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REPORT REVIEW WRITING.

TOPIC: POLYETHYLENE BIODEGRADATION BY BACTERIAL STRAINS FROM THE GUTS OF PLASTIC EATING BACTERIA

INTRODUCTION:

Polyethylene is a very common petrolium based platic product which we used everyday.there is around approximately 380 million tonnes of plastic produced per annum globally.
however the contrast between the durability and short time period of this product leads to the accumulation of polyethylene waste in the enviorment which is the major concern nawdays.
According to the facts, there are around 8 million metric tonnes of plastic end up in the oceans every year. Several features of PE make it resistant to biodegradation. Among these features are a) PE's highly stable C−C and C−H covalent bonds; b) its high molecular weight, which makes it too large to penetrate the cell walls of microbes; c) Its lack of eadily oxidizable and/or hydrolyzable groups; and d) its highly hydrophobic nature(1). Since the early 1970s, tests on the biodegradation of virgin PE (unpretreated and without any additives) have been performed under natural environmental conditions, including soils, seawater, sludge, and compost, which harbor a multitude of diverse microbial communities.(2) These studies have concluded that the biodegradation of virgin PE is extremely limited in selected mixed microbial communities. It is believed that the environmental degradation of PE can be achieved by photo- or thermo oxidation and then through the biological

activity of microorganisms.(3,4). When PE samples are pretreated with UV light or thermo-oxidation, depolymerization of the PE chains occurs and results in the formation of low molecular weight products, including alkanes, alkenes, ketones, aldehydes, various alcohols, and fatty acids, which are then further degraded by select microorganisms.(3,6) The pretreatments either decrease the hydrophobicity of the polymer, making it more compatible with microorganisms, or introduce groups such as CO or −OH, which are more prone to microbial degradation.(7) In contrast, almost no biodegradation of PE through the biological activities of select microorganisms can be observed without pretreatments. (3) The poor biodegradation of virgin PE has been attributed to the lack of specific plastic-degrading microorganisms with long carbon chain oxidization and depolymerization capabilities. Certain researchers have assumed that the time for microbial evolution, which is required for plastic degradation, might be much longer than the history of PE applications.(8) To date, researchers are still attempting to discover PE-degrading microorganisms through tests involving plastic-waste-contaminated soils, landfills, compost, and marine life and have isolated several bacterial strains (SI Table S1)(8-20). Some of these strains have shown a moderate ability to use virgin PE as a carbon source

based on the characterization of biofilm formation on PE films, weight loss of PE materials, surface deterioration, and changes in themechanical and thermal properties of PE (SI Table S1). For example, it has been reported that a Pseudomonas strain causes weight losses of up to 20% in the tested PE within 120 days.(20) Additionally, a Canadian Web site has reported the isolation of two novel bacteria that rapidly degrade 22% or more of the tested PE weight over a six week period. (20) but these reports of polyethylene degradation is not confirm due to failure in providing the additional reports or evidence such as change in surface hydrophobicity, formation of oxidised carbonyl group,or the release of low molecular weight product. No report on the deposits of the aforementioned bacteria in any culture collection center has been provided. More studies are needed to confirm PE degradation by microorganisms via sequential two-step reactions, which have been achieved by the synergistic actions of physicochemical oxidation and through the biological activity of microorganisms, as proposed by Albertsson(3) . Plastic is one of the few new chemicals material which cause environmental issues.polyethylene,polystyrene, polypropinylchloride are main components which are used in manufacturing of plastic. these compound are resistant to biodegradation and persist in soil environment for a very long period of

time. these plastic are extremely hazardous .they release toxic chemicals which contaminate the food .(21) plastic is one of the few new chemical material which pose environmental problems .some micro-organisms such as bacteria ,fungi ,and acitinomycetes are involved in biodegradation of both natural and synthetic plastic.(22)

METHODS AND PROCESS:

1) BY:- Jun Yang,*,†, // Yu Yang,†, // Wei-Min Wu,‡ Jiao Zhao,§ and Lei Jiang†.(2014)

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SUMMARY:

In this research , a PE film is purchased with no catalyst and additives were added. the molecular weight ,average molecular weight, number-average molecular weight were noted. now, the PE film is cut for incubation in agar .the PE material weight, disinfected in 75% ethanol and then air dried in a laminar flow clean bench prior to use. once the waxworms had chewed enough of the PE bags to cause significant observable damage.

