Biosynthesis of P(3HB-co-LA) from Renewable Carbon Source by Metabolically Engineered *Escherichia coli*

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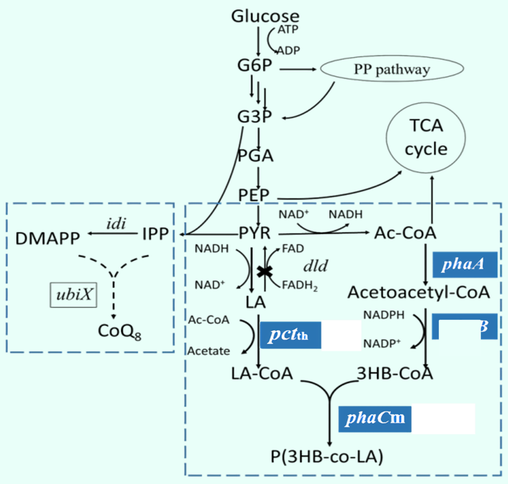
Abstract

P(LA-co-3HB) has been considered as a good alternative to petroleum-based plastic as it possesses several desirable properties such as biodegradability, biocompatibility, compostability, and low toxicity to humans. P(LA-co-3HB) can be produced in engineered *Escherichia coli* harboring the genes encoding LA-polymerizing enzyme (LPE) and monomer-supplying enzymes. In this study, the LA content in the polymer was increased through the strategies of weakened respiratory chain, promoter engineering and carbon source optimization. The results showed that weakened respiratory chain level can increase the component of LA in P(3HB-co-LA) from 5.1 mol% to 14.1 mol%; After optimization the expression of pct_{th} via promoter engineering, the LA component in the polymer can be further increased to 19.5 mol%. When the xylose was used instead of glucose as carbon, the LA content in wild strain was increased significantly, the highest LA content reached 30.6 moL%. Then, mixed sugar (glucose: xylose = 7:3) and lignocellulosic hydrolysate were used as carbon resource, respectively, the LA component of the polymer is improved. This study suggested that lignocellulose as a abundant and low-cost source can be used to produce the Lactic acid-based polymer.

Introduction

- ✓ P(LA-co-3HB) with the properties of biodegradability and biocompatibility is resource-saving and envioronment-friendly as a alternative to petroleum-based plastic [1].
- ✓ Xylose as carbon can increase the LA content of P(LA-co 3HB).
- ✓ lignocellulosic hydrolysate from the maize straw has the potential to be anabundant and inexpensive source of xylose for the production of value-added products.

Strategy



➢ **Weaken respiratory chain:**

deletion of UbiX to decrease the co-enzyme Q8 level, weakened respiratory chain, increase lactic acid content

➢ **Promoter engineering:**

strengthen the transcription of the key gene pct_{th}

➢ **Carbon source optimization:**

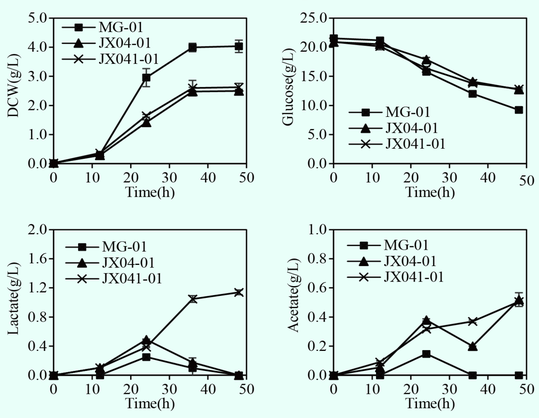
lignocellulosic hydrolysate, an agricultural waste, as a anabundant and inexpensive source advantageous for lactic acid-based polymer

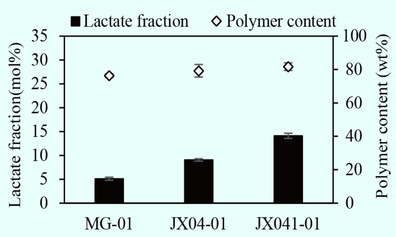
Strains and plasmids

Strains and plasmids	Description
Plasmids	
pTrc99aABC	pTrc99a containing the codon-optimized *phaC*m gene from *Pseudomonas fluorescens* strain 2P24, *phaA* and *phaB* gene from *Ralstonia eutropha*
pBADpct_{th}	pBAD33 containing the codon-optimized pct_{th} gene from *Clostridium propionicum* DSM 1682
pBAD-P*ldhA*-pct_{th}	pBAD33-P*ldhA* containing the codon-optimized pct_{th} gene from *Clostridium propionicum* DSM 1682
pBAD- P*trc*-pct_{th}	pBAD33-P*trc* containing the codon-optimized pct_{th} gene from *Clostridium propionicum* DSM 1682
Strains	
MG1655	Wild-type
MG-01	MG1655 carrying pTrc99aABC and pBADpct_{th}
JX04-01	JX04 carrying pTrc99aABC and pBADpct_{th}
JX041-01	JX041 carrying pTrc99aABC and pBADpct_{th}
MG-02	MG1655 carrying pTrc99aABC and pBAD-P*ldhA*-pct_{th}
JX04-02	JX04 carrying pTrc99aABC and pBAD-P*ldhA*-pct_{th}
JX041-02	JX041carrying pTrc99aABC and pBAD-P*ldhA*-pct_{th}
MG-03	MG1655 carrying pTrc99aABC and pBAD- P*trc*-pct_{th}
JX04-03	JX04 carrying pTrc99aABC and pBAD- P*trc*-pct_{th}
JX041-03	JX041 carrying pTrc99aABC and pBAD- P*trc*-pct_{th}

Results

➢ Effect of weakening the respiratory chain on P(LA-co-3HB) biosynthesis





Deletion of *ubiX* and *dld* showed a positive effect on lactate content, the JX041-01 produced P(3HB-co-**14.1**mol%LA), the lactate content was **2.76**-fold of that produced by MG-01.

