Photoheterotrophic metabolism of volatil fatty acids in *Rhodospirillum rubrum*

UMONS biosciences
UMONS RESEARCH INSTITUTE FOR BIOSCIENCES

esa

De Meur Q.[+] – Moulin C. [+] – Wégria G. [*] - Leroy B. [+] – Wattiez R. [+]

[+] - Department of Proteomics and Microbiology of the University of Mons, Belgium
[*] - Materia-Nova Biotech , Ghislenghien, Belgium

Introduction

Rhodospirillum rubrum belongs to the paraphyletic group of purple non-sulfur bacteria, one of the most versatile in terms of metabolism. *R. rubrum* is able to grow autotrophically or heterotrophically, phototrophically or chimiotrophically, in presence or in absence of oxygen. Among these different metabolisms, photoheterotrophy allows *R. rubrum* to use light as energy source and volatile fatty acids as carbon source. This metabolism has made *R. rubrum* an ideal candidate for MELISSA loop, the autonomous ecosystem developed by the European Space Agency (ESA). Indeed volatile fatty acids are the final product of fermentative processes and thus the main effluent from the first compartment of the loop. Throught his photoheterotrophic metabolism, *R. rubrum* is supposed to remove these VFA from the system, and furthermore produces edible biomass.

We recently undertook the characterization of metabolic pathways involved in the VFA assimilation in *R. rubrum*, and particularly acetate, propionate, butyrate and mixtures of them. In a first approach, we determined the phenotypic aspect of bacterial growth. In a second approach we tried to reconstruct the assimilation pathway of these VFA using differential proteomic data obtained through UPLC-HRMS platform and label-free quantitative analysis.

Phenotypic approach for unique VFA

Propionate

When propionate is present as unique carbone source, *R. rubrum* shows a short lag phase but, over all, it never reachs levels of biomass obtain with succinate (used as a control in this study), exept if bicarbonate is added to the medium (fig. 1), highlighting the well known requirement of carboxylation in the propionate assimilation. (Clayton et al., 1957)

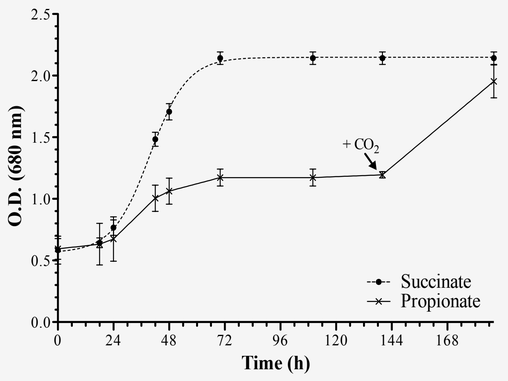


Fig. 1: Growth curves obtained when R. rubrum is cultivated in the presence of propionate, compared to succinate (control). Regardless the nature of the carbon source, each culture medium contains 125 mM carbon equivalent.

Acetate

Even if biomass reaches normal level (supposing a complete assimilation of the available carbon), *R. rubrum* undergoes a long lag phase when it uses acetate as unique carbon source (fig. 2A). This lag phase is supposed to be due to a redox imbalance induced by acetate assimilation.

Consumption of acetate from the culture supernatants was also assessed by HPLC (fig. 2B). These analyses confirmed the complete assimilation of acetate at the end of the exponential growth phase.