The biofilm formation on PE film determines its potential biodegradation because a biofilm enables the microorganisms to efficiently utilize the nonsoluble substrate. The cavities on the surfaces of the PE sheets had maximum depths of approximately 0.3 and 0.4 μm after inoculation with strains YT1 and YP1, respectively (Figures 3d and f). This type of deep cavity has not been reported by other researchers working on the characterization of PE-degrading microorganisms.26,3. PE’s degradation efficiency can be directly measured by the weight loss of samples, while the material’s depolymerization can be documented by the shift of its molecular weight and the release of lower molecular weight compounds. This study is the first to report the presence of PE degradingbacteria in the guts of waxworms, which eat PE films. PE degradation was confirmed not only by the bacterial growth on PE films,

which acted as sole carbon source and the PE substrate's weight loss, but also by identification of key reactions similar to those in photo- and thermal-based pretreatments, such as changes in surface topography, decreases in hydrophobicity, the formation of carbonyl groups, decrease in molecular weight, and release of soluble daughter products. The soluble daughter products were not identified in this study, and future study will be needed to identify the daughter and/ or intermediate products of PE degradation and the associated metabolic pathways.

2) **BY**:- EMILY FLASHMAN,UNIVERSITY OF OXFORD.

In 2016 , scientists from japan tested different bacteria from a bottle recycling plant and found that ideonella sakaiensis 201-F6 could digest the plastic used to make single use drinks bottles , polythene terephthalate (PET) . It works by secreting an enzyme known as PETase . This splits certain chemical bonds in PET, leaving smaller molecules that the bacteria can absorb , using the carbon in them as a food source.

Although other bacterial enzymes were already known to slowly digest PET , the new enzyme had apparently evolved specifically for this job. This suggests it might be

faster and more efficient and so have the potential for use in bio recycling.

As a result, several teams have been trying to understand exactly how PETase works by studying its structure . In the past 12 months , groups from korea , china have all published work showing the structure of the enzyme at high resolution and analysing its mechanisms.

These papers show that the part of the PETase protein that performs the chemical digestion is physically tailored to bind to PET surfaces and works at 30 degree celcius , making it suitable for recycling in bio-reactors . Two of the teams also showed that by subtly changing the enzyme's chemical properties so it interacted with PET differently made it work more quickly than the natural PETase.

Using enzymes from bacteria in bio-reactors to break down plastic for recycling is still easier said than done . The physical properties of plastics make them very difficult for enzymes to interact with.

The PET used indrinks, bottles has a semi-crystallinestructure, which means the plastic molecules are tightly packed and difficult for the enzyme to get to . The latest study shows thst the enhanced enzyme probably worked well because the part of the molecule that is involved in the reaction is very accessible , making

it easy for the enzyme to attack even the buried PET molecules.

3) BY:- Aneta K. Urbanek, Waldemar Rymowicz, and Aleksandra M. Mirończuk

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DEGRADATION OF PLASTIC IN COLD MARINE HABITS;

There is a different method through which biodegradation of plastic is possible as noted previously, once plastic wastes enter the marine environment, the large particles of plastic are first fragmented to form microplastics or nanoplastic particles (Debroas et al. 2017; van Sebille et al. 2016). This multistage process is influenced by a variety of biotic and abiotic factors (Pauli et al. 2017). It means that microbial attachment on the surface and formation of biofilms depend not only on microorganisms'

abilities but also on the properties of the material and the surface structure (Donlan 2002) such as the surface roughness, topography, surface free energy, surface electrostatic interactions, and surface hydrophobicity (Rummel et al. 2017). Particularly, the increase in degradation rate by raising the temperature and humidity may be crucial. Different variations in sea temperature are expected to affect the rate of plastic degradation due to acceleration or inhibition of chemical reactions (O'Brine and Thompson 2010). Hence, the biomass of a fouling community influenced by different surface characteristics and environmental conditions (Pauli et al. 2017) is not always the same. It was suggested that bacterial adhesion to the plastic surface depends on the physicochemical surface and bacterial properties rather than on biological processes (Artham et al. 2009). At the same time, biotic and abiotic factors have an influence on released products (van Sebille et al. 2016).

