Quantification of cofactors and polyhydroxyalkanoates in the non-sulphur purple bacteria *Rhodospirillum rubrum* S1H

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1. Introduction

Rhodospirillum rubrum is a non-sulphur purple bacterium well known for its huge metabolic versatility. Previous studies in the lab revealed the bacterial production of polyhydroxyalkanoates (PHAs) in different cultivation conditions (*fig.* 1). PHAs are bacterial biodegradable polymers which could be used to replace petroleum non degradable plastics. Several bacteria are known to produce those compounds with high yield . However, the industrial process is curbed by its production cost largely imputed to the cost of the carbon source. Henceforth, the use of waste coming from wastewater treatment like volatile fatty acids (VFAs, *e.g.* acetate, butyrate, propionate or valerate) would be a good solution to solve this issue. In our case, the hypothesis would be that a deregulation of the intracellular redox balance induces the production of PHA (*fig.* 2).

Here, we have developed new methods in one hand to quantify intracellular PHA content and determine the proportion of each monomer in the polymer and in the other hand to extract and quantify the different cofactors in the cell.

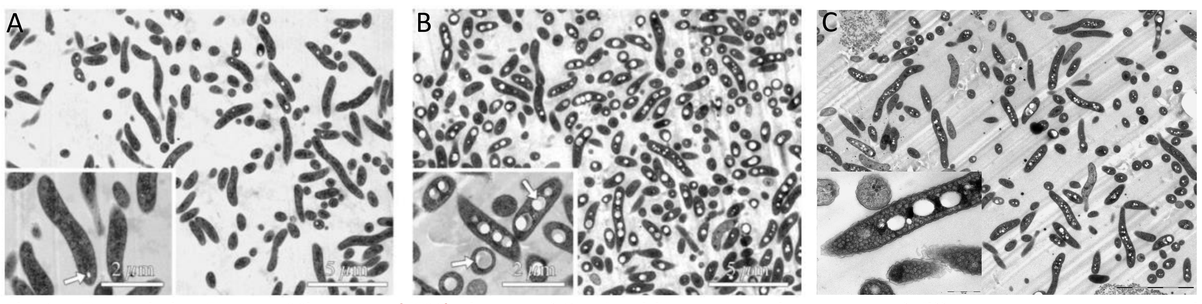

Fig. 1 : Transmission Electron Microscopy (TEM) pictures of *Rhodospirillum rubrum* in presence of different carbon sources showing PHA granules. a) succinate, b) acetate, c) butyrate

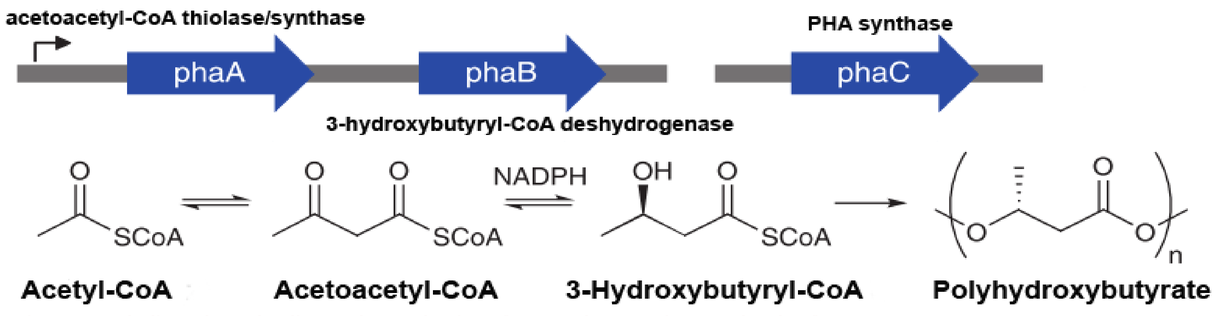

Fig. 2 : Metabolic pathway leading to the production of PHB and consuming a molecule of NADPH

2. PHA quantification

The polymer extraction implies their methanolysis *via* methanol and H_2SO_4 yielding the methyl ester species followed by solubilisation in chloroform (*fig.* 3).

