Rhodospirillum rubrum S1H: Unravelling the Volatile Fatty Acids Assimilation in the MELISSA Loop

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Purple non-sulfur (PNS) bacteria are well known for their remarkable metabolic versatility which allows them to occupy a broad range of environments. They are especially able to achieve a phototrophic metabolism through which they can assimilate volatile fatty acids (VFAs). Rhodospirillum rubrum S1H was subsequently selected by the European Space Agency to colonize the second compartment (C2) of its bioengineered life support system (i.e. MELISSA loop) and to remove VFAs from the effluents produced by the liquefying first compartment (C1), avoiding accumulation of dead-end metabolites.

The ethylmalonyl-CoA pathway was recently highlighted in Rs. rubrum by our laboratory as being involved in the assimilation of acetate, the most abundant VFA produced by the C1. Below are reported our results for a better understanding of the phototrophic metabolism of butyrate, the second most abundant VFA in the C1 effluent.

Bacterial Growth Analysis: Dependence to Carbonate

Bacterial growth analysis of Rs. rubrum showed a strong dependence on the presence of carbonate in the culture medium. Furthermore, transmission electron microscopy also highlights an increased presence of carbonate in the culture medium. It is hypothesized that butyrate assimilation may play a role in the increase of carbonate accumulation.

Biosynthesis and Degradation of the Valine as a new anaplerotic pathway

As reported for the assimilation pathway (Zhang et al., 2015), we also observe enzymes of the ethylmalonyl-CoA (EMC) pathway, which allows Rs. rubrum to assimilate the carbon source. The carbon flow of the process is:

\[ \text{Butyrate} + \frac{4}{3} \text{CO}_2 \rightarrow \text{Acetyl-CoA} + \text{NADPH} + \text{H}^+ \rightarrow \text{Propionyl-CoA} + \text{NADP}^+ + \text{H}^+ \rightarrow \text{Methylmalonyl-CoA} + \text{NAD}^+ + \text{H}^+ \rightarrow \text{Acetoacetyl-CoA} + \text{NAD}^+ + \text{H}^+ \rightarrow \text{Acetyl-CoA} + \text{NADP}^+ + \text{H}_2 \]

The reduced 

In order to determine if these two pathways, which present different global balance, are used simultaneously or at different stages of the growth, a second proteomic analysis was performed using purified cell extracts. Different phases of growth in presence of butyrate were chosen to compare the abundance of proteins involved in butyrate assimilation and metabolism. This proteome analysis resulted in the identification and quantification of 1804 proteins.

Multiple copies of orf gene – Long term adaptation of strains used in MELISSA?

During our mutants experiment on the orf gene, we highlighted a probable duplication of the selected gene. Indeed, after the mutagenesis process, we were not able to simplify a specific region of the orf gene on several mutant strains that were resistant to neomycin and for which we checked by PCR the correct insertion of the homologous region. A possible explanation is that the homologous region is duplicated on the DNA plasmid of the transformed strain. We translated the orf gene on the P16 biomass of the highest copy number (i.e. 3.85 copies/observed by PCR).

To assess the orf copy number heterogeneity, the competent strains were grown in liquid cultures and plasmidic orf amplified bands were analyzed. The amount of orf was quantified by qPCR. Non-acclimated strains showed an orf expression of 48 copies/fg DNA. Acclimated strains showed an increase in orf copy numbers, with a maximum of 184 copies/fg DNA. Consequently, accurate quantification could be linked to orf copy number variation and monitoring cells in acetate condition seemed to present a sensitive fluorometric tool for the high copy number orf.

These results might question the genetic stability of MELISSA copies of the orf gene in our strain could then evolve from an adaptation to our culture conditions. Consequently, we have compared our data with available MELISSA strains, another number of copy occurred from 1 to 10 (Supplementary).

The phototrophic metabolism of butyrate in Rs. rubrum seems to involve multiple pathways. Our proteomic analyses suggest that one fraction of butyrate is converted to acetyl-CoA while the other one is likely assimilated through the EMC pathway and the VbSD pathway. Further investigations, especially metabolites and fluxes analyses, will provide a better understanding of these assimilation pathways and their relative importance, as well as interactions between acetate and butyrate assimilation routes.

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