À la carte synthesis of polyhydroxyalkanoates using *Pseudomonas putida*: exploiting the possibilities within the Synthetic Biology

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INTRODUCTION

Bio-based materials are sustainable alternatives to oil-derived plastics. Within this group, polyhydroxyalkanoates (PHAs) are biodegradable polymers that possess similar properties to conventional plastics. PHA accumulation is triggered by nutritional imbalance conditions. In this study, the model bacterium *Pseudomonas putida* KT2440, a mcl-PHA producer, was used as a chassis for the heterologous production of tailored scl-PHAs. With this aim, synthetic operons containing genes from scl-PHA producing bacteria like *Cupriavidus necator*, *Rhodospirillum rubrum* or *Pseudomonas pseudoalcaligenes* were assembled with Modular Cloning (MoCLo). This technology is based on Golden Gate (GG) reaction to allow the efficient building of polycistronic genetic constructions.

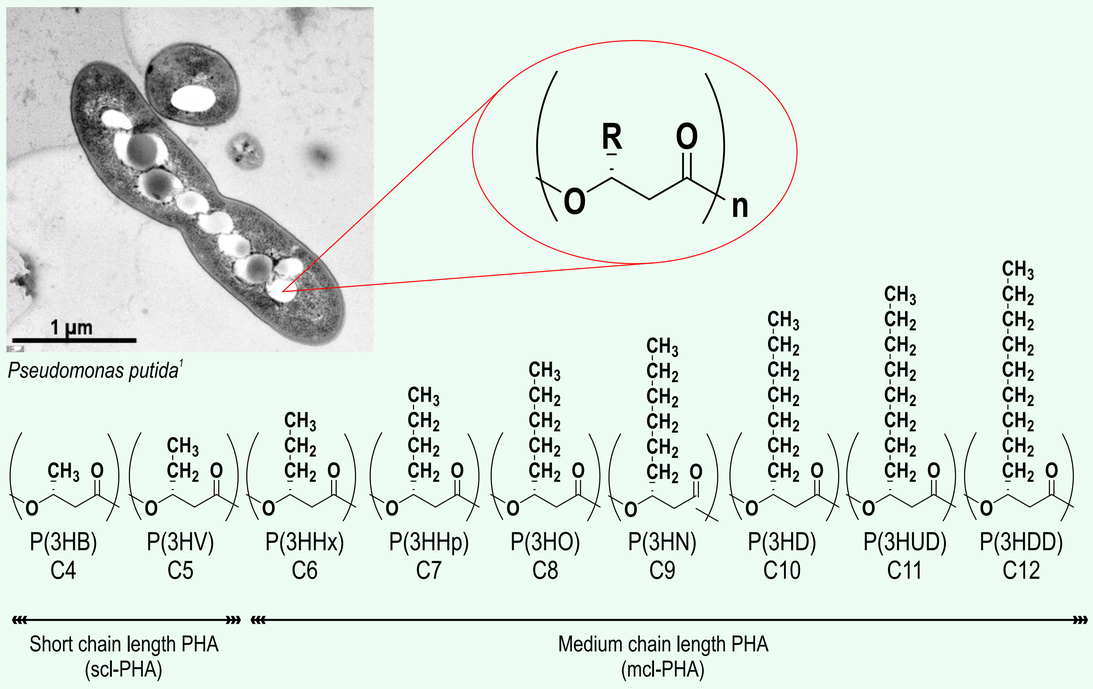


Figure 1. Monomeric composition of the PHA. The physicochemical properties of the PHA strongly depend on its monomeric composition[2]. Regarding the number of carbons of the monomers, we can classify PHAs into scl-PHAs, which tend to be stiff and brittle with a high degree of crystallinity, and mcl-PHAs, more elastic with low tensile strength and low melting point.

