Conclusions: Walnut consumption led to shifts in gut microbial composition and induced the expression of beneficial genes and pathways that have further health implications and provides possible explanations for reduction of CVD risk factors.

This study was funded by The California Walnut Commission. This research was also supported by the Penn State Clinical and Translational Research Institute, Pennsylvania State University Clinical and Translational Science Award and NIH/National Center for Advancing Translational Sciences (grant no. UL1TR000127).

103110, https://doi.org/10.1016/j.jbc.2023.103110

Abstract 1733

Metformin Degrading Bacteria: Genomes, Metabolic Products, and Transcriptional Regulation

Anna Schultz, *Hamline University*

Anthony Dodge, Wackett Lawrence, Betsy Martinez-Vaz

Metformin is one of the most prescribed pharmaceuticals worldwide, mainly for treating type 2 diabetes. This pharmaceutical is a major anthropogenic pollutant that accumulates in waterways at extremely high rates, negatively affecting aquatic life. A novel metformin-biodegrading bacteria known as *Pseudomonas mendocina* strain MET was recently isolated from a wastewater treatment plant. The genome of this bacterium encodes two novel enzymes, GbuAB and GuuH, essential for metformin degradation. The goal of this study was to conduct a complete genomic and physiological characterization of *Pseudomonas mendocina* strain MET and investigate the transcriptional regulation of the enzymes involved in metformin degradation. We hypothesize that strain MET can grow in and metabolize compounds structurally similar to metformin, and that this drug induces the expression of genes encoding metformin degrading enzymes. Metformin and structurally related compounds were used in growth studies to evaluate the metabolic capabilities of *P. mendocina* strain MET. Whole genome sequencing and bioinformatics analyses were performed to examine the DNA regions encoding the metformin-degrading enzymes and to carry out comparative genomics among closely related bacteria. Total RNA extractions and quantitative RT-PCR studies were done to investigate the transcriptional regulation of the GbuAB and GuuH enzymes. The results demonstrated that *Pseudomonas mendocina* strain MET had the ability to completely metabolize metformin and other biguanide compounds, including 1-N-methylbiguanide, biguanide, guanylurea, and guanidine, into equivalents of carbon dioxide and ammonia. Quantitative RT-PCR analysis revealed that the genes encoding the metformin-degrading enzymes were expressed at approximately the same level when cells were grown in this compound and in diverse nitrogen sources, including NH4Cl, biguanide, and 1-N-methylbiguanide. This observation suggests a constitutive rather than tightly regulated gene expression. This research provides genomic and metabolic insights into metformin biodegradation by *Pseudomonas mendocina* strain MET, which can aid in the development of biotechnological applications to reduce the levels of metformin in the environment.

NSF Chemistry of Life Processes Grant Number 2203751.

103111, https://doi.org/10.1016/j.jbc.2023.103111