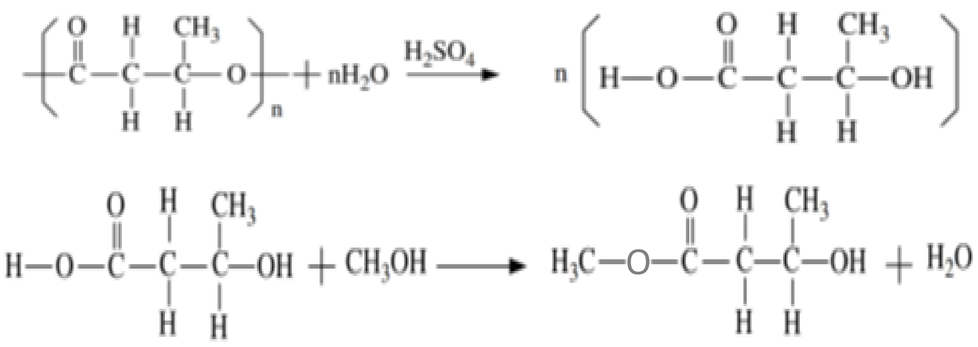

Fig. 3 : Methanolysis occurring during the extraction of the polymer yielding to its hydrolysis and the production of the methyl ester species

After some GC-parameters optimisation, compounds of interest have been successfully separated.

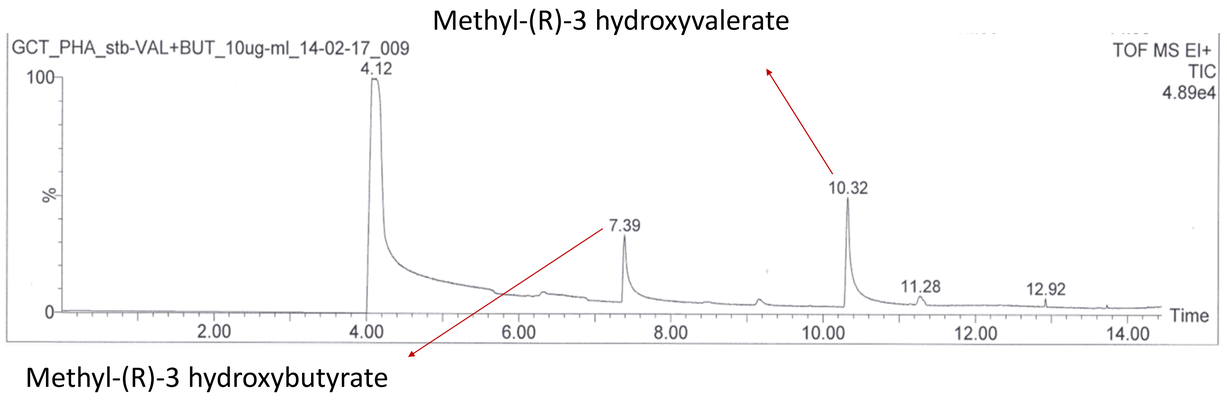

Fig. 4 : Retention time of the different standard (methyl-(R)-3 hydroxybutyrate and methyl-(R)-3-hydrovalerate) injected at 10 ng on column(*fig.* 3).

The determination of dynamic range is a crucial step in order to insure the reliability of the future quantification. This dynamic range has been done for the two most common methyl ester species. The dynamic range indicates that injected quantity comprised between 2 and 10 ng is suitable for the quantitation of both compounds (*fig.* 4).

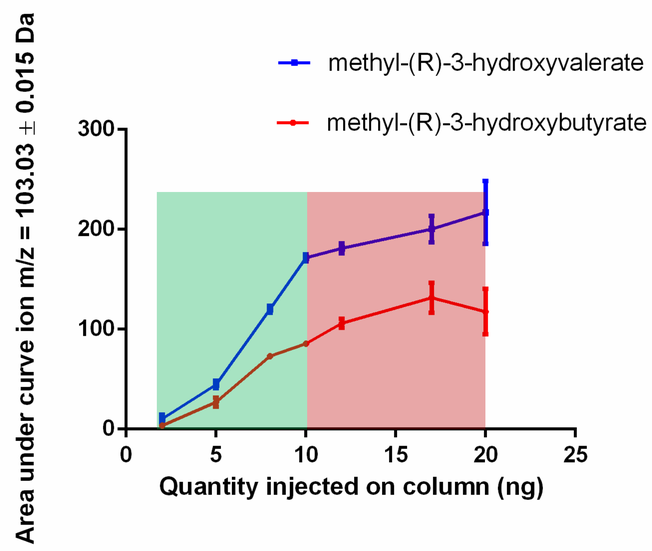

Fig. 5 : Graph describing the dynamic range of both methyl ester species. The green area depicts the range of quantity on column where the quantitation is reliable.

