Quantification of polyhydroxyalkanoates in the non-sulphur purple bacterium *Rhodospirillum rubrum* S1H

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Rhodospirillum rubrum is a non-sulphur purple bacterium well known for its huge metabolic versatility. Previous studies in the lab revealed its production of polyhydroxyalkanoates (PHAs) in different growth conditions (*Figure* 1). PHAs are bacterial biodegradable polymers which could be used to replace petroleum non biodegradable plastics.

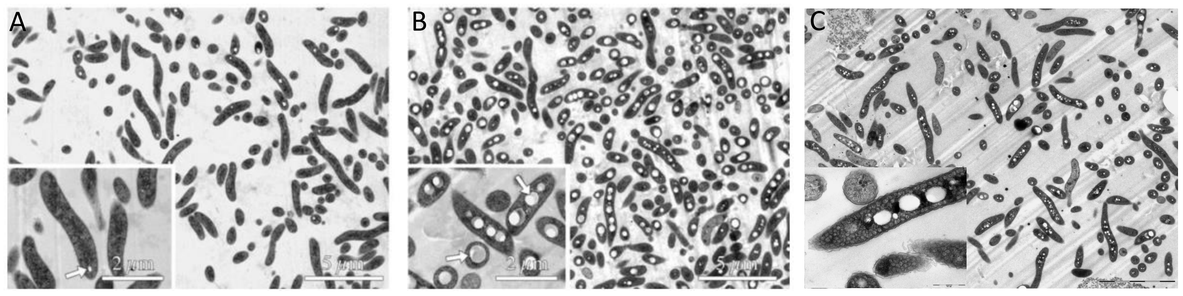

Figure 1 : Transmission Electron Microscopy (TEM) pictures of *Rhodospirillum rubrum* in presence of different carbon sources showing PHA granules. A) succinate, B) acetate, C) butyrate

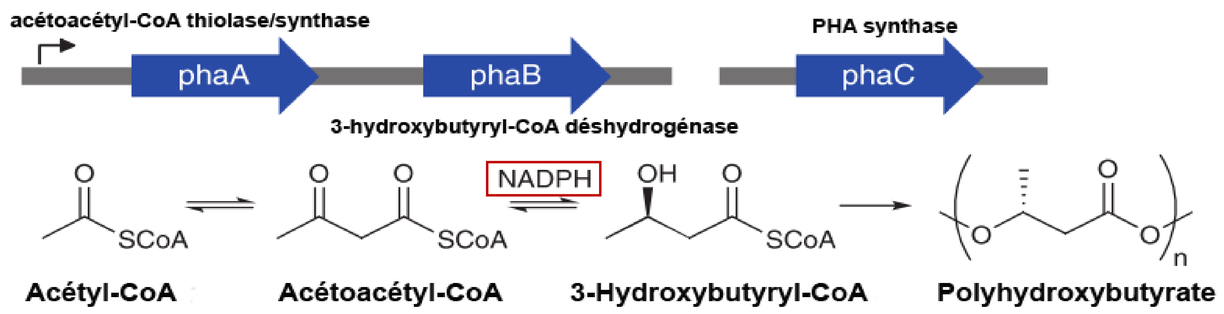

Figure 2 : Metabolic pathway leading to the production of PHB and consuming a molecule of NADPH

Nowadays, the industrial process is curbed by its production cost largely attributed to the carbon source. Henceforth, the use of waste coming from wastewater treatments like volatile fatty acids (VFAs, *e.g.* acetate, butyrate, propionate or valerate) could be a good solution to solve this issue. In our case, the hypothesis would be that a deregulation of the intracellular redox balance, induced by the use of a reduced carbon source, leads the production of PHA (*Figure* 2). Indeed, this mechanism is supposed to be driven by an excess of NAPDH in the cell.

Here, we have studied the production of co-polymer of poly(hydroxybutyrate-*co*-hydroxyvalerate (P(HB-*co*-HV)) , a high added value compound, but the non-sulfur purple bacterium *Rs. rubrum*.

