastic debris, little is known about the interaction of plastics with fungi which are also important degradation factors for complex organic matter in the marine environment, in fact recent research and studies have shown that some fungal strains found in the oceans such as *Zalerionmaritimum* has the ability to decompose polyethylene (Zeghal*etal.,* 2021).

Bacterial isolates decomposing HDPE have been identified and most of these bacterial isolates belong to the genus *Bacillus*spp and *Pseudomonas spp*Recent studies indicate that isolated bacterial strains can be used as environmentally friendly as they represent a safe way to dispose of polluting plastic waste *(Devietal., 2019).*

Materials and methodsof work:

Plastic Hydrolyzing Bacteria Isolation:

22 soil samples were collected from different areas in Anbar province, including soils planted with various field crops and plastic landfills in order to isolate bacteria that have the ability to analyze plastics from the soil, (1 g) was taken from the soil after cleaning and purifying it from impurities and conducting a series of decimal scarfs on it in distilled water and used the method of pouring dishes as it took (1 ml) of dilution IV 10-4 and V 10-5 Each was placed in sterile petri dishes after which both the pure PVC (polyvinyl chloride) medium and the pure PS polyester acar medium which was prepared by dissolving the substances 500 mg of K 2 HPO 4 , 400 mg of KH_2PO_4 and 100 mg of NaCl and 20 mg of CaCl 2, 200 mg of $(NH\ 4)_2$ SO 4 ,20 mg of MgSO 4 , 12 mg of $FeSO_4$ and100 mg of MnSO 4 3.0g of Poly Vinyl Powder Substance (PVC) Pure Chloride and 15 g of Akar-Akarevi 1000 ml of distilled waterMaroof *etal.*, (2021), stirred the dishes towards and

plastic not only increases the problems of waste disposal and landfill but also leads to the release of carbon dioxide as well as persistentorganic pollutants. When combustion causing pollution and global warming, common plastic disposal methods have proven insufficient to effectively manage plastic waste so there is an increasing focus on the use of effective microorganisms for biodegradable synthetic polymers *Kale et.al*., 2015) .

Population growth and industrial activity in the world have led to an increase in pollution caused by human activities and plastic pollution has become a concern , so plastic products must be recycled or made biodegradable Chemical and natural processing methods are often energy consuming and expensive in addition to that they are not environmentally friendly and often produce toxic by-products. Biological treatment is a suitable option because it is cost-effective and economical and environmentally friendly The production and consumption of plastics is increasing every day and as a result more microorganisms are exposed to these non-biodegradable polymers so the study of microorganisms effective in analyzing plastic products and increasing the understanding of their vitality can pave the way for effective and possible plastic processing processes (Delangiz*et.al.,* 2022) .

The increasing accumulation of plastic waste has become a serious environmental and social problem that is necessary to develop innovative approaches to the disposal of plastic waste . In recent years reports have emerged of the biodegradability of synthetic plastics by microorganisms or enzymes and these reports provide the possibility of developing the bioremediation technology of plastic waste (Ru *etal.*, 2020).

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bags reached 5.5 cm and based on the results of these analysis, only one isolation was selected that was the most efficient of the three, namely the bacterial isolation with the local symbol C1, which reached the diameter of the colony and the transparent area of decomposition 4.5.

Identification of Bacterial Isolates

The elected bacterial isolation with the highest decomposition rate was diagnosed depending on its phenotypic characteristics involved (colony's shape, height, color, edge and texture) and then studied the characteristics of bacterial cells microscopically after dyeing them with Gram's stain dye according to Holt *etal.,* (1994) After which the diagnosis was confirmed using the Vitek 2 compact system equipped by the French company BioMerieux.

Election of the best local carbon source used by elected isolation:

