PUBLIC ABSTRACT

NOVEL INSIGHTS INTO SUGAR AND SUCCINATE METABOLISM OF ACTINOBACILLUS SUCCINOGENES FROM STRAINS EVOLVED FOR IMPROVED GROWTH ON LIGNOCELLULOSE HYDROLYSATE SUGARS

By

Nikolas Robin McPherson

A wide variety of important chemicals are currently produced from petroleum, using very well-refined processes and a large industrial infrastructure. However, petroleum processing has a number of hazardous and otherwise negative impacts on the environment, as well as on human health. Additionally, the supply and price of oil into the future are uncertain. Thus, renewable substrates can help extend the current supply of oil and eventually replace it. Succinate is a specialty chemical currently produced from maleic anhydride from petroleum processing that can also be produced from renewable substrates by microorganisms. Sustainable production of succinate by microbial catalysis is an attractive alternative to petrochemical succinate because it could use the existing industrial infrastructure and be scaled up to meet the demands of this $15 billion commodity chemical market. Lignocellulose, the polymeric material that makes the stiff cell walls of plants, is a major potential feedstock source for conversion to succinate. Primarily made of sugars, lignocellulose from agricultural waste or from bioenergy crops could become a viable option for succinate production using organisms able to grow on its sugar components. Of particular interest is the bacterium Actinobacillus succinogenes, which is among the best natural succinate producers in terms of production rate and yield. It also grows on a wide variety of carbohydrates, including the major sugars that make up lignocellulose.

A. succinogenes grows well on glucose, the most common sugar in lignocellulose, but does not grow as quickly with the other lignocellulosic sugars. I have evolved strains of A. succinogenes for faster growth on xylose, the second most common sugar in lignocellulose and second most abundant sugar on the planet, as well as arabinose and galactose. Further, I evolved strains for optimal growth and succinate production from lignocellulose hydrolysate, the sugar and byproduct solution that results from treating lignocellulose to release its sugars. Many of the

evolved strains of A. succinogenes produce more succinate than the parental strain, even though the evolution process only selected for growth. Genomic analyses in the new strains identified mutations that shed light on the physiological traits that could have resulted in faster growth. RNA sequencing of the xylose-evolved strains identified changes in transcription that provided additional insights into xylose fermentation and growth in these strains. For example, many genes encoding the enzymes responsible for converting sugars to succinate in the xylose-evolved strains were upregulated. By contrast, genes that encode enzymes that redistribute carbon molecules toward byproducts were downregulated. I also evolved a strain that can grow on galactose, a sugar that the parental strain cannot use as sole carbon and energy source. The final evolved strain also grew faster on xylose, arabinose, and lignocellulose hydrolysates. I also engineered the parental A. succinogenes strain with a mutation from a xylose-evolved strain. This strain produced 40% more succinate than the parental A. succinogenes strain, although its growth rate was less than half the growth rate of the parental strain.

In summary, I have generated several strains of A. succinogenes for improved growth and succinate production from lignocellulosic substrates. I have also identified and characterized mutations responsible for some of the phenotypes and have provided novel insights into the factors that control fast growth and succinate production. This knowledge sets the foundation for further genetic improvements in A. succinogenes and other bacteria needed to efficiently convert renewable substrates into succinate.

ABSTRACT

NOVEL INSIGHTS INTO SUGAR AND SUCCINATE METABOLISM OF ACTINOBACILLUS SUCCINOGENES FROM STRAINS EVOLVED FOR IMPROVED GROWTH ON LIGNOCELLULOSE HYDROLYSATE SUGARS

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A wide variety of industrially vital chemicals are currently produced from petroleum, using very well-refined processes and a large industrial infrastructure. However, petroleum processing has a number of hazardous and otherwise negative impacts on the environment, as well as on human health. The supply and price of oil into the future are uncertain as well, and supplementing oil with feedstocks from renewable sources can help extend the current supply of oil and eventually replace it. Succinate is a specialty chemical currently produced from maleic anhydride from petroleum processing. If bio-based succinate could compete with the cost of maleic anhydride, it could replace maleic anhydride in a $15 billion commodity chemical market, taking advantage of the existing chemical production infrastructure. A major potential feedstock source for conversion to succinate is lignocellulose from agricultural waste or from bioenergy crops. The bacterium Actinobacillus succinogenes is one of the best natural succinate producers and it grows on a wide variety of carbohydrates, including the major sugars in lignocellulose. A. succinogenes grows well on glucose, the most common sugar in lignocellulose,

but does not grow as quickly on other lignocellulosic sugars.

I have evolved strains of A. succinogenes to grow faster on xylose, the second most common lignocellulosic sugar, as well as on arabinose, galactose, and lignocellulose hydrolysates. Many of the evolved strains produce more succinate than the parental strain as well, even though the evolution process did not specifically select for succinate production. The evolved strains were resequenced to identify the mutations accumulated during evolution. RNA sequencing of the xylose-evolved strains helped identify changes in transcript levels and was used to refine our conclusions about the xylose-evolved (X) strains. I discovered that the genes that encode many glycolytic enzymes were upregulated in at least one X strain, several genes

encoding succinate production enzymes were upregulated, while genes that encode enzymes that redirect fluxes from the succinate pathway to other fermentation products were downregulated. During the directed evolution process, I obtained a strain of A. succinogenes that can grow on galactose, a sugar that the base strain cannot use. The final evolved strain grew faster than the wild-type strain on xylose, arabinose, and lignocellulose hydrolysate, and could grow on galactose. I determined that A. succinogenes will co-consume glucose and xylose, but that xylose represses arabinose consumption. After directed evolution, though, arabinose represses xylose consumption. Finally, I used multiplex transformation to introduce mutations from the evolved strains into the wild-type strain. The first strain produced, using the xylose symporter mutation from a xylose-evolved strain, produced 40% more succinate than wild-type A. succinogenes, even though it grew at less than half the speed.

In summary, I have evolved a set of A. succinogenes strains that grow faster on lignocellulose sugars and some have a higher succinate yield, I know the location and nature of their mutations and have RNA sequencing data for the xylose-evolved strains. I have conducted numerous additional experiments to characterize sugar consumption in A. succinogenes and what causes the evolved strains to be able to grow faster and produce more succinate. My results lay solid groundwork for future work with A. succinogenes and other bacteria being grown on sugars

and synthesizing succinate.