Impact of redox stress on the growth of Rhodospirillum rubrum S1H

Paloma Cabecas Segura[1], Chiara Magnani[2], Anne-Lise Hantson[3], Audrey Tanghe [4], Florence Allert [4], Jean-Marie Raquez[2], Ruddy Wattiez[1], Baptiste Leroy[1]

[1]Department of Proteomics and Microbiology, Research Institute for Biosciences, University of Mons, Mons, Belgium

[2]Departement of Polymeric and Composite, Research **Institute** for Materials Science and Engineering, University of Mons, Mons, Belgium

[3] Department of Chemical and Biochemical Engineering, Research Institute for Biosciences , University of Mons, Mons, Belgium

[4] Materia Nova, Parc Initialis, Mons, Belgium

Introduction

Rhodospirillum rubrum is an α-proteobacteria that is known for its great metabolic versatility. Purple non-sulfur bacteria are well-studied for their ability to grow under photoheterotrophic conditions using energy from light and various volatile fatty acids (VFAs) as carbon and electron sources. Our previous studies revealed a production of polyhydroxyalkanoates (PHAs) when different VFA are used as carbon sources (*Figure* 1). PHA are bio-sourced, biodegradable polymers that could be used to replace traditional oil-based plastics.

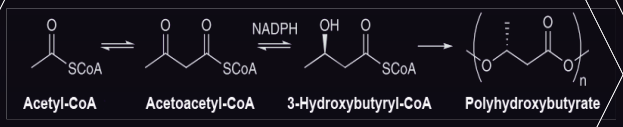

Figure 2: Metabolic pathway leading to the production of PHB and consuming a molecule of NADPH [1]

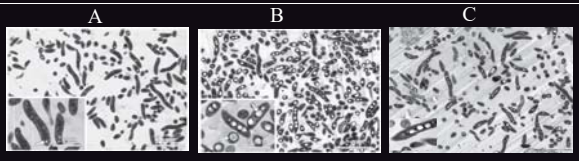

Figure 1 : Transmission Electron Microscopy (TEM) pictures of *Rhodospirillum rubrum* in presence of different carbon sources showing PHA granules. (A) succinate, (B) acetate, (C) butyrate [2]

PHAs are used as carbon or energy storage inclusions when a nutrient (N, S, P) is lacking. However, in our case, PHAs production by *Rhodospirillum rubrum* is observed under nutrient available conditions. Our hypothesis is that PHA production could be used as an electron sink because of the consumption of NADPH during the process (*Figure 2).* Moreover redox balance is known to be an important parameter of photoheterotrophic growth on reduced substrate such as VFA. In the prospect of industrial application, there is a need to better understand how carbon source impact the redox balance of the cell and by extension the PHA production.

1. Impact on growth

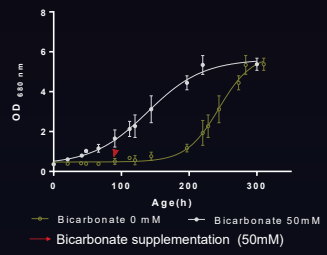

Figure 3 : *Rhodospirillum rubrum* S1H growth monitoring when hexanoate is used as carbon source

The growth on reduced substrate such as hexanoate seems to be inhibited in the absence of bicarbonate in the medium. (*Figure 3*)

The presence of bicarbonate can help reducing the redox-stress, as the fixation of CO_2 has been described as a central redox cofactor recycling mechanism in bacteria.

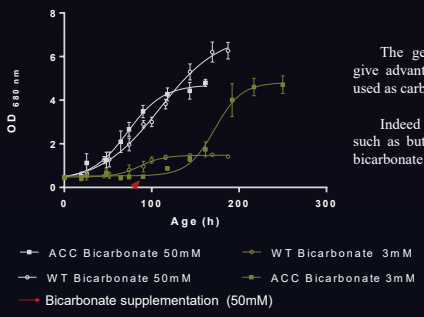

Figure 5 : *Rhodospirillum rubrum* S1H (WT) or acetate competent strain (ACC) growth monitoring when butyrate is used as carbon source with 3mM of bicarbonate or an excess of bicarbonate.

The gene amplification and over expression give advantage to the strain only when acetate is used as carbon source .