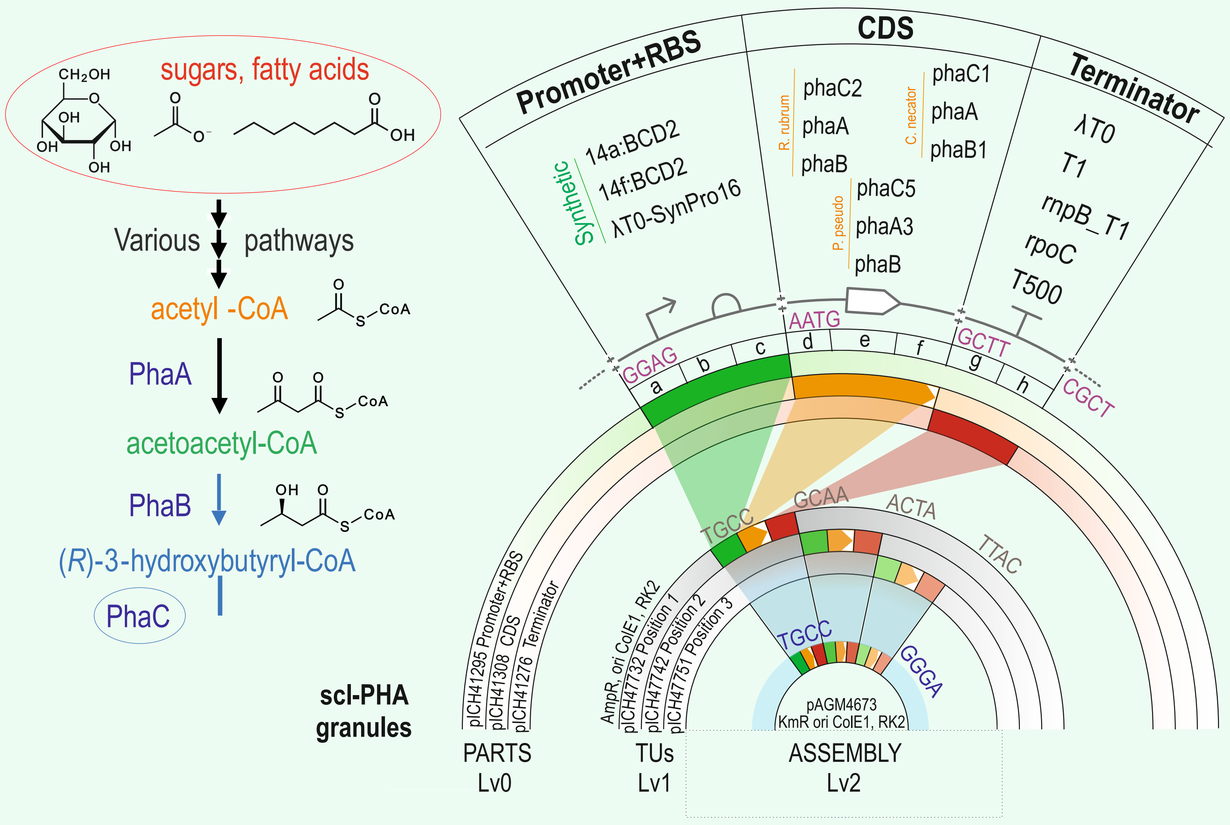


Figure 2. A. Route for scl-PHA production. B. GG/MoClo assembly cloning schema of levels 0, 1 and 2 as visualized by concentric plasmid constructs for the creation of a typical assembly containing three transcription units (TUs).

