The Effect of Microplastics on Restriction Enzymes

Ethan Li

Introduction:

In our modern world, plastics are involved in almost every facet of our everyday lives due to their durable and versatile uses. Plastics will break down into microplastics through physical, or chemical means and unfortunately, with the prevalence and convenience of plastics, comes the issue of microplastics and other chemicals from the plastic, entering our environment, food, and also our bodies as well. For example, in the npr study on plastic baby bottles (3. Godoy, Study: Plastic Baby Bottles Shed Microplastics When Heated. Should You Be Worried?), the milk containers often used for baby bottles can leach thousands of bits of microplastic into the milk the baby is drinking when the container is heated. Along with this, plenty of microplastics can also enter your food when it is heated in a plastic takeout container.

Additionally, the chemicals attached to microplastics, such as Bisphenol A (BPA), phthalates, and per- and polyfluoroalkyl substances (PFAS), which can be leached into our food and enter our bodies, have been also linked to insidious health issues.

Once these microplastics (MP's) have gone into the human body, they are suspected to cause a plethora of substantial health issues, including gut issues, cardiovascular issues, and even DNA and neural damage among many other possible harms. As shown in the ACS study,(4. Li et al. Potential Health Impact of Microplastics: A Review of Environmental Distribution, Human Exposure, and Toxic Effects), MP's have been shown to enter the lungs of people through inhalation of airborne plastic particles, and thus can easily be the cause of the lung cancer which MP presence has been linked to in humans and along with this, Mp's have also been shown to increase harmful gut bacteria. MP exposure has also been shown to cross the blood-brain barrier and cause cognitive decline similar to dementia.

Lastly, in an experiment testing the effect of microplastics on the immune system in human cells (6. Rubio et al. Biological effects, including oxidative stress and genotoxic damage, of polystyrene nanoparticles in different human hematopoietic cell lines), it was also found MP's cause genotoxicity.

One molecule, though not found in humans, that still interacts with DNA is the restriction enzyme EcoRI, which can be genotoxic due to its function of cutting DNA. EcoR1 is an enzyme commonly used in experiments with DNA as it cuts certain types of plasmid DNA in only one spot, hence making it genotoxic. Despite being genotoxic, this enzyme is beneficial during DNA replication due to the fact plasmid DNA needs to be cut and unraveled in order to replicate. Any enzyme that cuts DNA also has the potential to damage the genome, though the damage can be a positive thing and necessary for replication later on.

Another study involving microplastics effect on a different enzyme (carbonic anhydrase) (5. Polo et al. Interaction of Micro- and Nanoplastics with Enzymes: The Case of Carbonic Anhydrase), shows that microplastic have the potential to alter the output of another enzyme, carbonic anhydrase, that is also present in humans. Because microplastics have been shown to have a complex relationship with other enzymes and alter their output, it is also possible for it to alter the output of restriction enzymes by inhibiting the enzymes that commonly interact with

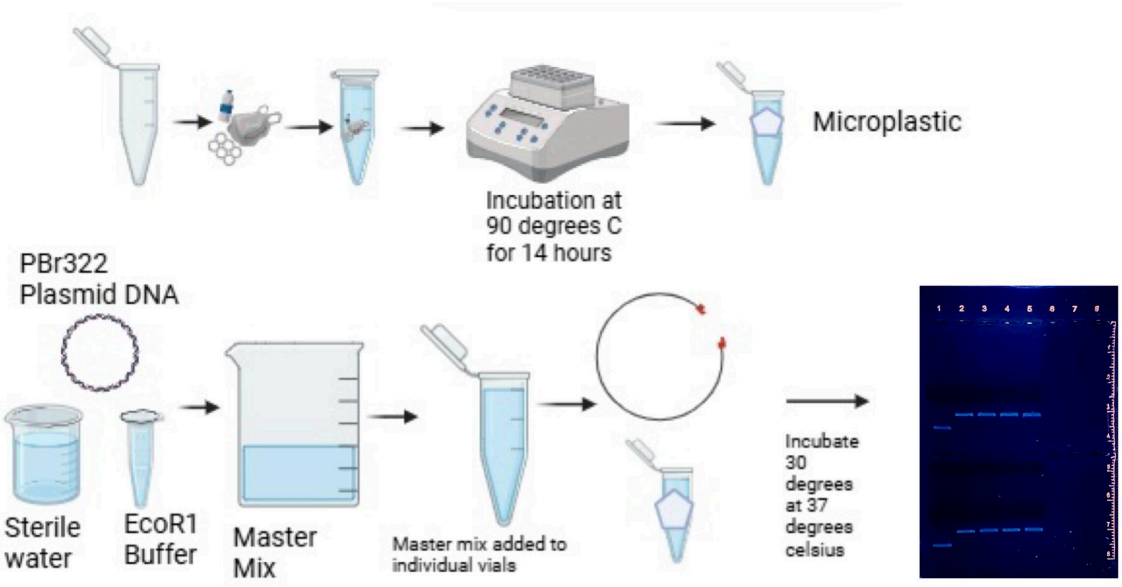
DNA. Because of this, the effect of microplastics, which have a possibility to negatively impact DNA, is being tested on EcoR1 to see whether or not it has an effect on the restriction enzyme that regularly interacts with DNA. Despite not being found in humans, it operates in a similar role to enzymes that can be found in humans, such as deoxyribonuclease.

