

High-Throughput Isolation of Bacteria to Dissect Corrinoid Based Interactions in Soil

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Project Goals: The goal of this project is to gain a comprehensive understanding of microbial metabolic interactions in soil through the study of corrinoids as model metabolites. Corrinoids are a family of structurally diverse cofactors that includes vitamin B₁₂. They are produced by a subset of the bacteria that require them, and thus are shared metabolites. Importantly, bacteria typically can use more than one corrinoid but display distinct preferences for specific structures. For these reasons, we hypothesize that corrinoids are key metabolites that contribute to shaping microbial communities in soil. By using this shared, structurally diverse family of metabolites, we are studying microbial interactions across scales, from the whole community to individual isolates.

Microbial communities in soil comprise much of Earth's microbial diversity and are major drivers of biogeochemical cycles. Interactions between microbes are essential determinants of community structure, function, and dynamics. Disentangling these interactions is crucial for understanding the soil microbiome's global role. However, the physicochemical and taxonomic complexity of the soil microbiome poses a challenge. By focusing on corrinoids as model metabolites, we aim to mechanistically study complex metabolic interactions between soil microbes. Corrinoids are a family of at least 16 structurally diverse cofactors that includes vitamin B₁₂. They are shared nutrients, required by an estimated 86% of all sequenced bacteria but produced by only 36% of bacteria. Importantly, bacteria typically can use more than one corrinoid, but display distinct preferences for specific corrinoid structures. For these reasons, we hypothesize that corrinoids are key nutrients in soil and that corrinoid composition can impact microbial community structure.

By isolating soil microbes on media supplemented with different corrinoids, we will address two hypotheses. First, because bacteria are known to have distinct preferences for specific corrinoids, we hypothesize that corrinoids influence culturability, and we expect to isolate distinct taxa on each corrinoid condition. Second, because corrinoids other than B₁₂ have never been used in growth media, species that prefer them have likely remained uncultured, leading to a pervasive bias across microbiology. To test these hypotheses, we did a high-throughput isolation of soil microbes on media supplemented with one of six corrinoids. We found that adding a corrinoid to the medium results in an increase in isolated soil bacteria. This finding supports our hypothesis that a significant number of soil bacteria are corrinoid-dependent. 158 isolates are currently

being identified by 16S rRNA amplicon sequencing and will be experimentally characterized as corrinoid producers, auxotrophs, or independent. These isolates and knowledge of their corrinoid metabolism will be crucial for future steps in this research aimed at predicting and testing corrinoid-sharing interactions.

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