OPINION

The *rseC* gene's essential function for autotrophy and the significance of a functional electron balance for nitrate reduction in *Clostridium ljungdahlii* are shown by genetic evidence

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ABSTRACT

Conversion from gaseous sources like carbon dioxide and hydrogen. In a previous study, autotrophy was lost when RNF-complex genes were disrupted, although glycolysis still allowed for heterotrophy. Additionally, it was demonstrated that nitrate supplementation might remove the energy restriction during autotrophy, leading to increased cellular growth and ATP output. Here, we deleted the putative RNF regulator gene rseC, the putative nitrate reductase gene cluster, and the RNF complex-encoding gene cluster rnfCDGEAB using CRISPR-Cas12a. The entire loss of autotrophy caused by the deletion of either rnfCDGEAB or rseC may be recovered by plasmid-based complementation of the lost genes. We noticed a transcriptional inhibition of the RNF-gene cluster in the rseC-deletion strain during autotrophy, and we looked into the distribution of the rseC gene acros-s Acetogenic bacteria. We contrasted the autotrophic and heterotrophic development of our three deletion strains with either ammonium or nitrate in order to investigate nitrate reduction and its relationship to the RNF complex. During autotrophy but not during heterotrophy, the *rnfCDGEAB* and *rse*C deletion strains failed to decrease nitrate as a metabolic activity in non-growing cultures. While the nitrate reductase deletion strain was unable to reduce nitrate, it was nevertheless able to grow in all of the test conditions. Our findings show how crucial the rseC gene is for autotrophy and also help us understand how nitrate reduction is related to energy metabolism.

Key Words: *Plasmid; Autotrophic; Acetogenic; Complex Encoding*

INTRODUCTION

Acetogenic bacteria (acetogens) use combinations of the gaseous substrates carbon dioxide, carbon monoxide, and hydrogen as carbon and energy sources to sustain autotrophic development, including Clostridium ljungdahlii. The Wood-Ljungdahl route permits carbon fixation for autotrophic development in acetogens. Overall, the Wood-Ljungdahl pathway is thought to be nature's most energy-effective method of carbon fixation. Two molecules of carbon dioxide are broken down into one carbonyl group and one methyl group via the Wood-Ljungdahl pathway, where they are joined with coenzyme A to form the essential metabolite acetyl-coenzyme A. While carbon monoxide can enter the pathway directly to supply the carbonyl group, the electrons for these reductions can alternatively come through the oxidation of hydrogen or carbon monoxide. Acetyl-coenzyme A is directed into anabolism for cellular proliferation for carbon fixation. Acetyl-coenzyme A is changed into acetate for energy conservation, and acetate is then phosphorylated at the substrate level to produce cellular energy. For every mole of acetate produced, one mole of ATP is produced. However, in the first step of the process, one mole of ATP is used to activate the formate to formyl-tetrahydrofolate after carbon dioxide has been reduced to formate. Therefore, the Wood-Ljungdahl pathway alone has a net energy balance of zero. Through membrane-coupled phosphorylation, the

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bacteria's autotrophy generates all the cellular energy they need for anabolism. Theoretically, *C. ljungdahlii* may produce a maximum of 0.63 moles of ATP per mole of acetate for anabolism via membrane-coupled phosphorylation in the presence of carbon dioxide and hydrogen. Thus, at the thermodynamic limit of life, cellular energy conservation occurs during autotrophy.

The RNF-gene cluster rnfCDGEAB in *C. ljungdahlii* encodes the RNF complex. Although *C. ljungdahlii* RNF complex is crucial for energy conservation during autotrophy, little is known about the regulation and gene expression control of the RNF-gene cluster that codes for it. According to transcriptome studies with *C. ljungdahlii*, the RNF complex is significantly increased during autotrophy and is subject to rigorous gene expression control. Unknown regulatory processes underlie this. But in *C. ljungdahlii*, the tiny gene *rse*C, which the conserved protein domain family *Rse*C *Muc*C is found in the gene *rse*C. Positive transcriptional regulators are found in different microorganisms and belong to the domain family *Rse*C *Muc*C. The one representative, *Rse*C, was discovered to be involved in Salmonella Typhimurium's thiamine production as well as *Escherichia coli's* oxidative stress response. The other representative, MucC, was discovered to be important in the control of Azotobacter vinelandii and Pseudomonas aeruginosa production of alginate. It is situated right upstream of *rnf*C, is likewise substantially expressed during autotrophy and exhibits the same expression pattern as *rnf*C. Others discovered a potential terminator region between the *rse*C and *rnf*C genes and a transcription start site for *C. ljungdahlii* upstream of the *rse*C gene. This demonstrates that rseC is expressed independently of the RNF-gene cluster transcripts. The conclusion drawn from all of this is that the rseC gene product is intimately related to the RNF complex and may play a role in the control of autotrophy in *C. ljungdahlii*.

It has been noted that *C. ljungdahlii* is able to relate the reduction of nitrate to the synthesis of ATP during growth with carbon dioxide and hydrogen, whereas autotrophy in acetogens results in low cellular energy outputs. As a result, there was no longer an energy restriction during autotrophy, and the biomass production was much larger. This was corroborated in a bioreactor study, where the biomass outputs were significantly higher with nitrate, but this led to stochastic crashes of the continuous bioreactor cultures, which suggested that NADH serves as a source of the electrons needed for nitrate reduction.