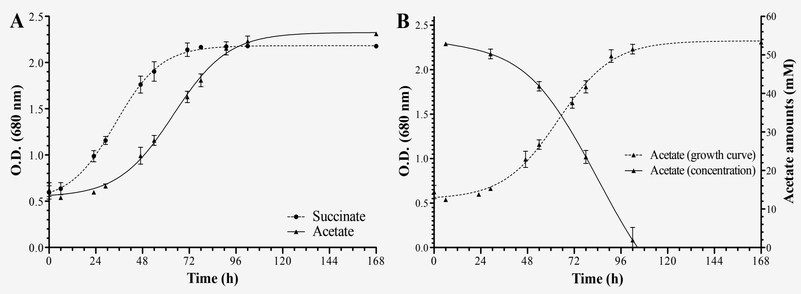


Fig. 2: (**A**) Growth curves obtained when R. rubrum is cultivated in the presence of acetate, compared to succinate (control). Regardless the nature of the carbon source, each culture medium contains 125 mM carbon equivalent.
(**B**) Evolution of the concentration in acetate in the culture mediumduring the culture growth.

Butyrate

With butyrate, the nature of the inocculum seems to influence the behavior of the culture. When *R. rubrum* comes from a succinate preculture, it reaches a higher plateau than if it comes from butyrate preculture (fig. 3A). This effect totally disappears when inocculum is rinsed. Something from the succinate preculture seems to help *R. rubrum* to grow in presence of butyrate.

However, butyrate is never completely assimilated by R. rubrum. In the best case, only half of the initial amount of butyrate is consumed (fig. 3B). This inhibition is still misunderstood. It could be due to metabolite in limitant concentration, or more probably to a redox imbalance since butyrate is a particularly reduced carbon source.

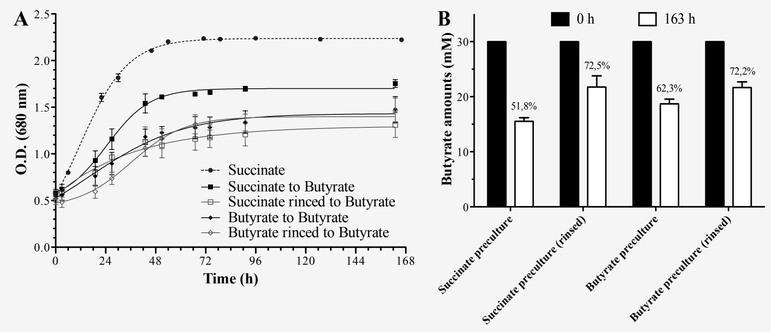


Fig. 3: (**A**) Growth curves obtained when *R. rubrum* is cultivated in the presence of butyrate, compared to succinate (control). Regardless the nature of the carbon source, each culture medium contains 125 mM carbon equivalent.
(**B**) Evolution of the concentration in butyrate at the begining and the end of the growth phase.

Phenotypic approach for mixes VFA

7:2:1 Mix

The assimilation of VFA supplied as a mixture was explored following VFA proportions defined by the VITO & EPAS technical note 71.9.4 (ESA; 2005): 70% of acetate, 20% of butyrate and 10% of propionate. *R. rubrum* seems to growth more efficiently with a mixed carbon source than with VFA used as single carbon sources: there is no lag phase and cultures reach O.D. at least as good as O.D. of culture in succinate.

All the available carbon is completely consumed, but the three VFA are not simultaneously assimilated: propionate and acetate are immediatly assimilated, unlike butyrate which is not assimilated during the first 24 hours.

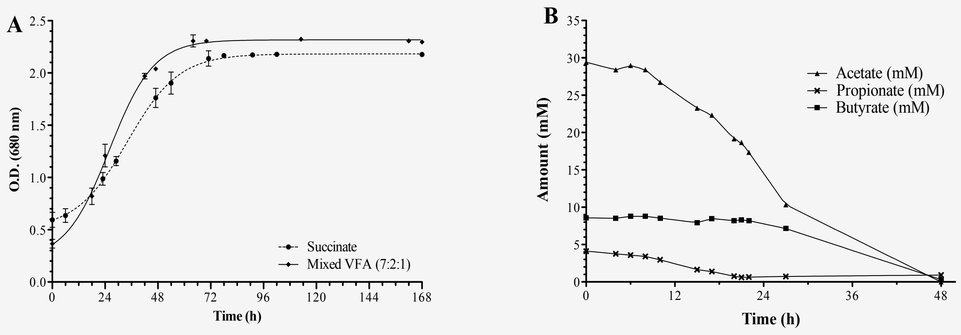


Fig. 4: (**A**) Growth curves obtained when R. rubrum is cultivated in the presence of acetate, butyrate and propionate in a 7:2:1 proportion mix (125 mM carbon equivalent) compared to succinate (control).
(**B**) Evolution of the concentration in acetate, butyrate and propionate in culture medium during the culture growth.

