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Identification of Solar-Powered N₂-Fixing Cyanobacteria from Native Grasslands for Soil Health and Bio-Nitrogen Fertilizer Application in South Dakota Crop Fields

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Summary:

Nitrogen fertilizer is one of the most limiting and expensive inputs in agriculture production. Unfortunately, current fossil fuel-dependent ammonia production is both energy-intensive and environmentally-damaging. An economically-practical and environmentally-friendly solution for the production of ammonia is urgently needed. **Aerobic** nitrogen-fixation by some cyanobacteria is a uniquely solar-powered process, which offers distinct advantages over the **anaerobic** nitrogen-fixation by other N₂-fixing bacteria, such as *rhizobia*. Thus, solar-powered cyanobacterial N₂-fixation provides a greater promise for applications in agriculture in comparison to all other N₂-fixing bacteria that cannot use solar energy, and also require anaerobic condition that is detrimental to its practical application.

Solar-powered N₂-fixing cyanobacteria have been playing critical roles in flourishing a healthy soil that harmonize the soil biological, chemical, and physical properties to sustain huge annual biomass production (without applying any nitrogen fertilizer) in native grasslands and native forests. Cyanobacteria can contribute at least **30 Kg N (\approx 65 Kg urea)·ha⁻¹·season⁻¹** as well as abundant organic matter to the soil; which is significant, not only benefiting for the farmers but also for soil health. This project is to survey and isolate solar-powered N₂-fixing cyanobacteria in South Dakota native grasslands and native forests, so that we can put them back to crop fields where the solar-powered **N₂-fixing cyanobacteria may be extinct** due to heavily applied chemically nitrogen fertilizer. Knowledge gained from this project will help develop N₂-fixing cyanobacteria as a producer of nitrogen-fertilizer in soil, and also providing recommendation for selecting non-till cropping systems. The long-term goal of this project is to enable these isolated N₂-fixing cyanobacteria in crop fields as *in situ* “**solar-powered living N-fertilizer factories**” to reduce current cost for N-fertilizer for framers and also improving soil health for crop fields.

Relevancy to SD agriculture production: 1) reducing the No. 1 cost of chemical nitrogen fertilizer while improving soil health and soil fertility; 2) There have been many research on **Non-Solar Powered N₂-fixing bacteria** world-wide, but to our best knowledge, there is no such research on **Solar-Powered N₂-fixing Cyanobacteria** in world-wide. We are truly a pioneer in developing “**solar-powered living N-fertilizer factories**” in crop fields.

Objectives:

1. Isolate solar-powered, constitutive nitrogen fixing cyanobacteria from native grasslands and native forests across SD (Year 1 to Year 2) and validate the N₂-fixing capability.
2. Down-select the most productive of these isolates in terms of growth and N fixation (perhaps 2-4 isolates) by determining and quantifying the N₂-fixing capability (Year 2 to Year 3)
3. Laboratory test and then greenhouse trials first in sterile soil and then in actual soil (Year 2 to Year 3). Evaluate different application rates and assess cyano growth, nitrogen fixation, and plant responses. For these trials cyano can be grown in the lab. The trials could be repeated in the same soil over several years to track what happens over time when the cyano are just inoculated in the first cycle. In other words, how long will the cyano last? From this information we should be able to determine how various application rates will benefit N availability and crop production. This cost/benefit analysis will serve as the basis for making a go/no go decision regarding commercial potential.

Preliminary Results:

- 1) **We collected more than 244 topsoil samples** (1-2 cm depth) from different native grasslands, No-till cropping system and Badlands in SD.
- 2) From the **244** topsoil samples, **we have isolated at least 13 new potential N₂-fixing cyanobacterial strains** that are able to reproducibly grow well on BG11₀ agar plates (without any combined nitrogen), suggesting that these **13** strains can fix nitrogen using solar energy. Acetylene reduction assay are being used for further verification of their nitrogen fixing ability, So far, four strains have been verified to have nitrogen fixing ability. We are working on isolating genomic DNA from these four strains with verified nitrogen fixing ability for genomic sequencing to identify novel nitrogen fixation genes.
- 3) Microscopy verified that six of them are filamentous cyanobacteria, and seven of them are unicellular cyanobacteria/microalgae. The other 9 potential N₂-fixing cyanobacteria or microalgae strains are currently under test for nitrogenase activity to confirm its N₂-fixing ability.

Potential Impacts:

1. SD corn fields (total 2,326,945 hectares or Ha) apply an average of 143 Kg N (\approx 306.43Kg of urea) Ha⁻¹ annually. Total cost of 0.705M tons of urea (\$345.36/ton) is \$243.5M/year for SD corn fields only. **Cyanobacteria** in native soil of SD can produce 30Kg of fixed N Ha⁻¹ annually, which accounts for \approx **21%** of applied N-fertilizer and can save \approx **51 million US dollars** only for N-fertilizer in corn production of SD.

2. Using N₂-fixing cyanobacteria in soil to produce bio-N-fertilizer on site will greatly improve both the corn economics and soil health. Not only can cyanobacteria contribute combined nitrogen and abundant organic matter, but also cyanobacteria providing other benefits, such as forming *biological soil crusts to improve soil moisture and stability by building rich, porous sponge like soil and prevent soil erosion, which greatly increase nutrient capacity, resistance and resilience of farmlands to extreme weather events, consequently reduce fertilizer costs and eventually increase farmers' profitability.*
3. The findings from this project may promote no-till farming for cropping systems in South Dakota and lead a practical application of N₂-fixing cyanobacteria as a bio-solar producer of nitrogen-fertilizer

Changes in projects/ Personnel: N/A

Products: The first research paper titled “Isolating solar-powered N₂-fixing microbes from topsoil of native grasslands in South Dakota” was published.

Wang N, S Tian¹, L Gu, L Xu, Y Qiu, T Van Den Top, J. L Gonzalez-Hernandez, M Hildreth, S Li, **R Zhou** (2018) ISOLATION OF POTENTIAL PHOTOSYNTHETIC N₂-FIXING MICROBES FROM TOPSOIL OF NATIVE GRASSLANDS IN SOUTH DAKOTA. *Proceedings of the South Dakota Academy of Science*. 97:117-128.

Budget and Expenditures:

Initial budget: \$69,816
Expenditures so far: ≈\$55,000