To determine the best local carbon source used by bacteria as the only source of carbon and energy, three local carbon sources were used, namely nylon bags, water bottles and water cups, where the center of the solid nylon bags, the center of the hard water bottles and the medium of the hard water cups were prepared by adding3.0 mg of each of the nylon bags, water bottles and water bags to 500 mg of K 2 HPO 4 and 400 mg of KH_2PO_4 100 mg of NaCl, 20 mg of CaCl 2, 200 mg of $(NH_4)_2$ SO 4, 20 mg of MgSO 4 , 12 mg of $FeSO_4$ and100 mg of $MnSO_4$ 15 g of Agar-Agar 1000 ml of distilled water, then sterilized the mediums with the repellent and poured the media after cooling them into petri dishes and left to harden, then planted the elected bacterial isolation by making a circle 1 cm in diameter in the center of the dish by three repeaters for each source and incubated at a temperature of 30° C and for 5 The day after that, the best environmental residue was elected counterclockwise for the purpose of ensuring the homogeneous distribution of the sample in the dish, after which the dishes planted at a temperature of 30° C were incubated for 6 days, those isolates were re-purified by replanting (sub-culturing)) after several transitions on the pure PVC (polyvinyl chloride) medium and pure PS polyester acar medium to obtain pure single colonies, then the selected insulation on both mediums was sifted by planting them in the form of a circle (1cm) in the center of the dish and incubated at a temperature of 30° C for 5 The day after that it was revealed the bacteria's ability to analyze the plastic by measuring the diameter of the colony or the transparent area around the developing colony in the center of the dish.

Competency Test of Elected Isolations:

The most efficient isolation in plastic analysis was elected using the method of measuring the diameter of the colony or the transparent area around it, as the ten selected and dissolved plastic isolates were replanted on the pure PVC (polyvinyl chloride) agar medium and the pure PS polyester acar medium in the form of a circle (1cm) in the center of the dish and incubated for 5 days at a temperature of 30 After that, the diameter of the colony or the transparent area around the colony was measured in measuring the growth efficiency of the isolates used, and three isolates were elected, which were the largest diameter among the other isolators, these three isolates were replanted on three environmental waste mediums, namely the center of solid nylon bags, the center of solid water bottles and the center of solid water cups in the same way as the previous by making a circle of 1 cm in diameter and showed high growth efficiency on all mediums and it turned out that the highest rate The diameter of growth on the center of the solid nylon

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based on their growth density on the insulation medium. After the transplant and incubation of these bacterial isolates on the pure PVC (polyvinyl chloride) medium and the pure Acaralbolester PS medium, the colonies appeared with clear growth diameters on both mediums, as the bacteria showed a good ability to grow in the two mediums and exploit plastics in it as the only source of carbon and energy, and a discrepancy appeared in the diameter of the colonies on both mediums and that the variation in the growth of bacterial isolates is due to the difference in their ability to consume plastics as well as to the environment from which these bacteria were isolated.

that bacteria used as the sole source of carbon and energy according to the measurement of the diameters of bacterial colonies.

Results and discussion:

Isolation of plastic-decomposing bacteria:

The results of the primary isolation in Table (1) showed obtaining 6 bacterial isolates capable of growing efficiently on the pure PVC (polyvinyl chloride) medium and pure PS polyester acar medium out of 30 bacterial isolates isolated from 22 soil samples brought to the laboratory, these isolates varied on their ability to grow and use plastics used in the study as the sole source of carbon and energy

Table (1) showing the diameters of plastic decomposition in secondary screening

Pronounced like	Symbol of isolation	Diameter of transparent area (cm)
1	C1	4.5
2	CH	3.8
3	F2	3.1
4	G1	3.3
5	N2	3.4
6	R1	2.4
7	T	2.0
8	X1	2.1
9	Y	2.3
10	S	2.0

analysis varied as the diameter of the colony and the transparent area ranged from 4.5–2)) cm, 10 isolates were elected that were the most efficient in plastic analysis Table(1), then those isolates were replanted and the same conditions were applied to them after which three bacterial isolates that carry local g-rheumatozoaC1 - N2 -CH were elected in their ability to analyze plastics and the three isolates were replanted under the same conditions and the average diameter of the transparent area around

Test the efficiency of the selected isolates in plastic analysis:

After obtaining pure single colonies that have the ability to analyze plastic waste, replant the 30 selected bacterial isolates on the pure PVC acar medium and the pure PS polyester medium by making a circle 1 cm in diameter at the center of the dish and incubated for 5 days to choose the most efficient insulation in plastic analysis by measuring the decomposition diameters of these isolates whose efficiency in plastic

growth efficiency on all media and it was found that the highest growth diameter rate on the center of nylon bags amounted to 4.5 cm and based on the results of these decomposition only one isolation with high efficiency in plastic analysis was chosen , namely C1.

the colony was 3.8 -3.4 -4.5)) cm respectively and also replanted these three isolates on three environments of environmental waste, namely the center of nylon bags, the center of water bottles and the center of water cups in the same way as the previous by making a circle with a diameter of 1 cm and showed high

Figure (1) shows the secondary screening of the selected isolates on the purePVC medium

bears the characteristics of the bacteria *Kytococcucsedentarius* Table (2), (3) and this was confirmed by the diagnosis of isolation using the VITEK2 Compact device .