Thus if it could be found that microplastics significantly affect the function of EcoR1, it can be a sign that a similar concept is worth testing with human DNA and enzymes. Due to the correlation between microplastics and genotoxicity and the proven effects of microplastics on enzymes, it could be possible that microplastics affect an enzyme directly related to DNA damage, with one of those being the restriction enzyme EcoR1. Because of this correlation between microplastics and restriction enzymes it is possible that microplastics affect the cutting of plasmid DNA by the restriction enzyme EcoR1. This correlation will be determined by using gel electrophoresis and comparing lanes with plasmid DNA, plasmid DNA cut by EcoR1, and plasmid DNA cut by EcoR1 and inhibited by microplastics.

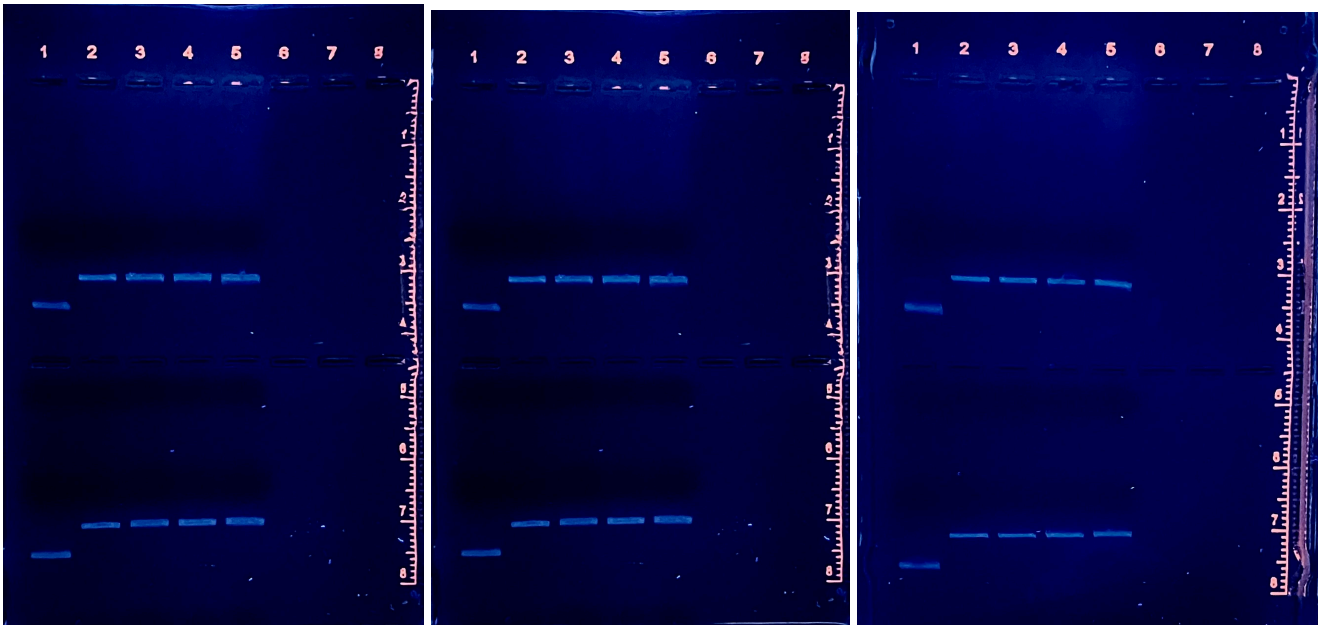
In the end, microplastics are present everywhere due to the prominence of plastic use today and there are many potential health hazards with Dna damage being one of them. EcoR1 is a restriction enzyme in plasmid DNA also responsible for causing DNA damage, so it seems to be worth testing if there is a relation between microplastic presence and restriction enzyme activity. This experiment sets out to find how microplastics affect the cutting of plasmid DNA by the restriction enzyme EcoR1 with the results being measured through Gel electrophoresis.

