PHB production increases by *algD*, *pycA* and *rsmA* inactivation in *Azotobacter vinelandii* under different carbon sources

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Introducction. *Azotobacter vinelandii* produces *Poli-β -hydroxybutyrate* (PHB); it is used as a biodegradable thermoplastic (Anderson and Dawes 1990). Their production depends in the availability of Acetil-CoA as biosynthetic precursor on PHB synthesis. It was reported that inactivation of the gene *pycA* increases PHB accumulation in solid medium (Segura ans Espín. 2004). The biosynthetic operon *phbBAC*, responsible for synthesizing PHB, is under complex regulation by PhbR and RsmA proteins. PhbR is a transcriptional activator of the *phbBAC* operon. RsmA protein is a negative post-transcriptional regulator controlling the expression of *phbR* and *phbB* (Hernández Eligio 2011). In this work, we made a strain with *pycA* and *rsmA* inactivation to improve the PHB production and, we assay different growth conditions to determine the most favorable production conditions in this strain. In particular, the effect of different carbon sources on the accumulation of PHB in a mutant *algD pycA rsmA* was tested.

Methodology.

A. *vinelandii* strain E modifications as follow: *algD* mutation to prevent the alginate production was performed by insertion of a streptomycin cassette. *pycA* mutant was constructed by a kanamycin cassette insertion. Finally, the last mutation in the *rsmA* gene was constructed by deleting the gene with a gentamicin cassette.

The constructed E*algDpycArsmA* strain was characterized by its accumulation of PHB when growing in a minimal and a rich medium added with carbon sucrose and fructose as carbon sources.

Results.

The triple mutant E*algDpyArsmA* was growing in two different carbon sources to study the differences in PHB accumulation. Figure 1 shows the PHB production in a rich solid medium (PY) added with sucrose, and Table 1 shows the data obtained when the mutant was grown in liquid-rich (PY) and minimal medium (Burk) added with sucrose or fructose as carbon source.

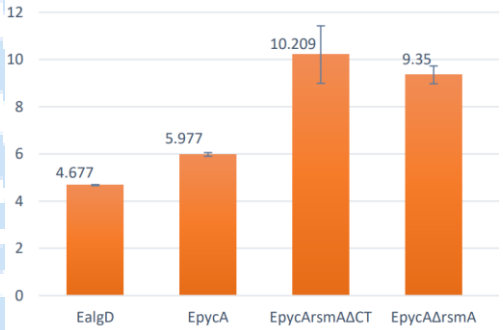

Figure 1. PHB accumulation in PY-sucrose solid medium. The PHB accumulation is expressed inμg PHB/ mg protein.

Table 1. PHB accumulation in liquid rich media PY added with different carbon sources.

Strain	Medium	μg PHB / mg protein
E*algD*	PY-sucrose	0.006
E*pycA*	PY-sucrose	.024
E*rsmA*	PY-sucrose	.0052
E*pycArsmA*	PY-sucrose	.0022
E*algD*	PY-Fructose	7.4
E*pycA*	PY-Fructose	9.11
E*rsmA*	PY-Fructose	3.18
E*pycArsmA*	PY-Fructose	25.99

Conclusions.

The most favorable condition to produce PHB in the constructed triple mutant is established when it is grown in PY liquid medium added with fructose.

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