3. Cofactor quantification

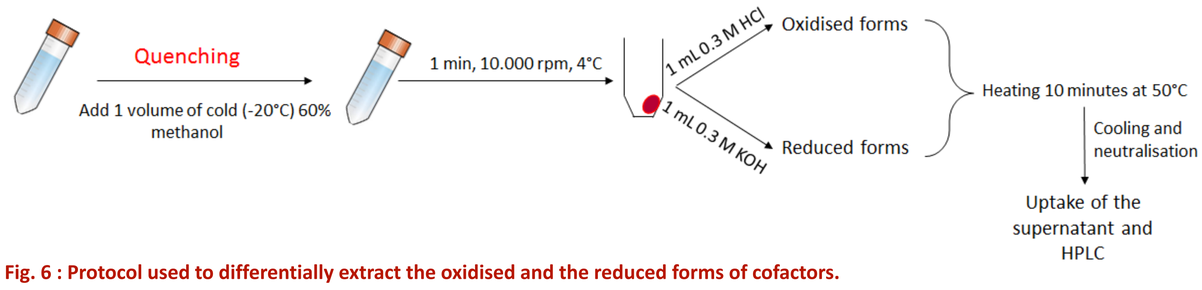

Fig. 6 : Protocol used to differentially extract the oxidised and the reduced forms of cofactors.

Cofactors have been differentially extracted by either 0.3M HCl (for oxidised forms) or 0.3M KOH (for reduced forms). This step was followed by a heating step before cooling and neutralising the solution. The supernatant was then taken (*fig.* 6).

The cofactor quantity was then monitored by HPLC using C18 column following the elution phase gradient exposed here (*table* 1).

Table 1 : Mobile phase gradient applied during the HPLC. A : 0.2M phosphate buffer, pH = 7.0 and 10 mM TBA ; B : 80% of A and 20% of methanol

Time (min)	Mobile phase (%A/%B)
0 to 2.5	100/0
5	80/20
10	60/40
13	0/100
17	0/100
20	100/0

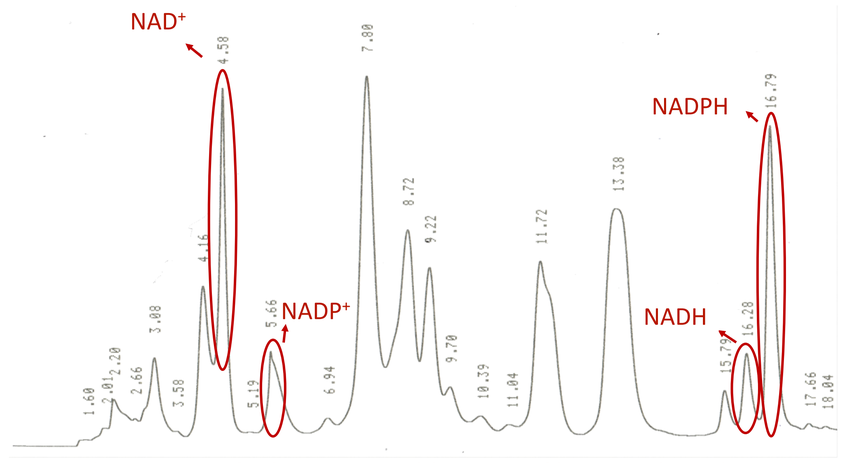

Fig. 7 : Chromatogram of cofactors extracted from 5 mL of biomass cultivated in presence of acetate

First experiments conducted at the lab seem to show that the protocol here above mentioned is suitable for cofactors extraction. Nevertheless, considering the number of peak, further studies are required to insure that the observed peaks are well related to cofactors (*fig.* 7).

4. Conclusion

The use of these methods will allow us to link the PHA production with the redox state of the carbon source and the link between this polymer production and the intracellular redox state. The study of these three elements and the connection between them is of first interest in order to fully understand the PHA production. This work is of particular importance in order to produce cheap microbial biodegradable plastics by using wastes issued from wastewater treatment.

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