Moreover, it was demonstrated that the weathering process is an important factor in degradation of plastic. Degradation mechanisms in the marine environment are not clear. As mentioned above, the attachment is a key initiation process for degradation (Webb et al. 2009). However, it was noted that even though bacteria can easily colonize plastic, there is no evidence of potential degradation during early attachment (Lobelle and

Cunliffe 2011). Nonetheless, O'Brine and Thompson observed biofilm appearance on the surface of four types of plastic: oxo-biodegradable *d2w* and EPI polyethylene bags, compostable BioBag bags and standard polyethylene bags. The biofilm formation occurred after 4 weeks of exposure in the shallow waters of the North Sea (O'Brine and Thompson 2010). Moreover, it was observed that the degradation of biodegradable bags was higher than polyethylene (PE) bags, with 100% degradation of the compostable material between 16 and 24 weeks. In turn, Eich et al. noted biofilm formation on the plastic bag surface after 15 days of exposure to the marine environment (Eich et al. 2015). The amount of biofilm increased significantly within 33 days on polyethylene and biodegradable plastic bags distributed to a shallow benthic and pelagic habitat. Due to differentiation between biofilm communities observed on both plastic types in different environments, the authors suggested that the early biofilm formation and composition are affected by the plastic type and habitat. Unfortunately, in their study, mechanical tests did not reveal a reduction in tensile properties of both plastic types within 1 month of exposure. However, scanning electron microscopy analysis revealed alterations in the surface of the biodegradable plastic. They noticed that the lack of clear changes in the properties of material could be caused by

too short a period of carrying out the experiment. Lobelle and Cunliffe (2011) noted that biofilm formation may be visible within 1 week. The hydrophobic features of polyethylene plastic food bags submerged at the sea-end of Queen Anne's Battery (UK) changed during an experiment lasting 3 weeks, but they did not observe polyethylene-degrading organisms. Additionally, their study shows that removing the visible biofilm from plastic reverses its physicochemical properties (Lobelle and Cunliffe 2011). In a recent study, biofilm formation on PS and PE was investigated (Oberbeckmann et al. 2017). Bacteria from this genus are known for their ability to utilize polycyclic aromatic hydrocarbons (PAHs). Thus, it was suggested that members of *Erythrobacter* might be able to degrade PAH associated with plastic (Oberbeckmann et al. 2017). Although the knowledge about microorganisms degrading plastic in cold habitats is poor, De Tender et al. (2015) investigated biofilm development on a plastic surface and suggested that factors modeling the biofilms may help to identify species potentially involved in biodegradation.

These observations highlight that the degradation process in the marine environment has not been studied sufficiently so far.

4) MARINE BACTERIA AS POTENTIAL CANDIDATE FOR BIODEGRADATION:

Most of our planet is permanently cold (< 5 °C) and uninhabited by humans. The reason for this is that more than 70% of Earth is covered by seawater, mostly deep ocean, of which two thirds has a remarkably constant temperature of approximately 2 °C (Russell 1990). Nevertheless, bacteria can exist under these unfavorable conditions. Microbial communities resistant to such conditions may show many unique features. Among a number of microbial abilities in cold areas, the ability to decompose plastic is mentioned increasingly. It was assumed that the increasing amount of plastic waste leaking to the oceans may provide a new substratum for benthic organisms (Pauli et al. 2017). It was shown that in seawater, plastic releases dissolved organic carbon, stimulating the activity of heterotrophic microbes (Romera-Castillo et al., 2018). Adaptation to new sources of carbon can create new features of microorganisms, particularly in the production of cold-active enzymes. The uniquely cold-adapted enzymes of polar microorganisms provide numerous opportunities for biotechnological exploitation and give new insights into a wide range of applied issues such as plastic pollution (Rampelotto 2014). Currently, enzymes from psychrophilic

