Final report (01/01/2018-12/31/2018)

Title: Identifying Solar-Powered N_2-Fixing Cyanobacteria in Topsoil for Bio-nitrogen Fertilizer Application in South Dakota

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Co-PIs: Lan Xu, Dept. of Natural Resource Management, College of Agriculture & Biological Sciences; **Liping Gu**, Dept. of Biology and Microbiology, College of Agriculture & Biological Sciences

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 N_2-fixing bacteria, such as *rhizobia*. Thus, solar-powered cyanobacterial N_2-fixation provides a greater promise for applications in agriculture in comparison to all other N_2-fixing bacteria that cannot use solar energy, and also require anaerobic condition that is detrimental to its practical application.

Solar-powered N_2-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 nitrogen fertilizer) in native grasslands and native forests. Cyanobacteria can contribute at least **30 Kg N (≈ 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 N_2-fixing cyanobacteria in South Dakota native grasslands and native forests, so that we can put them back to crop fields where the solar-powered **N_2-fixing cyanobacteria may be extinct** due to heavily applied chemically nitrogen fertilizer. Knowledge gained from this project will help develop N_2-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 N_2-fixing cyanobacteria in crop fields as *in situ* "**solar-powered living N-fertilizer factories**" to reduce current cost for N-fertilizer and also improving soil health for crop fields.

There have been many research on **Non**-Solar Powered N_2-fixing bacteria world-wide, but to our best knowledge, there is no such research on **Solar-Powered N_2-fixing Cyanobacteria** in world-wide. We are truly a pioneer in developing "**solar-powered living N-fertilizer factories"** in crop fields.

Objectives:

1) Collecting all cyanobacterial species from native grasslands or native forests.
2) Screen for solar-powered N_2-fixing cyanobacteria isolated from native grasslands or native forests.
3) Evaluating the diversity and abundance of photosynthetic N_2-fixing cyanobacteria
4) Determining the N_2-fixing capability of the interested strains for future application

Results:

1) **We collected 144 topsoil samples** (1-2 cm depth) from different native grasslands in SD. We placed 3-5 grams of each of homogenized topsoil into a 35-ml small mouth bottle filled with 20-ml autoclaved mineralized water (AA/8N medium) with combined nitrogen. These bottles were incubated under continuous light (ca. 50 $\mu E/m^{-2} \cdot s^{-1}$) at 28-30 °C. When "green" cultures appeared in 10 days, we transferred them into fresh AA/8N medium to isolate the cyanobacteria from the soil. The green cultures were then streaked onto $BG11_0$ (free of combined nitrogen) agar plates.

2) From the 144 topsoil samples, **we have isolated six potential N_2-fixing microbial strains** that are able to reproducibly grow well on $BG11_0$ agar plates (without any combined nitrogen), suggesting that these six strains can fix nitrogen aerobically.

3) Microscopy verified that two of them are filamentous cyanobacteria, and four of them are unicellular green microbes. The six potential N_2-fixing microbial strains are currently under test for nitrogenase activity to confirm its N_2-fixing ability.

Potential Impacts:

1. SD corn fields (total 2,326,945 hectares or Ha) apply an average of 143 Kg N (≈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 **≈21%** of applied N-fertilizer and can save **≈ 51 million US dollars** only for N-fertilizer in corn production of SD.

2. Using N_2-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 N_2-fixing cyanobacteria as a bio-solar producer of nitrogen-fertilizer

Changes in projects/ Personnel: N/A

Products: The paper "ISOLATION OF POTENTIAL PHOTOSYNTHETIC N_2-FIXING MICROBES FROM TOPSOIL OF NATIVE GRASSLANDS IN SOUTH DAKOTA" has been published in Proceedings of the South Dakota Academy of Science, Vol. 97 (2018), 117-128

Budget and Expenditures:

Funded budget: $54000
Available fund: $0 by 12/31/2018

Final report (01/01/2019-12/31/2020)

Identification of Solar-Powered N_2-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 N_2-fixing bacteria, such as *rhizobia*. Thus, solar-powered cyanobacterial N_2-fixation provides a greater promise for applications in agriculture in comparison to all other N_2-fixing bacteria that cannot use solar energy, and also require anaerobic condition that is detrimental to its practical application.

Solar-powered N_2-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 (≈ 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 N_2-fixing cyanobacteria in South Dakota native grasslands and native forests, so that we can put them back to crop fields where the solar-powered **N_2-fixing cyanobacteria may be extinct** due to heavily applied chemically nitrogen fertilizer. Knowledge gained from this project will help develop N_2-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 N_2-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 N_2-fixing bacteria world-wide, but to our best knowledge, there is no such research on **Solar-Powered N_2-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 N_2-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 N_2-fixing capability (Year 2 to Year 3)
3. Laboratory test on Agar-plates and qatar sands, and then move to greenhouse trials first in sterile soil and then in actual soil (Year 3 to Year 4). 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.

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. We placed 3-5 grams of each of homogenized topsoil into a 35-ml small mouth bottle filled with 20-ml autoclaved mineralized water (AA/8N medium) with combined nitrogen. These bottles were incubated under continuous light (ca. 50 $\mu E/m^{-2} \cdot s^{-1}$) at 28-30 °C. When “green” cultures appeared in 10 days, we transferred them into fresh AA/8N medium to isolate the cyanobacteria from the soil. The green cultures were then streaked onto $BG11_0$ (free of combined nitrogen) agar plates to screen for potential light-powered, N_2-fixing cyanobacteria

2) From the **244** topsoil samples, **we have isolated at least 15 new potential N_2-fixing cyanobacterial strains** that are able to reproducibly grow well on $BG11_0$ agar plates (without any combined nitrogen), suggesting that these **15** strains can fix nitrogen using solar energy. Acetylene reduction assay confirmed at least 5 strains have great nitrogenase activity.
3) We investigated the impacts of the isolates (those are confirmed to have N_2-fixing ability) on plant growth using **sterilized Qatar-sands without any N-fertilizer** in growth chamber trials. The preliminary results (**Fig. 1**) are very promising! Inoculation of the isolated N_2-fixing cyanobacteria with corn seedlings greatly improving the corn growth (**B:** in below **Fig. 1**) compared to negative control (**A:** without cyanobacteria, the other nutrients are identical to **B**). The data for plant height, dry weight, fresh weight is currently under collecting.

Figure 1. The isolated, solar-powered N_2-fixing cyanobacteria can provide fixed nitrogen to greatly support the corn growth
A: Sterilized Qatar-sands only
B: Sterilized Qatar-sands and inoculated with one isolated N_2-fixing cyanobacteria

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Changes in projects/ Personnel: N/A

Products: Two papers below were published.

1) 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.

2) Young J, L Gu, W Gibbons, **R Zhou** (2021) Harnessing Solar-Powered Oxic N2-fixing Cyanobacteria for the **BioNitrogen Economy**. In ***Cyanobacteria Biotechnology***, First Edition. ed: Paul Hudson, Publisher: WILEY-VCH GmbH. (in press).