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学位論文内容の要旨

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Application of microbial consortium and recombinant microorganisms in polyethylene degradation

（微生物群集および組換え微生物を用いたポリエチレンの分解）

Polyethylene (PE) waste has become a major problem of today. Although there have been reports of microorganisms and potential enzymes capable of degrading PE, current understanding of the degradation process and the practical application is limited. A microbe possessing all the necessary PE-degrading enzymes and effectively degrading and mineralizing PE has yet to be isolated. Individual microbes, therefore, with limited metabolic functions, may be less efficient in breaking down PE when used alone. As a potential solution to this limitation, one approach involves creating a microbial consortium with a specialized member for each enzyme. Another approach entails genetically engineering a suitable host bacterium to express these target enzymes.

1. Consortium strategy for PE biodegradation

In this strategy, an artificial bacterial consortium was constructed to degrade PE. Bacterial strains for the consortium were selected based on their reported ability to degrade PE, as found in the literature. Enzyme activity of various PE-degrading enzymes, such as laccases, lipases, esterases, and alkane hydroxylases, with a focus on their extracellular expression, was assessed for these strains. Based on these results, an artificial bacterial consortium was constructed and used for PE degradation.

PE pellets were incubated with the consortium culture for 200 days. Scanning electron microscopy (SEM) of PE samples showed multilayered biofilms and surface-embedded bacteria. SEM images of PE samples after biofilm removal, revealed surface deterioration and pitting. Fourier-transform infrared spectroscopy (FTIR) analysis further supported biodegradation by revealing the production and consumption trends of PE oxidative products. Next-generation sequencing (NGS) analysis showed *Rhodococcus erythropolis* to be the dominant bacteria in both the culture broth and on the PE samples. Additionally, the colonization and biofilm formation of *R. erythropolis* was confirmed by SEM analysis of PE samples treated with its pure cultures. The results of this study indicate the potential

of an artificial microbial consortium for PE degradation and also demonstrate the suitability of *R. erythropolis* as a host for the genetic engineering strategy.

2. Genetic engineering strategy for PE biodegradation

In this strategy, for effective PE depolymerization, the constructed host must express the introduced enzymes extracellularly and be able to effectively colonize the PE surface to ensure efficient localized enzymatic degradation. Based on the consortium study *R. erythropolis* was chosen as the host due to its colonization ability. *R. erythropolis* was observed to produce esterase, lipase, and alkane hydroxylase efficiently, but its laccase production was relatively weak. Therefore, laccase was chosen for heterologous expression in this study.

Specifically, three extracellular putative laccase genes from *Streptomyces* spp. were selected and cloned from their genomes. These include EpoA from *S. griseus* and two multicopper oxidase family protein genes, MCO1 and MCO2 from *S. jumonjinensis*. The three putative laccase genes with their original signal peptide were cloned into the expression vector. Additionally, to improve the recombinant protein expression in the host and to overcome any potential signal peptide (SP) - gene incompatibility issues, the native SP of the *Streptomyces* putative laccase genes was replaced with that of the *Rhodococcus* SP as necessary. Six modified *R. erythropolis* twin-arginine translocation (TAT) SP, linked to abundantly expressed proteins from the *R. erythropolis* proteomics study, were used. Furthermore, additional protein transporter elements were incorporated into the vector to balance the recombinant protein production and secretion in the host. These vectors were then introduced into the *Rhodococcus* host. Clones were assessed for extracellular enzyme expression using the laccase substrates, ABTS and guaiacol.

The three *Streptomyces* laccases were successfully expressed extracellularly in the *Rhodococcus* host. Enzyme expression levels differed among various SP-gene combinations, even when using the same SP. These results highlight the importance of SP-gene compatibility in heterologous protein secretion studies. Notably, the modified *Rhodococcus* SP improved the extracellular expression of the introduced genes in cases where the original SP-gene combinations did not perform well. Furthermore, for a future PE degradation study, a *Rhodococcus* recombinant was selected for each of the three putative laccase genes with the best performing SP-gene combination. This study highlights the potential of *R. erythropolis* as a host for expressing PE-degrading enzymes and the applicability of its recombinants in PE biodegradation.