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## Enhancing Bacterial Plastic Degradation Using Genetic Engineering: A Sustainable Solution for Bangladesh's Plastic Disposal

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### Introduction

Each year, over 400 million tons of plastic are synthesized worldwide, of which Bangladesh contributes approximately 800,000 tons. Favored by its low cost and durability, it has become deeply incorporated in our daily lives- but these same properties make it the most persistent polluter. It is estimated that only 10% of plastic waste is recycled, 14% is combusted, while the remainder is dumped into landfills, ultimately entering the natural environment [1] and causing widespread ecological harm. Despite decades of attempts at traditional recycling and waste management strategies, plastic pollution remains one of the world's pressing environmental challenges. On the bright side, recent scientific breakthroughs have revealed naturally occurring bacteria such as *Ideonella sakaiensis*, capable of breaking down common plastics like polyethene terephthalate (PET). However, this process is extremely slow and not viable for large-scale applications for countries like Bangladesh. This is where genetic technology offers hope. By modifying metabolic pathways or enhancing gene expression, engineered microbes can become powerful tools for sustainable waste management and addressing the pressing issue of plastic pollution.

### Importance of this research

Plastic pollution is slowly engulfing the ecosystems worldwide, and Bangladesh is no exception- limited recycling and poor waste management make the matter worse. Natural bacterial degradation does exist, but it's far too slow to be implemented on a large scale. Genetically enhanced microbes can offer a faster, scalable option, with potential for practical and socially acceptable application in Bangladesh.

### Aim

This research proposal investigates the feasibility and challenges of enhancing bacterial plastic degradation through genetic engineering, while also focusing on public awareness, perceptions, and a potential roadmap for future implementation.

### Research Questions

1. How can bacterial plastic degradation be enhanced through genetic engineering?
2. How feasible is it to enhance bacterial degradation in Bangladesh?

### Literature Review

Bacterial degradation of plastics has gained significant attention due to its potential as a sustainable solution. In 2016, Dr Kohei Oda and Dr Kenji Miyamoto, upon collecting and screening 250 samples

of PET debris, identified *Ideonella sakaiensis* 201-F6 as capable of using polyethylene terephthalate (PET) as its primary source of carbon and energy for metabolic processes. When grown on PET, this strain produces two enzymes: PETase, which hydrolyzes PET into an intermediate, and MHETase, which further breaks this intermediate into environmentally benign monomers, terephthalic acid and ethylene glycol. In laboratory conditions, a thin PET film was nearly fully degraded within six weeks at 30 degrees Celsius, highlighting the potential of microbial approaches for managing plastic waste under controlled conditions. [2] While PET accounts for a significant share of plastic waste, it is only one of the 7 major plastic types. Current bacterial degradation is strictly limited to PET, leaving the other types unaddressed. Moreover, for plastic to be completely harmless, it needs to be broken down to its basic elements, such as carbon and hydrogen. Plastic-eating bacteria, however, typically stop at producing smaller monomers. Consequently, although larger plastic pieces are removed, these monomers can exist in the environment, causing harm to human and animal health. [3] Furthermore, natural bacterial degradation is slow and inefficient. Even under controlled laboratory conditions, *Ideonella sakaiensis* takes weeks to degrade a thin PET film, and its effectiveness decreases even further with high crystallinity plastics, mixed waste, and real-world environmental conditions. To make naturally occurring plastic-degrading bacteria practical, they must be bioengineered to work hundreds or even thousands of times faster. Significant progress has been made in this area: in 2018, researchers in the United Kingdom and the United States modified bacteria to break down plastics within days rather than weeks. By October 2020, the process was further enhanced by combining the two bacterial enzymes into a single 'super enzyme', greatly increasing degradation efficiency. [4] One of the key organisms involved in PET degradation is *Ideonella sakaiensis*, a bacterium naturally capable of breaking down PET plastic. [5] Modern tools such as CRISPR-Cas9 now allow scientists to modify microorganisms to enhance enzyme activity and plastic-degrading efficiency, for example, by increasing biofilm formation to capture microplastics. [6]

### Methodology

This study will adopt a mixed-methods design, combining laboratory-based quantitative experiments with qualitative expert insights. The quantitative phase will provide measurable evidence of whether CRISPR-Cas9 can enhance bacterial plastic degradation, while the qualitative phase will explore the broader implications, including biosafety and scalability.