Acknowlegments

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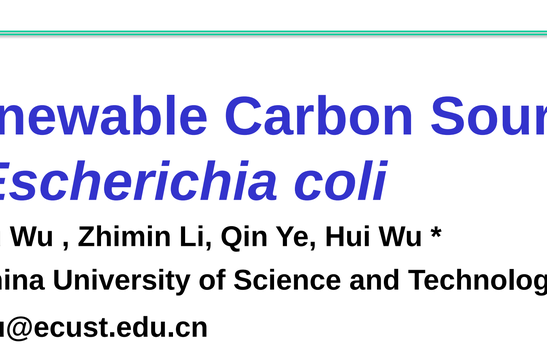
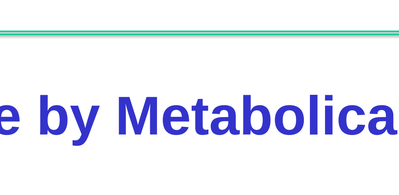
➢ Utilization of promoter engineering of pct_{th} to improve P(LA-co-3HB) biosynthesis

✓ Effect of *trc* promoter





When the *trc* promoter was was used to express pct_{th},, the LA content of JX041-03 reached **19.5mol%.**

➢ Biosynthesis of P(LA-co-3HB) through different carbon resources

✓ Effect of xylose

Strain	Carbon source	Polymer content (wt%)	LA fraction (mol%)
MG-01	Glucose	76.3	5.1
	xylose	76.1	24.5
JX04-01	Glucose	79.2	9.1
	xylose	38.0	2.7
JX041-01	Glucose	81.7	14.1
	xylose	28.6	7.5
MG-02	Glucose	82.2	3.6
	xylose	70.1	6.3
JX04-02	Glucose	62.9	6.9
	xylose	42.7	2.5
JX041-02	Glucose	64.0	13.4
	xylose	33.6	7.9
MG-03	Glucose	83.1	10.0
	xylose	72.0	30.6
JX04-03	Glucose	70.0	5.9
	xylose	18.8	5.5
JX041-03	Glucose	69.8	19.5
	xylose	22.7	11.3

When xylose was used as carbon resource, the wild type strains' LA content of copolymer has been improved obviously, especially MG-03 reached **30.6mol%.**

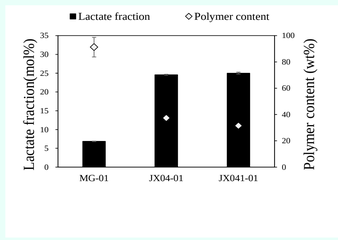
✓ Effect of glucose and xylose as co-carbon resource (G:X=7:3)

Strain	Carbon source	Polymer content (wt%)	LA fraction (mol%)
MG-01	Glucose	76.3	5.1
	mixture	72.0	12.0
JX04-01	Glucose	79.2	9.1
	mixture	38.4	10.3
JX041-01	Glucose	81.7	14.1
	mixture	35.5	13.0
MG-02	Glucose	82.2	3.6
	mixture	90.5	1.5
JX04-02	Glucose	62.9	6.9
	mixture	42.0	8.3
JX041-02	Glucose	64.0	13.4
	mixture	26.8	15.3
MG-03	Glucose	83.1	10.0
	mixture	88.0	5.4
JX04-03	Glucose	70.0	5.9
	mixture	53.7	8.8
JX041-03	Glucose	69.8	19.5
	mixture	51.5	13.3

When the mixture(glucose:xylose=7:3) was used, most strains' LA content in the copolymer were increased.

➢ Biosynthesis of P(LA-co-3HB) through lignocellulosic hydrolysate

Strain	Carbon source	Polymer content(wt%)	LA fraction (mol%)
MG-01	Glucose	76.3	5.1
	Hydrolysate	91.2	6.9
JX04-01	Glucose	79.2	9.1
	Hydrolysate	37.4	24.6
JX041-01	Glucose	81.7	14.1
	Hydrolysate	31.5	25.0



The lignocellulosic hydrolysate is mainly composed with gluose(69%) and xylose(31%), results shows it is advantageous for producing the lactic acid-based polymer.

Conclusions

- ✓ Construct the heterologous synthetic pathway of P(3HB-co-5.1mol%LA)
- ✓ Weaken the respiratory chain, the production of P(3HB-co-14.1mol%LA)
- ✓ Strengthen the promoter of the key enzyme, the production of P(3HB-co-19.5mol%LA)
- ✓ After the carbon optimization, the production of P(3HB-co-30.6mol%LA)
- ✓ Innovatively used the lignocellulosic hydrolysate, an agricultural waste, we synthesize degradable, biocompatible copolymer

References

[1] Yang T H , et al. Biotechnology & Bioengineering, 2010, 105(1):150-160.

[2] Wu H , et al. Metabolic Engineering, 2015, 28:159-168.

[3] Choi S Y , et al. Nature Biotechnology, 2016, 34:435-441.