RESULTS

1. Generating *P. putida* chassis for a customized scl-PHA production

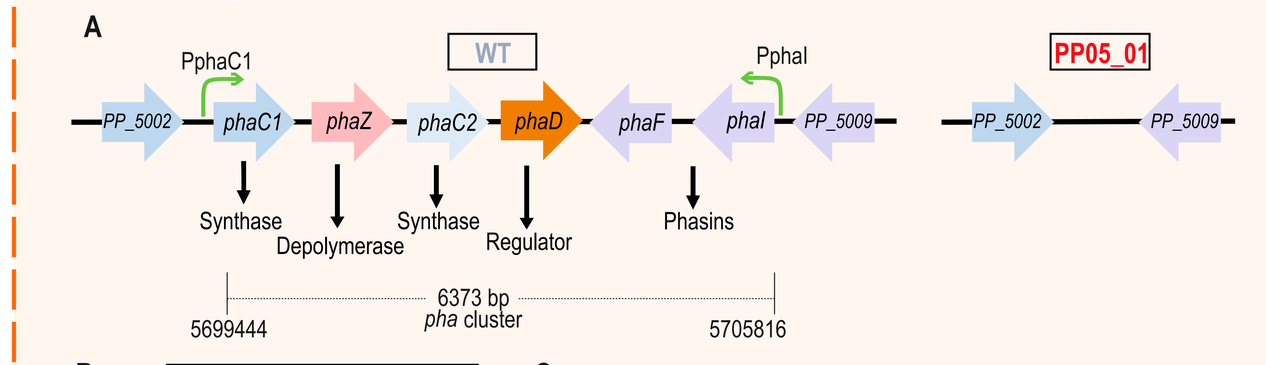


Figure 3. A. The generation of the PP05_01 strain was achieved by deleting the *pha* cluster in *P. putida*. **B.** CFU of wt and PP05_01. **C.** TEM images of wt and PP05_01 grown 0,1N M63 supplemented with 15 mM octanoate.

2. scl-PHA expression assemblies constructed by GG/Moclo

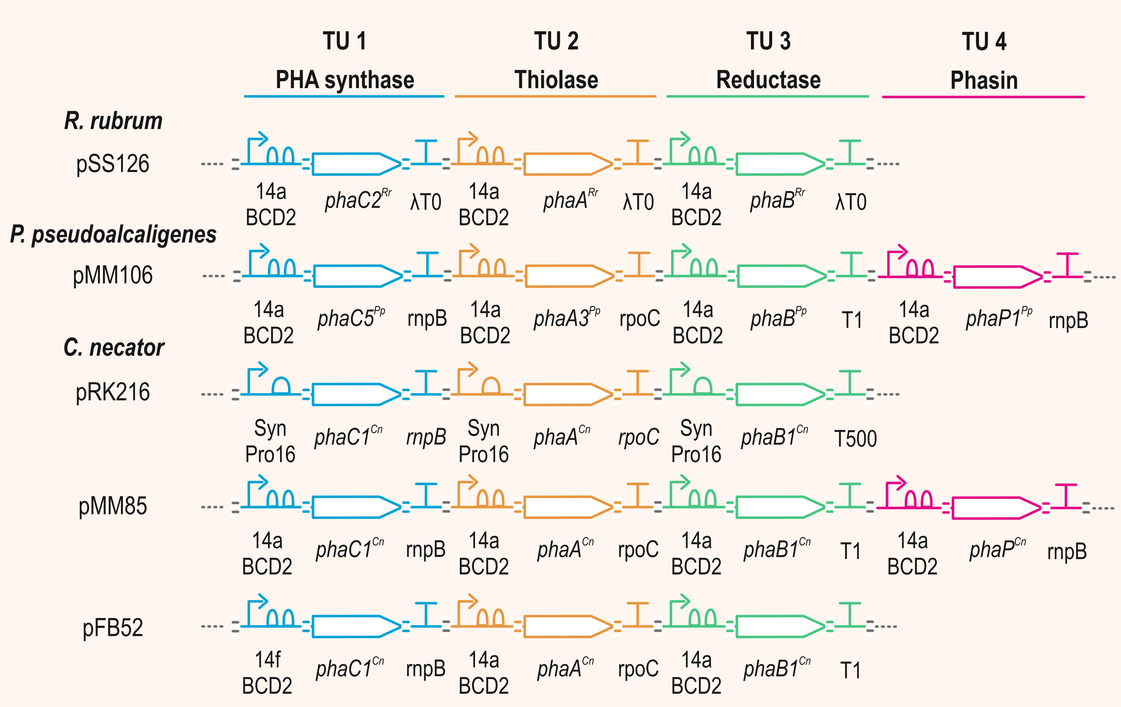


Figure 4. Genetic design schematics of GG/MoClo level 2 plasmid assemblies for the expression of scl-PHA machinery from different species.