#### Phase 1: Quantitative (Laboratory Experiments)

The bacterium *Ideonella sakaiensis* (*I. sakaiensis*) will be selected as the primary organism due to its natural ability to produce PETase, the enzyme responsible for PET plastic degradation. As a comparative reference, *Escherichia coli* (*E. coli*) will also be included to ensure that any observed PET degradation is specific to *I. sakaiensis* and its PETase activity, rather than being caused by any experimental factors, contamination, or general bacterial presence.

Gene modification will be carried out using CRISPR-Cas9, a well-established gene-editing tool. The goal is to increase the activity of PETase in *I. sakaiensis* to improve plastic degradation. In this method, a guide RNA (gRNA) directs the Cas9 enzyme to a specific DNA sequence in the PETase gene, allowing a precise cut. The bacterium then repairs the cut in a way that enhances PETase activity. This approach ensures that modifications are specific and controlled, allowing measurable improvements in PET degradation while keeping the procedure feasible and understandable.

In this study, several experimental groups and controls will be used to ensure the results are reliable and meaningful. The first control group will consist of wild *Ideonella sakaiensis*, which will remain unmodified, thus providing a baseline for PET degradation. Control Group 2 will undergo the same experimental handling as the experimental group, but without introducing any gene edits. This

ensures that any differences observed in PET degradation are due to the genetic modification itself and not the experimental procedure. The experimental group will consist of *L.sakaiensis* strains modified using CRISPR-Cas9 to enhance PETase expression. Additionally, a reference strain of *E.coli* will be included, subjected to the same procedures, but serving as a negative control. Since *E.coli* does not naturally produce PETase, it will help confirm that observed PET degradation is specific to PETase activity rather than other factors.

All groups will be cultured under identical conditions to maintain consistency. This includes the same temperature and incubation time, pH and nutrient medium composition, and amount and size of PET films. Measurements will be taken over a 14-day period, while noting down the percentage of PET degradation, weighing the films before and after incubation, enzyme activity and bacterial growth rates. For statistical validation, a one-way ANOVA will be applied to compare PET degradation rates and enzyme activity across all groups, using a significance threshold of  $p < 0.05$ . This design ensures that all relevant variables are controlled, allowing any observed differences to be confidently attributed to CRISPR modifications.

#### Phase 2: Qualitative ( Expert Perspectives)

To complement the laboratory findings, 5-7 experts in microbiology and environmental science will be consulted. Semi-structured interviews will be conducted to explore important areas such as the environmental safety of genetically modified bacteria, the industrial feasibility and scalability of applying such microbes, and potential ethical concerns and public acceptance. In addition to conducting interviews, experts will complete a short Likert-scale questionnaire. The qualitative data from the interviews will be analyzed to identify recurring patterns and insights, while Likert responses will be summarized using mean and standard deviation, with a Chi-square test applied if comparisons between different expert groups are necessary.

#### Potential Roadmap

A step-by-step roadmap will be necessary to apply this research in real life. The initial phase will focus on laboratory studies, where the modified bacteria can be tested for safety and efficiency in breaking down plastic. If these results are successful, the next stage will involve small-scale controlled trials in contained environments to check whether the bacteria remain safe and effective outside the lab. Following this, the method could be extended to a larger scale, such as in waste management facilities, where its impact on reducing plastic pollution can be properly measured. Financial support for this stage could come from international bodies, such as the World Bank, which has a proven record of supporting environmental and climate-related initiatives in Bangladesh. Finally, to ensure long-term success, awareness programs and community outreach would help people understand the benefits and safety of the technology.

#### Conclusion

In conclusion, this study combines laboratory experiments, expert insights, and a step-by-step roadmap to explore how genetically modified *L.sakaiensis* can improve plastic degradation. Using clear experimental and control groups, along with statistical analysis, ensures that the results will be reliable. At the same time, consulting experts and considering public understanding allows the study to address safety, ethical concerns, and social acceptance. Overall, this approach provides a practical and responsible plan for moving from lab research to real-world solutions that could help reduce plastic waste in Bangladesh.

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