Metabolic Connections between Carbon Utilization and Iron Scavenging by Soil *Pseudomonas* species Revealed by Cellular ^{13}C-Metabolomics

LUDMILLA ARISTILDE,[1*] CAROLL M. MENDONCA,[1] ANNE P. WERNER,[1] SHO YOSHITAKE[1]

[1]Department of Biological and Environmental Engineering, College of Agriculture and Life Sciences, Cornell University (*correspondence: ludmilla@cornell.edu)

Aerobic bacteria including the nutritionally versatile and ubiquitous *Pseudomonas* species are subjected to iron (Fe) limitation in their environmental niches due to the low solubility of Fe hydroxides and oxides. Ample Fe supply is required to meet the demand for Fe-containing catalytic cofactors in metabolic enzymes. To overcome Fe limitation, aerobic bacteria secrete high-affinity Fe-scavenging molecules or siderophores, which are expensive to the carbon metabolism. We have combined stable-isotope tracers with cellular metabolomics to investigate the strategic balance between carbon utilization, metabolism, and siderophore biosynthesis employed by soil *Pseudomonas* cells to survive Fe-deficient growth media. Here we present our experimental findings with two soil *Pseudomonas* species (*P. putida* and *P. protegens*). We uncovered an Fe-dependent hierarchy in carbon utilization of carbohydrates (i.e. glycolytic substrate) versus short-chain carboxylic acids and aromatics (i.e. gluconeogenic substrates). By contrast to Fe-replete cells, which exhibited no preference between the two substrate types, Fe-limited cells preferentially assimilated gluconeogenic substrates over glycolytic substrates. Stable isotope-assisted metabolomics with ^{13}C tracer experiments revealed that, in response to Fe limitation, the cells reprogrammed metabolic pathways to achieve (1) enhanced carbon fluxes towards metabolite precursors for siderophore biosynthesis, (2) increased secretion rates of siderophores, and (3) promoted mobilization of available Fe from the dissolution of Fe-bearing minerals. Our findings thus demonstrate that cellular reprogramming in soil *Pseudomonas* species result a favorable coupling between Fe scavenging from iron minerals and preferential carbon utilization (Figure 1).

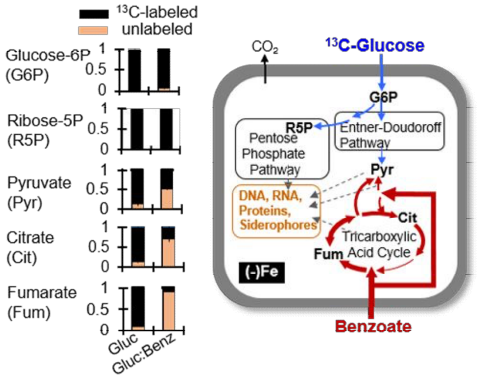

Figure 1. ^{13}C-labeling of metabolites capture carbon assimilation of glucose (Gluc) and unlabeled benzoate (Benz) into distinct metabolic pathways.