**Probing the central carbon metabolism of *Rhodobacter sphaeroides* by transposon mutagenesis**

**Emily Smith**, Jordan Allen, Kathleen Sandman, Marie Asao, and Birgit E. Alber

Department of Microbiology, The Ohio State University, Columbus, OH

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**Introduction**

*Rhodobacter sphaeroides* is a metabolically diverse photosynthetic bacterium that is capable of utilizing a wide variety of carbon substrates. Recently, the ethylmalonyl-CoA pathway, which is required during the growth on acetate to replenish the intermediates of the tricarboxylic acid cycle, was discovered in *R. sphaeroides*. While this discovery was an important step in understanding the central carbon metabolism of this organism, much is still unknown. In this study we examined acetate metabolism and further explored the central carbon metabolism by identifying genes required for lactate metabolism in *R. sphaeroides*.

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**The central carbon metabolism of *Rhodobacter sphaeroides***

**Legend**

Blue figures are essential precursor metabolites to amino acids, fatty acids, and sugars. These precursor metabolites are required for cell biosynthesis. They are continually removed from the central carbon metabolism and must be replenished.

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**Random Transposon Mutagenesis**

The plasmid (pMW15) (Lamb et al.) containing transposon with a kanamycin-resistance gene cassette (aph) was mobilized from the donor E. coli to the recipient wild-type *R. sphaeroides* to obtain random transposon mutants of *R. sphaeroides*.

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**Methods**

In each mutant, the randomly inserted transposon retained the aph(3′) gene and the mutation was placed on the selective medium mutant succinate with kanamycin to select for transposed *R. sphaeroides* mutants. Kanamycin sensitivity of transconjugants and non-conjugating recipient cells was determined.

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**Screening**

Screening revealed several lactate and acetate mutants which indicated transposon insertion into genes necessary for lactate and acetate assimilation within the central carbon metabolism of *R. sphaeroides*.

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**Transposon insertion identification via plasmid DNA isolation and E. coli cell transformation**

The genes of the *R. sphaeroides* genome that were interrupted by transposon insert were cloned and identified by DNA sequencing.

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**Plasmid DNA sequencing**

Plasmid DNA was isolated through neutralization. The isolated plasmid DNA was then gel purified and submitted for DNA sequencing.

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**Liquid growth experiment**

Growth of acetate mutants on acetate and succinate substrates was assessed for different carbon substrates.

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**Acknowledgments**

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**Conclusion**

Mutant lactate metabolism of *R. sphaeroides*:

- Five mutants were isolated for negative growth on lactate. Of these, five mutants, all contained mutations in the pyruvate carboxylase gene. The experiments showed compromised growth on lactate and therefore confirm the need for pyruvate carboxylase in lactate assimilation.
- Slow growth on lactate, pyruvate, and D-malate despite the mutation in pyruvate carboxylase, may suggest that an additional enzyme is replenishing the tricarboxylic acid cycle. It is possible that this enzyme is phosphoenolpyruvate (PEP) carboxylase which converts pyruvate to oxaloacetate. However, further experiments are needed to confirm the role of PEP carboxylase.

Mutant acetate metabolism of *R. sphaeroides*:

- Experimentation confirms the involvement of methylmalonyl-CoA mutase (mcm) in the ethylmalonyl-CoA pathway as evidenced by impaired growth of acetate mutants (T10-18) on acetate and propionate-butyrinate.
- Slow growth on T10-48 on acetate in liquid culture experiments may suggest that methylmalonyl-CoA mutase plays a role in acetate assimilation into the TCA cycle intermediate succinyl-CoA. However, the ability to grow on acetate without mcm indicates that acetate can alternatively be assimilated into the TCA cycle along the ethylmalonyl-CoA pathway. Experimentation suggests that these mutants with functional [methylmalonyl-CoA]-CoA-ligase (mcm1) and propionate-CoA-carboxylase/hydrolase (wcaC) can alternatively assimilate acetate into the TCA cycle intermediate L-malate.
- Furthermore, no growth of the acetate mutant T10-48 on propionate-butyrate in liquid culture experiments suggest that propionate-CoA mutase is involved in the ethylmalonyl-CoA pathway after the alternative pathway described above. This indicates that *R. sphaeroides* has only one pathway of carbon assimilation from propionate and that it requires ren to produce the TCA intermediate succinyl-CoA.
- No growth of the UDP-glucose-6-dehydrogenase mutant (T02-101) on acetate in liquid growth experiments suggests that the transposon mutation may have an effect on the transcriptional level of a neighboring gene, which may be involved in acetate assimilation. Moreover, the gene may not be UDP-glucose-6-dehydrogenase or the problem of acetate assimilation may be due to an additional mutation in T02-101. All in all, the function of UDP-glucose-6-dehydrogenase in acetate assimilation is unknown and provides a means for future experimentation.

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**Literature**


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