3. Heterologous scl-PHA production in *P. putida* engineered chassis

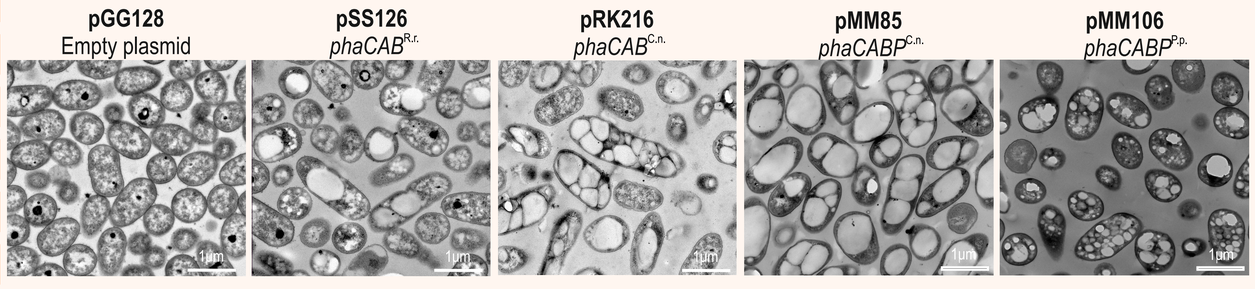
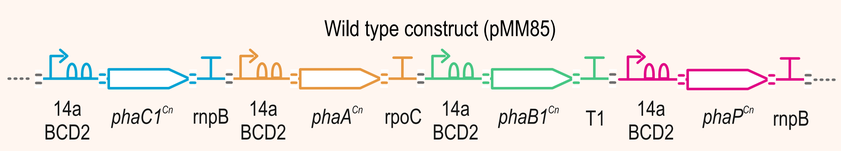


Figure 5. Transmission electron microscopy images of heterologous scl-PHA production using PP05_01 engineered strain harboring the corresponding PHA machinery genes for 24 h.

Table 1. Properties of heterologous PHA production in *P. putida*. GC-MS analysis of PHA content after 24 of growth in 0.1 N M63 15 mM octanoate.

Strain	Total CDW (g/L)	PHA (%CDW)	%C4 (mM)
pGG128; empty plasmid	0.4 ± 0.1	N.D.	N.D.
pSS126; *phaCAB*$^{R.r.}$	0.6 ± 0.1	24.8 ± 1.4	99.6 ± 0.1
pRK182; *phaCABP*$^{R.r.}$	0.6 ± 0.03	25.4 ± 1.6	100.0
pRK216; *phaCAB*$^{C.n.}$	0.8 ± 0.1	56.9 ± 10.6	99.9 ± 0.0
pMM85; *phaCABP*$^{C.n.}$	1.3 ± 0.1	84.3 ± 0.1	98.5 ± 0.5
pMM106; *phaCABP*$^{P.p.}$	0.7± 0.02	44.9 ± 0.08	100.0

4. A panel of PHBV co-polymers production



Conditions	Total biomass (g/L)	PHA (%CDW)	PHA (g/L)	Residual biomass (g/L)
(20mM) Glucose	0.99 ± 0.01	39.95 ± 3.76	0.39 ± 0.03	0.59 ± 0.04
Glu+1 mM propionic	0.95 ± 0.04	33.00 ± 1.49	0.31 ± 0.00	0.64 ± 0.04
Glu+5 mM propionic	0.98 ± 0.11	34.61 ± 3.84	0.34 ± 0.08	0.64 ± 0.03
Glu+1 mM undecenoic	1.08 ± 0.17	44.26 ± 4.00	0.48 ± 0.12	0.60 ± 0.05

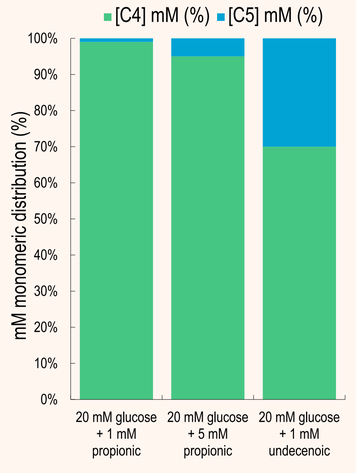


Figure 6. Panel of PHBV co-polymer production. The PP05_01 strain harboring the wild type PHA production operon from *Cupriavidus necator* (pMM85) was grown using 20 mM glucose and several co-feeding scenarios were assessed. At the conditions tested, we reached a panel of 0.6 to 18% C5 composition.

CONCLUSIONS

1. The *P. putida* PP05_01 strain was successfully constructed. This deletion mutant strain lacks the entire PHA operon, and therefore, it is unable to produce mcl-PHA.
2. Orthologous expression of scl-PHA gene assemblies was achieved in the PP05_01 by means of Moclo technology.
3. A panel of PHBV co-polymers with controlled monomeric composition was achieved by a co-feeding strategy using glucose and different odd fatty acids.

REFERENCES

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