microorganisms are raising interest for many industrial applications due to ongoing attempts to decrease energy demand (Yadav et al. 2017). Lower temperature needed for the growth at which enzymatic activity is maintained may be a huge advantage in the degradation process due to reduction of electric energy usage for heating. Thus, potentially microorganisms from cold habitats could be employed in open area landfill. Among the prominent microbial agents being used for biodegradation, species belonging
to *Pseudomonas*, *Streptomyces*, *Corynebacterium*, *Arthro bacter*, *Micrococcus*, and *Rhodococcus* are mentioned most often (Pathak and Navneet 2017); the microorganisms have also been found in cold environments. Besides *Pseudomonas and Micrococcus*, bacterial isolates
from *Polaromonas*, *Micrococcus*, *Subtercola*, *Agreia*, *Leifs onia*, *Cryobacterium*, and *Flavobacterium* were isolated from the cryoconite of three glaciers located in northwest Spitsbergen. Moreover, 12 of the isolated strains were able to produce lipase (Singh et al. 2014), an enzyme that hydrolyses ester bonds in lipids and in some polyesters (Jaeger et al., 1995). Extracellular lipase activity was also detected in microbial strains isolated from Arctic sea ice of the Canada Basin. Here, microorganisms were identified as belonging to the

genera *Colwellia*, *Marinomonas*, *Pseudoalteromonas*, *Pseudomonas*, and *Shewanella*. Interestingly, the relative lipase activity was still detected at 0 °C in 20–40% and 10–30% of psychrophilic and psychrotolerant strains, respectively (Yu et al. 2009). The finding of cold-adapted bacterial strains with lipase activity is potentially important in view of the biodegradation process because several lipases hydrolyze polyesters such as PCL (Tokiwa and Calabia 2004; Pathak and Navneet 2017). Furthermore, it can be expected that other enzymes secreted by bacteria isolated from cold environments will show biodegradable activity. Potential enzymes that could be described as biodegradable include the lipases mentioned above, depolymerases (PHA depolymerases, PHB depolymerases, PLA depolymerases, PCL depolymerases), esterases, proteinases (proteinase K against PLA), cutinases, ureases, and dehydratases (Pathak and Navneet 2017). The biodegradability rate could be increased by supplementing polymers with additives which affect their thermal sensitivity and UV-absorbing capacity. Chemically sensitive polymers are more available for microbial attachment (Pathak and Navneet 2017). It was demonstrated that bacteria able to grow at − 1 °C release the greatest quantities of proteases (Huston et al. 2000), which proved the huge potential capacity for enzyme production by polar bacteria. Despite

these facts, we still lack information about the possibility of biodegradation of petrochemical plastic such as PCV or PET. However, in a recent study, a new enzyme, PETase, produced by *Ideonella sakaiensis*, has been characterized (Austin et al., 2018). Thus, it clearly shows that we still lack full information about the microbial potential for plastic degradation.

RESULT AND CONCLUSION :

Preliminary Screen of PE-Degrading Isolates.

Eight bacterial strains were isolated during the enrichment of the gut contents of the PE-eating waxworms when using PE as a sole carbon source (SI, Figure S1). Screenings for PE-degrading microorganisms among these isolates were subsequently performed in terms of the colonization on the PE film sheets in the CFBAM plates, the changes in tensile strength of the PE sheets, the floating behavior of the PE pieces in the LCFBM,and the WCA of the PE surfaces. The Gram-positive strain YP1, identified by 16S rDNA sequencing as Bacillus sp., grew as relatively thick, opaque colonies visible to the naked eye on the PE film sheet.

The Gram-negative strain YT1, identified as Enterobacter asburiae, grew as visible, thin translucent colonies (Figure 1a and Table1). The other six isolated bacterial strains did

not develop visible colonies on the PE film sheets. In addition, no visible colonization occurred on the two control plates: a) the CFBAM with a PE film sheet but without inoculation or b) the CFBAM without a PE sheet but with inoculation. After a 28-day incubation period, all of the incubated PE film sheets were collected to test their tensile strength. The samples spread with the culture of Bacillus sp. YP1 or E. asburiae YT1 exhibited tensile strength decreases of over 50% compared to that of the sterile controls, while the samples with the other six study. Sequences of the two strains were deposited in GenBankunder references KJ466896 to KJ466897..

