PRODUCTION OF PHB IN *E. coli* PTS- STRAINS DURING COUTILIZATION OF TWO CARBON SOURCES

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Key words: Polyhydroxybutyrate, Escherichia coli, coutilization

Introduction. PHB has been extensively researched as potential substitute for petroleum-derived plastics (1). Its biosynthesis is regulated by high NADPH, low CoASH and high acetyl-CoA concentrations (2). Our group has developed *E. coli* strains lacking the PTS system (PB11) and derivatives inactivated in *pykAF* and *ppsA* genes, in which the carbon catabolite repression exerted by glucose was released. When these strains, which express the *phbBAC* operon from *A. vinelandii*, are cultivated in a medium containing glucose and acetate as carbon sources, they are able to supply reducing power (NADPH) through glucose assimilation and acetyl-CoA by acetate catabolism for redirecting them to biosynthesis of PHB. In this work we evaluated the capability of these strains as potential producers of PHB.

Methods. We used a two-stage batch culture for PHB production. First, the strains were grown in medium containing glucose + acetate for biomass generation and in the second stage (production) the remaining content of the flasks was washed and re-inoculated using different treatments for accumulation of PHB: 1) medium containing glucose + acetate 2) medium containing glucose 3) medium containing acetate. Samples were taken to determine cellular dry weight (CDW) by lyophilization, PHB content by GC analysis and substrate consumption by HPLC analysis.

Results. The mutant strains PB11 *pykAF*-/pBAC and PB11 *pykAF-ppsA*-/pBAC were not able to accumulate significant quantities of PHB (Table 1-3) in the different treatments evaluated in this work, probably due to an imbalance in precursor concentrations. Nevertheless the extent of PHB accumulation was significant in parental strain PB11/pBAC, which represents 28% of its CDW during coutilization of glucose and acetate.

Table 1. Comparison of accumulation of PHB by mutant strains using two carbon sources.

Glu+Ace	PHB content %	CDW mg/ml
PB11/pBAC	27.95 ± 2.91	2.02 ± 0.29
PB11*pykAF*-/pBAC	2.32 ± 0.85	1.57 ± 0.13
PB11*pykAF-ppsA*-/pBAC	ND	1.40 ± 0.18

Table 2. Comparison of accumulation of PHB by mutant strains using glucose.

Glucose	PHB content %	CDW mg/ml
PB11/pBAC	2.16 ± 0.71	1.28 ± 0.08
PB11*pykAF*-/pBAC	0.57 ± 0.03	0.59 ± 0.05
PB11*pykAF-ppsA*-/pBAC	ND	0.61 ± 0.04

Table 3. Comparison of accumulation of PHB by mutant strains using acetate.

Acetato	PHB content %	CDW mg/ml
PB11/pBAC	16.93 ± 3.76	1.32 ± 0.02
PB11*pykAF*-/pBAC	1.28 ± 0.17	1.28 ± 0.09
PB11*pykAF-ppsA*-/pBAC	ND	0.63 ± 0.12

Conclusions. We have demonstrated that heterologous expression of *phbBAC* genes in *E. coli* PTS- was useful in the production of PHB during coutilization of glucose and acetate.

Acknowledgements. This work was supported by grant 105782 from CONACYT and IN205811 from PAPIIT.

References.

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