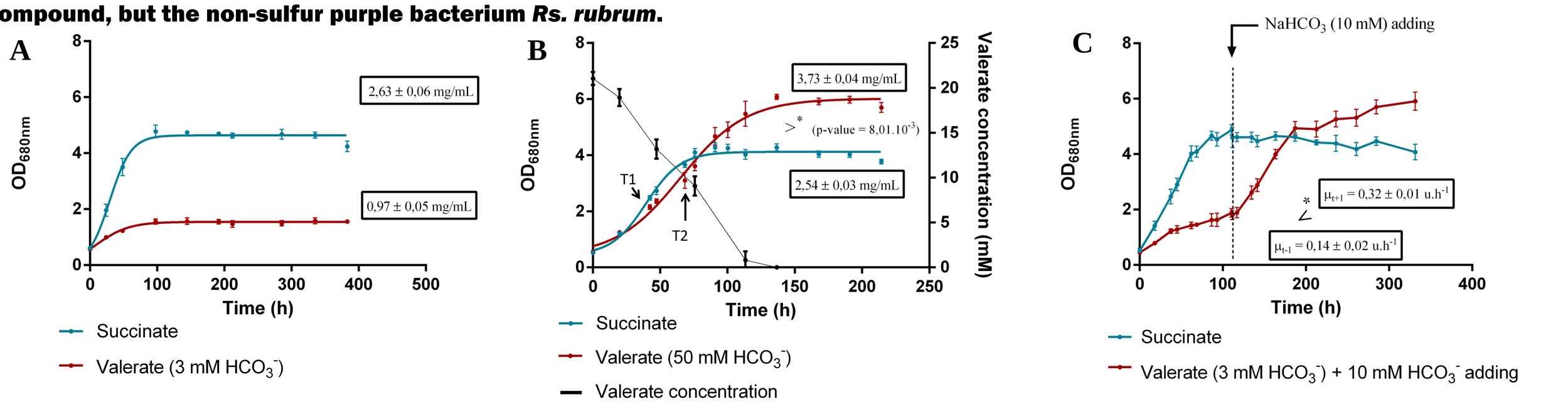

Figure 3 : Culture of Rs. rubrum cultivated in 25 mM valerate and 3 mM of CO_3^- (A). Culture of Rs. rubrum cultivated in 25 mM valerate and 50 mM of CO_3^{--}, black arrow represents the sampling time for MS/MS analysis (B). Influence of CO_3^{--} on Rs. rubrum in presence of valerate, black arrows indicate the pulse of 10 mM CO_3^{--} (C).

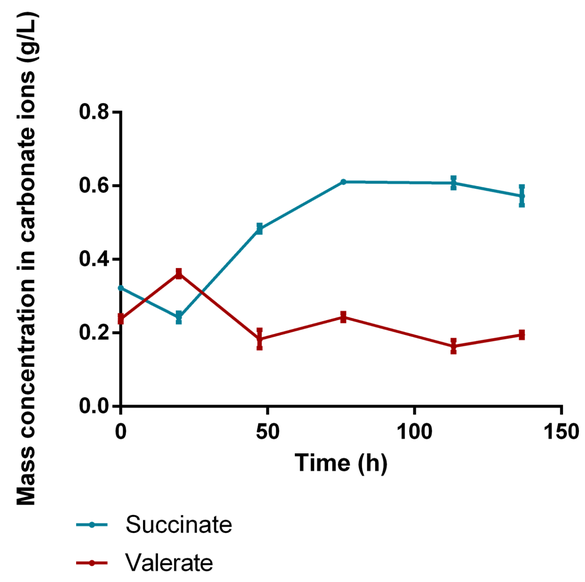

Figure 4 : Dissolved Inorganic Carbon (DIC) analysis realised culture cultivated on succinate and valerate (50 mM CO_3^-).

The dependence of carbonate ions on the assimilation of valerate has been shown (*Figure 3* and *4*). This type of observation has already been done by our group in butyrate and propionate condition. Several hypotheses could explain the phenomenon : I) the carbonate ions are needed in carboxylation steps occurring in the carbon source assimilation II) they could be necessary for the activity of Calvin cycle which act as an electron sink.