Methods:

- 0.05 g of Polyethylene Terephthalate, Polypropylene and Polystyrene were incubated at 90 degrees celsius in 5 ml of deionized water for 14 hours
    - Polyethylene Terephthalate taken from plastic kirkland water bottles
    - Polypropylene is from plastic cups from the solo cup company
    - Polystyrene taken from a fork from the solo cup company
- Experimental samples were made with 0.75 ug pBR322 DNA, 5.0 uL 10x EcoRI buffer (rCutSmart buffer), sterile water, 2.5 uL EcoR1, and 15 uL of microplastic sample prepared above to a total volume of 50 uL.
- A negative control (DNA) was made by including DNA and omitting enzyme and microplastic and replacing them with sterilized water.
- A positive control (EcoR1) was made by including DNA and EcoR1 omitting the microplastic sample and replacing it with sterilized water.
- All reactions were incubated at 37°C for 30 minutes
- Reactions were run using gel electrophoresis at 200 volts for 20 minutes



Data analysis:



In the end, six total trials of this experiment were run. In all above results, lane 1 contains the negative DNA control, lane 2 contains positive DNA control, and lanes 3, 4 and 5 contain the Polyethylene Terephthalate, Polypropylene and Polystyrene experimental trials respectively.

As seen in all six gels above, in lane 1, the negative control with only plasmid DNA migrates the furthest down the gel. On the other hand, in all six cases the positive control in lane

2 with plasmid DNA and also restriction enzyme migrated significantly less than the negative control.

This difference between negative and positive control trials can be attributed to the fact that when plasmid DNA is cut, the supercoiled nature of the DNA breaks down, thus causing the DNA to expand. Because of this, uncut plasmid DNA is more compact than cut plasmid DNA, and thus the uncut plasmid DNA will be able to migrate more easily through the gel compared to the cut plasmid DNA. This explains why the negative control migrated significantly further than the positive control.

When comparing lanes 3-5 –the experimental trials with microplastics– to the positive control, there is no difference seen. This data thus suggests that the DNA in the experimental lanes are equally cut by the restriction enzymes. Therefore it appears that the microplastics are not inhibiting the enzyme's cutting of plasmid DNA.

Because the experimental trials showed no significant difference from the positive control trial, it can be concluded that microplastics do not affect the ability of a restriction enzyme to cut plasmid DNA.

Discussion:

The reason EcoR1 was used is even though EcoR1 isn't directly involved in DNA splicing in humans, it can still be present in the gut microbiome due to its involvement in bacterial DNA cutting. This means that if microplastics affect their ability to cut DNA, it will still lead to changes in health outcomes in humans. Along with this, its function is still similar to that of enzymes involved in DNA replication, meaning there can be some inference drawn from this experiment in regards to human DNA.

Though in this experiment microplastics were not shown to affect the cutting of plasmid DNA by EcoR1, which would lead to the inference that microplastics likely would not inhibit human enzymes involved in replication, that may not necessarily be the case. It may be that microplastics simply do not affect restriction enzymes specifically but still affect the enzymes that interact with human DNA.

Also, it is possible the microplastic preparation method in this case was the issue. In previous experiments measuring release of microplastics in water with elevated temperature, it was seen that microplastics/nanoplastics were released with just 15 minutes of exposure to water at 80 degrees Celsius. By increasing the temperature and drastically increasing the duration, a significant amount of nanoplastics were expected to be released into the DI water; however, this is not certain as there was no way to verify the presence of microplastics.

In the end, this experiment suggests that microplastics do not affect the ability of the restriction enzyme EcoR1 to cut the plasmid DNA pBR322. Regardless, these results may still open the door to understanding the interactions between human DNA replication enzymes and microplastics.

Works Cited

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