Indeed when grow on other reduced substrates such as butyrate, the growth is still dependent of bicarbonate supplementation. (Figure 5)

2. Substrate adaption

When growing on acetate, *Rhodospirillum rubrum* is known to present a characteristic long lag phase, which is hypothesized to be due to redox unbalanced in the cell. In the lab acetate competent cells, strain of *Rhodospirillum rubrum* presenting an amplification of a genome fragment containing gene coding for key enzymes of the EMC such as the crotonyl-CoA reductase/carboxylase, have been obtained. [3]

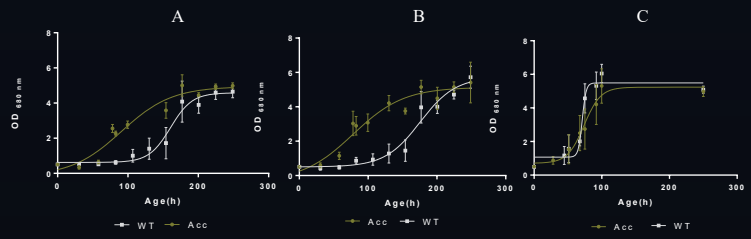

Figure 4 : Growth of *Rhodospirillum rubrum* S1H (WT) or the acetate competent strain (ACC) when acetate is used as carbon source without bicarbonate supplementation (A), with 3mM of bicarbonate (B) or with an excess of bicarbonate (C) .

For *Rhodospirillum rubrum S1H*, there in no lag phase when bicarbonate are present in excess. This is not the case for the acetate competent strain, that presents this growth phenotype whatever the concentration of bicarbonate. Both excess of bicarbonate and higher abundance of the crotonyl-coA reductase/carboxylase, could increase the flux in the EMC. Thus it seems to indicate that this mechanism may help the cell to manage redox-imbalance

3. Blend of carbon sources

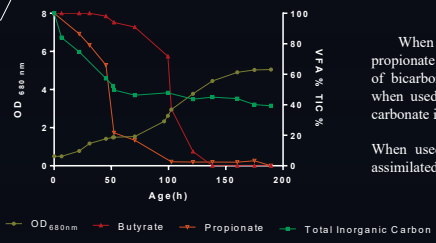

Figure 6 : *Rhodospirillum rubrum* Growth in presence of butyrate and propionate as carbon source.

When used as sole carbon source, propionate and butyrate required a large amount of bicarbonate to be completely assimilate but when used as a blend only a small amount of carbonate is needed.

When used as a blend, VFA are sequentially assimilated. (Figure 6)

Tableau 1: Ratio between carbonate consumption and propionate and butyrate assimilation when used as sole carbon source or as a blend

Carbon sources	Carbonate/Biomasse (mM/mM)	Carbonate/Biomasse (mM of C /mM of C)
Propionate	0.11	0.018
Butyrate	0.3	0.075
Mix :		
Propionate	1.74	0.58
Butyrate	0.21	0.052

Conclusion

Redox balance is one of the major parameter of photoheterotrophic growth on reduced substrate such as VFA. It seems that it could impact the production of biomass, by inhibiting the growth or leading to a long lag phase. Increasing the flux in the EMC pathway, by gene amplification or bicarbonate excess in the medium seems to be a mechanism to deal with this unbalanced. Blend of VFA also seems to impact the redox stress of the cell. Further investigations are needed to better understand this phenomenon and its impact on PHA synthesis.

Aknowledgments

This research is included in the PHASYN project supported by an ARC convention

Presenting author : paloma.cabecassegura@umons.ac.be
Corresponding author : baptiste.leroy@umons.ac.be

References

[1] : Bond-Watts, B. B., Bellerose, R. J., & Chang, M. C.. Enzyme mechanism as a kinetic control element for designing synthetic biofuel pathways. *Nature chemical biology*, **7(4)**, 222-227.(2011)

[2] : Leroy, B., De Meur, Q., Moulin, C., Wegria, G., & Wattiez, R.. New insight into the photoheterotrophic growth of the isocytrate lyase-lacking purple bacterium Rhodospirillum rubrum on acetate. *Microbiology*, **161(5)**, 1061-1072. (2015)

[3] : De Meur, Q., Deutschbauer, A., Koch, M., Wattiez, R., & Leroy, B.. Genetic Plasticity and Ethylmalonyl Coenzyme A Pathway during Acetate Assimilation in Rhodospirillum rubrum S1H under Photoheterotrophic Conditions. *Applied and environmental microbiology*, **84(3),** e02038-17. (2018)