Implications. To date, photo- and thermal-based pretreatments have been considered a critical step in the biodegradation of PE, and the studies of PE-degrading microorganisms have focused on plastic-contaminated environments, such as soils, compost, landfills, and marine environments. This study is the first to report the presence of PEdegrading bacteria in the guts of waxworms, which eat PE films. PE degradation was confirmed not only by the bacterial growth on PE films, which acted as sole carbon source and the PE substrate's weight loss, but also by identification of key reactions similar to those in photo- and thermal-based pretreatments, such as changes in surface topography,

decreases in hydrophobicity, the formation of carbonyl groups, decrease in molecular weight, and release of soluble daughter products. The soluble daughter products were not identified in this study, and future study will be needed to identify the daughter and/or intermediate products of PE degradation and the associatedmetabolic pathways. Further research should also focus on the ecophysiological relationship between the PE-degrading bacteria and waxworms, which may be mutually beneficial, similar to the microbial symbionts in cow rumens. The discovery of the presence of PE-degrading microorganisms in waxworm guts has provided a promising direction for further study of the microbial degradation of both PE and other plastics, along with new insights into the life cycle of synthetic plastics in nature. In addition, the isolation and characterization of more PE-degrading microorganisms from this source and a better
understanding of the enzymatic system involved in PE degradation could be helpful in the development ofremediation approaches for plastic wastes, which could eliminate plastic pollution concerns.

CONCLUSION FOR MARINE BACTERIA PROCESS:

Floating plastic wastes have a negative influence on marine species and ecosystems. However, there is still a

lack of precise knowledge about the quantity, sources, transport, accumulation, and role of plastics in the oceans. Fortunately, the scientific and public awareness of plastic as a global threat is rising. Numerous actions focus on tackling plastic accumulation by encouraging active involvement of consumers, producers, industry, and companies (Löhr et al. 2017). In 2016, for the first time, more plastic packaging waste was recycled than landfilled (region of European Union/Norway/Switzerland). Unfortunately, in many countries, landfill is still the first option of treatment for plastic waste (PlasticsEurope 2017). Thus, searching for new solutions is needed. Aside from the 3R strategy—reduce, reuse, and recycle plastic waste—which everyone is aware of, two more Rs should be considered: energy recovery and molecular redesign. Notably, the latter is seen as an emerging and very important element of the strategy (Thompson et al. 2009). Development of new bioplastic materials and their widespread application should help to reduce the impact of plastics on the environment. Typically, renewable raw materials instead of crude oil are used for their production, which saves valuable fossil resources and makes them more susceptible to waste

management by composting or anaerobic digestion to reduce the input into the environment. The research applies to both BP and conventional plastic and is focused on the microbial activity associated with biofilm development on plastic surfaces in the marine environment. As the effects of biofouling communities are poorly understood, interactions between plastics and microorganisms urgently need to be studied. Arctic microorganisms may have unique potential due to the environmental conditions of polar oceans, which differ from other marine ecosystems. Bacteria from these regions respond quickly to changing environmental patterns. Thus, the growing amount of plastic waste might force microorganisms to adapt to new substrates. Despite this possibility, the future of our planet depends on us and on our responsibility for the plastic waste problem. Natural adaptation of microorganisms might take too much time, and consequently the littering of the natural environment may be irreversible.

ABSTRACT:

Synthetic plastics present in everyday materials constitute the main anthropogenic debris entering the Earth’s

oceans. The oceans provide important and valuable resources such as food, energy, and water. They are also the main way of international trade and the main stabilizer of the climate. Hence, changes in the marine ecosystem caused by anthropogenic influences such as plastic pollution can have a dramatic impact on a global scale. Although the problem of plastics still remains unsolved, different ways are being considered to reduce their impact on the environment. One of them is to use microorganisms capable of degradation of plastic.

1.A particularly interesting area is the application of microorganisms isolated from cold regions in view of their unique characteristics. Nevertheless, the interactions between plastic and microorganisms are still poorly known.

2.Two bacterial strains capable of degrading PE were isolated from this worm's gut, Enterobacter asburiae YT1 and Bacillus sp. YP1. Over a 28-day incubation period of the two strains on PE films, viable biofilmsformed, and the PE films' hydrophobicity decreased. Obvious damage, including pits and cavities (0.3−0.4 μm in depth), was observed on the surfaces of the PE films using scanning electronmicroscopy (SEM) and atomic force microscopy (AFM).

The molecular weights of the residual PE films were lower, and therelease of 12 water-soluble daughter products was

also detected. The results demonstrated the presence of PE-degrading bacteriain the guts of waxworms and provided promising evidence for the biodegradation of PE in the environment.

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