1:1 Mixes

To highlight an eventual catabolic repression of the assimilation of butyrate by other VFA, three VFA mixes in fifty-fifty proportions were analysed. *R. rubrum* seems to growth more efficiently with these 1:1 VFA mixes than with VFA used as single carbon sources. However growth is always better when butyrate is not present in the mix, even better than 7:2:1 mix with a faster exponential growth phase (fig. 5A). Once butyrate is added to the mix, *R. rubrum* seems to growth slower than with 7:2:1 mix (fig. 5A & 5B). Surprisingly, when butyrate and propionate are used together (fig. 5C) culture reaches normal O.D. while when they are used as single carbon sources, propionate needs carbon dioxyde fixation for complete propionate assimilation, and butyrate stop growing for an unknow reason before complete assimilation of carbon.

Preliminary results of HPLC analysis of supernatants from these 1:1 mix cultures (data not shown) confirm observations made for assimilation in culture with 7:2:1 mix: acetate and propionate are immediatly assimilated while the butyrate concentration remain constant until other carbon sources are consumed. These results corroborate the inhibition of the butyrate assimilation by acetate and propionate.

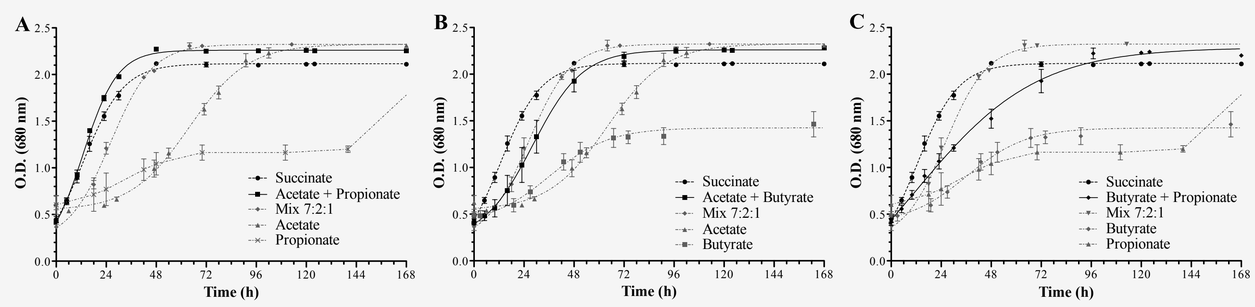
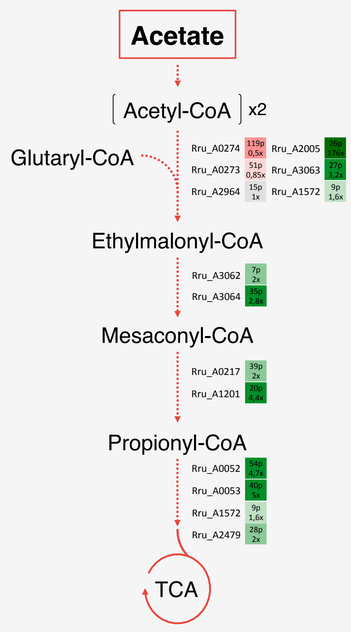


