論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Study on biosynthesis of polyhydroxyalkanoates with diverse structures
著者(和文)	MRAMAMOORTHI Sivashankari
Author(English)	M Ramamoorthi Sivashankari
出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第12577号, 授与年月日:2023年9月22日, 学位の種別:課程博士, 審査員:柘植 丈治,曽根 正人,林 智広,田中 祐圭,福居 俊昭
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第12577号, Conferred date:2023/9/22, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	ライフエンジニアリング　系　コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of　（工学　　）
学生氏名： Student's Name	SIVASHANKARI M RAMAMOORTHI	審査員主査： Chief Examiner	柘植　丈治

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

The utilization of petroleum-based plastics has experienced a steady increase in recent years, and this trend is projected to persist into the future. However, these plastics are profoundly unsustainable, spanning from their manufacturing stage to their eventual disposal, thus engendering substantial environmental issues and jeopardizing the planet's well-being. Biopolymers have emerged as a promising avenue to address these challenges due to their inherent sustainability. Particularly, polyhydroxyalkanoates (PHA) have garnered attention for their potential to replace conventional plastics, largely due to their capability to degrade in both soil and marine environments, a feature that distinguishes them from other biopolymers. Nonetheless, the prevalent variant of PHA, namely 3-hydroxybutyrate (3HB), poses material limitations for diverse industrial applications due to its elevated crystallinity and brittleness. Additionally, uncertainties persist regarding the precise fate of PHA during its biodegradation process. Clarification is needed regarding whether PHA undergoes complete biodegradation into carbon dioxide and water or undergoes fragmentation into smaller oligomers. Consequently, this doctoral thesis has been meticulously crafted to address these pressing concerns.

Chapter 1 serves as a comprehensive introduction, tracing the historical trajectory of PHA research over the past century. It identifies critical research gaps and outlines the driving motives behind this study. Meanwhile, Chapters 2 and 3 concentrate on the identification of novel PHA synthases (PhaCs) with broad substrate specificities. Recognizing the limitations of PHA's material attributes, especially the commonly occurring P(3HB) variant, these chapters explore copolymerization strategies to introduce additional monomers into the polymer structure. This strategic approach enables the creation of PHA-based biomaterials endowed with enhanced mechanical properties, rendering them suitable for industrial applications.

Chapter 2 presents an in-depth exploration of an enhanced version of $PhaC_{Ac}$, derived from *Aeromonas caviae*, capable of synthesizing poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) [P(3HB-*co*-3HHx)]. However, the incorporation of a low 3HHx fraction via $PhaC_{Ac}$ does not exert a significant impact on the overall material properties. Previous efforts have yielded a superior $PhaC_{Ac}$ double mutant, $PhaC_{Ac}$NSDG, demonstrating exceptional polymerization capacity towards 3HHx. The study in Chapter 2 delves into evolutionary engineering to generate an enhanced variant based on $PhaC_{Ac}$NSDG. The exploration of significantly superior $PhaC_{Ac}$NSDG variants is documented through random mutagenesis. Around 20,000 clones were initially obtained from primary Nile red plate screening, subsequently narrowed down to 54 putative mutants through meticulous secondary screening involving gas chromatography analysis. Ultimately, five mutation points were selected and individually studied to ascertain their potential in enhancing the desired monomer incorporation. Intriguingly, a triple point mutant emerged, encoding heightened incorporation of the second monomer compared to the existing premier NSDG enzyme.

In Chapter 3, the focus shifts to the search for naturally occurring PhaCs from diverse bacteria, considering their limited availability. In this study identifies PhaCs from various bacteria such as *Ferrimonas marina*, *Plesiomonas shigelloides*, *Shewanella pealeana*, and *Vibrio metschnikovii*. These newly identified PhaCs underwent a comprehensive screening process utilizing the $PhaC_{Ac}$ amino acid sequence as a template in BLASTP. This approach represents an innovative method for acquiring such enzymes from diverse bacterial sources. Notably, the PhaC originating from *P. shigelloides* demonstrates a notable performance, prompting subsequent efforts in protein engineering aimed at augmenting its attributes. The resultant mutant showed enhanced activity in specific monomer incorporations than the parent enzyme and also $PhaC_{Ac}$NSDG.

Chapter 4 undertakes a significant exploration into addressing the limited comprehension of PHA biodegradation. This entails the synthesis of highly carbon 13 (^{13}C)-labeled P(3HB), utilizing the stable isotope ^{13}C to trace the polymer's fate during biodegradation. The investigation spans both native and non-native PHA producers under varying nutrient conditions, establishing a robust framework. Notably, both categories of producers synthesized nearly 98% ^{13}C-labeled PHA, closely approximating the theoretical value based on the 98% purity of the employed ^{13}C-glucose under nutrient-limited conditions. Intriguingly, the native producer exhibited slightly reduced labeling ratios under nutrient-rich conditions compared to the non-native PHA producer. A plausible assumption is that the diverse metabolic pathways in the native PHA producer may enable it to utilize alternate carbon sources alongside the provided ^{13}C-glucose.

Lastly, Chapter 5 encapsulates a comprehensive summary of this study. This thesis decisively addresses the two predominant limitations impeding the commercial expansion of PHA. By enabling the synthesis of PHA with diverse structures, these findings aspire to significantly contribute to the eventual replacement of petroleum-based plastics with PHA. This transition holds the promise of fostering sustainability and environmental preservation on a broader scale. This research not only underscores PHA's potential as an alternative to petroleum-based plastics but also offers critical insights into various aspects. Through this extensive investigation, the thesis offers substantial contributions to the realm of sustainable material development and ecological conservation.