IMPROVING THE PRODUCTION OF POLYHYDROXYBUTYRATE IN *E. coli* BY INCREASING THE NADPH POOL AND ACETYL-CoA AVAILABILITY

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Introduction. The biosynthesis of polhydroxybutyrate (PHB), a biodegradable bioplastic, requires acetyl-CoA as precursor and NADPH as cofactor. *E. coli* had been used as a heterologous production model for PHB, but metabolic pathway analysis shows a deficiency to sustain high levels of NADPH and the acetyl-CoA is mainly converted to acetic acid by native pathways (1). The objective of this work was to boost the pool of NADPH, abolish the production of acetate and increase the production of PHB in *E. coli* through the heterologous expression of the $NADP^+$-dependent glyceraldehyde 3-phosphate dehydrogenase ($NADP^+$GAPDH) gene (*gapN*) from *Streptococcus mutans*, deleting the main acetate production pathway (AckA-pta) and over-expressing the PHB synthesis pathway from *Azotobacter vinelandii* (PhbA, PhbB and PhbC), respectively.

Methods. pTrc99A (GeneBank A13038) and pAcyc184 (GeneBank X06403) vectors were used to clone and express the genes for the PHB synthesis pathway (pPHBAv) and the *gapN* gene (pGapN), respectively. The *E. coli* strain MG1655 (Wild Type Strain = WTS) and their Δ*ackA-pta* derivative (MGΔ*ackA-pta*) were used to evaluate the increase in the NADPH and acetyl-CoA pools, as well as PHB production. The strains were characterized in flask containing 50 mL mineral medium-glucose (10 g/L, 37 °C and 300 rpm). The PHB synthesis was induced with 10 µM of IPTG when cultures reached a cell mass of 0.42 g/L. Glucose (GLC) and acetate (ACE) were analyzed by HPLC and PHB polymer was extracted, hydrolysed and quantified by gas chromatography. NADPH levels were assayed using an enzymatic kit.

Results. In comparison with the WTS, results showed that the specific growth rate (µ) decreased (18%) in the strain overexpressing *gapN* (MG1655/pGapN) (Table 1), however the specific glucose consumption rate (q_{GLC}) was similar for both strains. Acetic acid production was abolished in strain MG1655*ΔackA-pta*; but q_{GLC} was also reduced (19%) (Table 1).

Table 1. Summary of results from strains characterization

STRAIN	µ (h^{-1})	$Y_{ACE/GLC}$ (mol/mol)	q_{GLC} mmol/gh	$Y_{NADPH/GLC}$ (mol/mol)
MG1655	0.61	0.67	7.98	1.62
MG1655/pGapN	0.50	0.69	7.57	2.27
MGΔackA-pta	0.57	0	6.47	ND

Furthermore, $NADP^+$GAPDH enzymatic activity is not present in MG1655, while this activity in MG1655 overexpressing *gapN* was 0.937 $IU/mg_{PROTEIN}$, accordingly the yield of NADPH on glucose increased 40 % (Table 1).

Table 2. Summary of results for the strains producing PHB

STRAIN	q_{GLC} (mmol/gh)	$Y_{PHB/S}$ (g_{PHB}/g_{GLC})	$Y_{PHB/S}$ g_{PHB}/g_{CELL}
MG1655 pPHBAv	0.57	0.22	1.53
MG1655 pGapN/pPHBAv	1.42	0.31	3.16
MG*ΔackA-pta* pGapN/pPHBAv	1.21	0.36	3.38

The PHB yield on consumed glucose with the WTS carrying pPHBAv was 46 % of the maximum theoretical (0.48 g_{PHB}/g_{GLC}) (Table 2). Interestingly, glucose consuption increased when PHB synthesis was induced in the strains carrying pGapN (Table 2). Accordingly, the PHB yield (g_{PHB}/g_{GLC}) increased to 65 % and 75 % of the maximum theoretical in the strains MG1655/pGapN/pPHBAv and MG1655Δ*ackA-pta*/pGapN/pPHBAv, respectively. Moreover, the specific accumulation of PHB (g_{PHB}/g_{CELLS}) in these strains increased 2 and 2.2 times, respectively (Table 2). Such results indicate that increasing the availability of NADPH and abolishing the production of acetate allow increasing the intracellular accumulation of PHB.

Conclusions. This work shows that increasing the NADPH availability and eliminating the metabolism of glucose to acetate boost the production and specific production of PHB in engineered *E. coli*.

References.

Tyo K., Fisher C., Simeon F; Stephanopoulos G. 2010. Metabolic Engineering. 12:187.