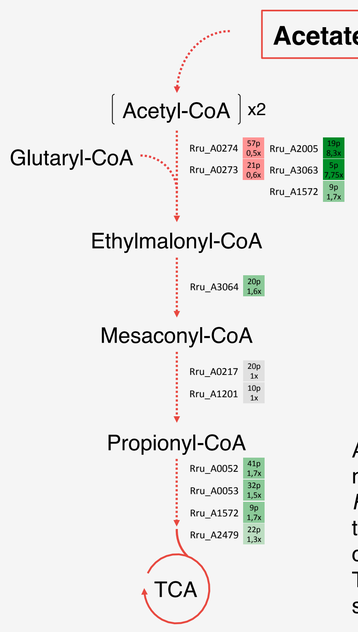
Fig. 5: Growth curves obtains when R. rubrum is cultivated in the presence of acetate and propionate (**A**), acetate and butyrate (**B**), or butyrate and propionate (**C**) in a 1:1 proportion mix (125 mM carbon equivalent) compared to succinate (control).

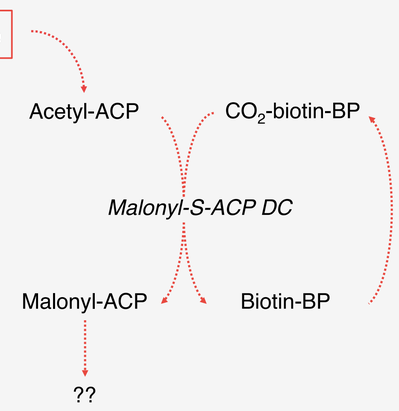
Proteomic approach

Proteomic data obtained by UPLC-HRMS about propionate assimilation confirmed the photoassimilation pathway constructed by Knight in 1962.

Unexpectedly, when acetate is the sole carbon source, proteomic analyses didn't confirmed the citramalte pathway of acetate assimilation reported by Berg & Ivanovsky in 2009. On the other hand, the ethylmalonyl-CoA pathway of acetate assimilation (proposed by Albert et al. in 2006 in *Rhodobacter sphaeroides*) seems to be use by our strain in our conditions since all the enzymes of this pathway were observed in higher abundance (enzymes quantified with a p-value < 0.05). Similar results were also observed in samples from culture with 7:2:1 mixed VFA for segment of the exponential growth phase where acetate is the sole assimilated carbon source.







Additionally, we also observed a probable new pathway of acetate assimilation when *R. rubrum* is cultivated at 35°C instead of the default 30°C, involving enzymatic complex of the malonate decarboxylase. This new alternative pathway needs further studies.

Conclusion & perspectives

***R. rubrum* seems to grow always higher and faster in presence of mixed carbon sources than if VFA are used as unique carbon source. Despite these promising results, butyrate seems to be the most problematic VFA because its assimilation is inhibited when other VFA are present in the culture medium. Since butyrate is the second most abundant VFA in the C1 effluents (which furthermore get into the C2 in a continuous flux), an accumulation of butyrate could occur in the C2, questioning the entire MELISSA loop.**

Our understanding of the photoheterotrophic metabolism of butyrate should be improved by future proteomic analyzes. The potential catabolic repression of this VFA will also be explore to better understand how other carbon source inhibit butyrate assimilation. And finally, the trail of redox imbalance will be simultaneously explored since this parameter seems to be a crucial feature of the VFA assimilation.

Acknowledgments

This projet is supported by European Space Agency (Prodex « Melgen-3 » project)

Clayton et al., 1957, **A carbon dioxide requirement for the metabolism of propionate in rhodospirillum rubrum**, Archiv für Mikrobiologie (25) ; Knight M. , 1962 , ***The Photometabolism of propionate by Rhodospirillum rubrum*** , The Biochemical Journal (84) ; Alber et al., 2006, **Study of an alternate glyoxylate cycle for acetate assimilation by Rhodobacter sphaeroides**, Molecular Microbiology (61) ; Berg I. A. & Ivanovsky R. N., 2009, **Enzymes of the Citramalate Cycle in Rhodospirillum rubrum**, Microbiology (78)