Diagnosis:

The results of implant, microscopic and biochemical examinations of the elected bacterial isolation and based on Holt *et.al.,* (1994) showed that the bacterial isolation given the local code C1

Table (2) Implant and microscopic qualities of elected bacterial isolation with local code C1

Pronounced like t	Diagnostic trait	C1	Pronounced like t	Diagnostic trait	C1
1	Shape	Pie	6	Height	Elevated
2	Color	White	7	Cell shape	spherical
3	Textures	solid	8	Cell aggregation	Pairs or quatrains
4	The Edge	Round	9	Tincture of Cram	Positive
5	Transparency	Dark	10	Production of dyes	Does not produce dyes

Table (3) Biochemical Tests for *Kytococcussedentarius*

N	Type of test	*Kytococcussedentarius*
1	Oxidase	-
2	Catalase	+
3	Indole	-
4	Methyl red	-
5	Voges-Proskauer	-
6	Motility	-
7	Urease	+
8	Simmon Citrate	-
9	Hymolysis	-
10	Gelatin hydrolysis	-
11	Arginine	+
12	Gram stain	+

(+) Presence of growth or positive for the test (-) Lack of growth or negative for the test

sheet of these bags and the increase in their surface area led to an increase in their exposure to decomposing bacteria which made them easier to consume by these bacteria, that the abundance of microplastics formed in the decomposition medium increases with the thinness and decreasing size of the plastic piece (Wilby, 2019).

This finding is consistent with several studies that have used nylon bags as a carbon source, if Kale *et.al.*, (2015) referred to the use of nylon bags as the best carbon source, and Patil (2018) also used polyethylene bags as the sole source of carbon and energy in his study.

Selection of the best environmental residue as a carbon source of *Kytococcucsedentarius* :

The results shown in Table (6-4) showed the variation of the isolation of *Kytococcussedentarius* in its ability to analyze plastic waste and in its ability to exploit carbon sources represented by nylon bags, water bottles and water cups, as it was found that nylon bags are the best used carbon source, which gave the highest rate of decomposition diameter, and amounted to 4.5 cm for the selected bacterial isolation.

This result is attributed to the type and nature of the materials from which transparent nylon bags are made and the

Figure (2) represents the diameter of the colony and the transparent area on the center of the solid nylon bags at a temperature of 30° C and for a period of incubation (5) days

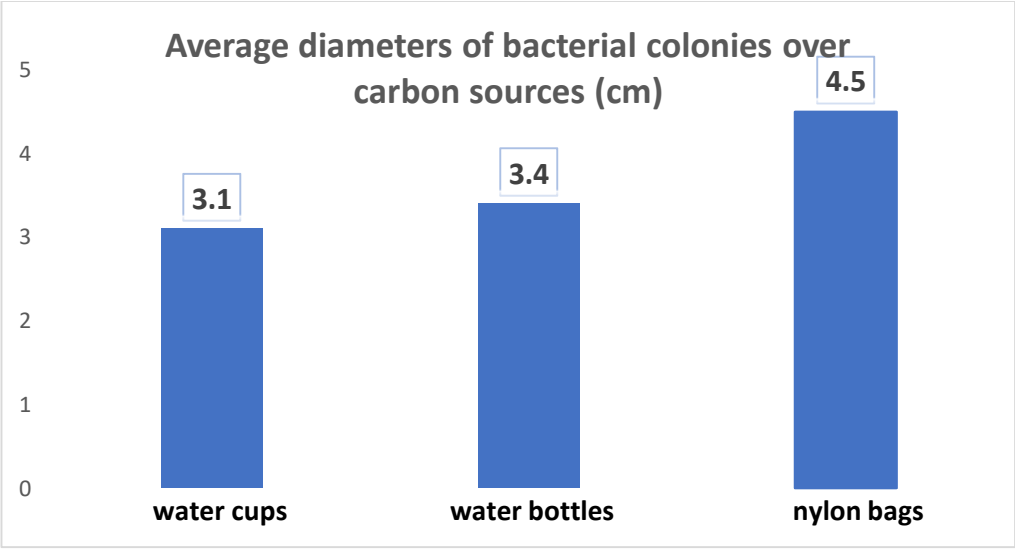

Figure 3: Rate of diameters of the bacterial colony on carbon sources

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