Proteomic analyses conducted on valerate culture have shown PHA related proteins with differential abundance compared to succinate condition (*Table 1*) This observation could reveal PHA production in those culture. PHA quantitation *via* GC-MS proved the PHA production in both condition. (*Figure 5*). Interestingly, PHAs produced in valerate condition are high PHV ratio co-polymers (21% HB/79% HV).

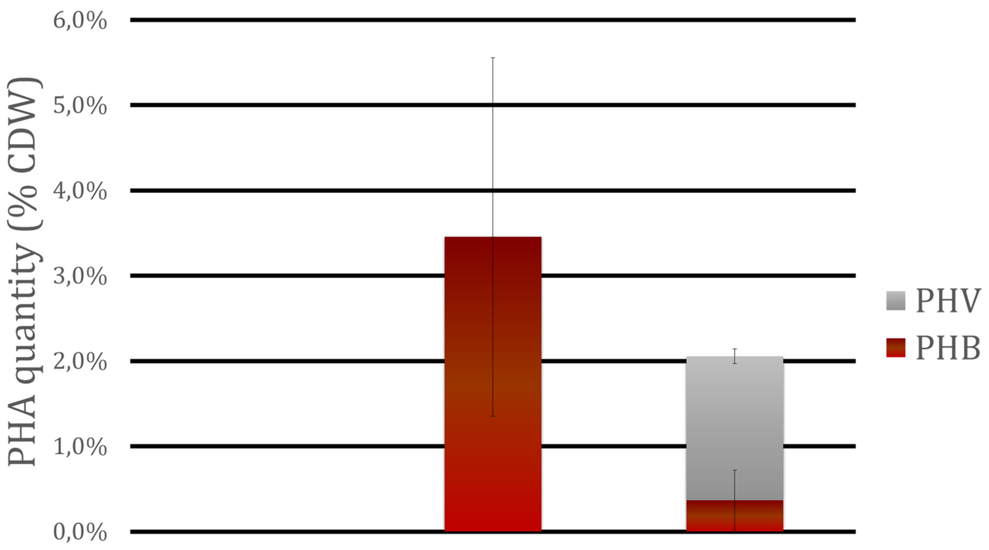

Figure 5 : Quantification of PHB and PHV content contained in cells in different carbon sources, PHA have been extracted *via* methanolysis

Table 1: PHA-related proteins showing differential abundance between valerate and succinate condition. T1 and T2 sampling are shown on *fig 3B*).

			Early exponential phase (T1)			Half of exponential phase (T2)		
Locus Tag	Description	Access number	Peptides	p-value	Abundance ratio (Val/Suc)	Peptides	p-value	Abundance ratio (Val/Suc)
Rru_A0273	3-oxoacyl-ACP reductase	Q2RXR7	5	3.5E-01	0.8	5	2.8E-07	0.47*
Rru_A2817	Phasin (family 2)	Q2RQI1	5	3.8E-04	48.24*	5	5.2E-04	41.46*
Rru_A3283	Phasin ApdA (PHB degradation activator)	Q2RP67	5	2.8E-02	2.06*	5	6.1E-09	4.41*
Rru_A3356	Polyhydroxyalkanoate depolymerase	Q2RNZ5	3	4.1E-03	0.58*	-	-	-

Take-away message

The **quantification of PHA** during the assimilation of different carbon source inducing different **intracellular redox state** is of first interest. The connection between the redox state and the PHA production is needed to fully understand the PHA production. This leads to the **production of cheap microbial biodegradable plastics** using VFAs derived from, for example, **wastewater treatments**. First results show promising results for the production of the co-polymer **P(HB-*co*-HV)** during the assimilation of **valeric acid.**

Acknowledgement: This work was partially financed by the FNRS and was supported by an ARC project. G. Bayon-Vicente is a F.R.I.A. PhD student. I kindly thank Quentin De Meur who provides me the different TEM pictures. Presenting authors: guillaume.bayon-vicente@umons.ac.be & Corresponding author: Ruddy.wattiez@umons.ac.be