Selecting high performance N_2-fixing cyanobacteria for agricultural soil amendment and biofilm formation

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Background

Biological soil crusts (BSCs)[1] play crucial roles in stabilizing soils of arid and semiarid regions, but their presence in temperate agricultural soils has received little attention.

Cyanobacteria are important components of BSCs because they fix both CO_2 and N_2. Although cyanobacteria are used in paddy rice production in Asia, the use of cyanobacteria to form BSCs in row crop agriculture is a new concept.

Annual growth of naturally occurring BSCs has been observed in diverse field plots at Penn State's research farm for the past 15 years. Direct application of N_2 - fixing cyanobacteria to agricultural soils could facilitate development of self-renewable BSCs.

Figure 1. BSCs in no-till maize plot at PSU Agronomy Farm. They are most abundant every fall and can survive through winter. Arrows are indicating BSCs.

Objective and Method

Objective: Select high-performance cyanobacteria that will grow well on soil surfaces, increase biological N_2 fixation, bind and stabilize soils, resist runoff, and reduce nutrient losses.

Table 1. Experimental methods were developed based on these three criteria.

Criteria	Method
N_2 fixation	Screen both commercial and local isolated cyanobacteria (filamentous, heterocystous) with N-free medium
High stability after application	Evaluate biomass stability by a 100ml H_2O flooding treatment to freshly applied cyanobacteria on sand surface
Robust growth on soil surface	Monitor cyanobacterial growth in N -imited soil microcosms using chlorophyll a measurments

Figure 2. Different cyanobacterial cultivation systems. From left to right are agar plates, flasks, soil microcosms, and photo-bioreactors.

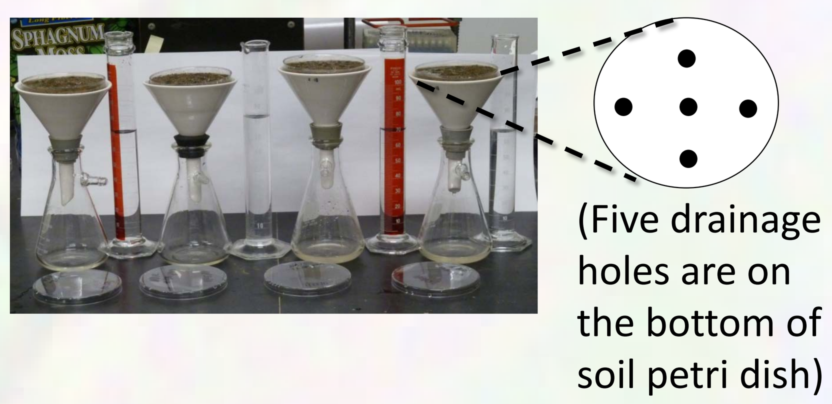

Figure 3. Experimental set up for the evaluation of biomass stability. Freshly applied cyanobacteria on sand surfaces were subjected to flow-through passage of 100ml H_2O. Biomass was recovered for quantification after 24 hrs.

Results

Criterion 1: N_2 fixation

4 commercial strains and 1 local cyanobacterial enrichment (designated 'DG') showed robust growth in N-free liquid medium.

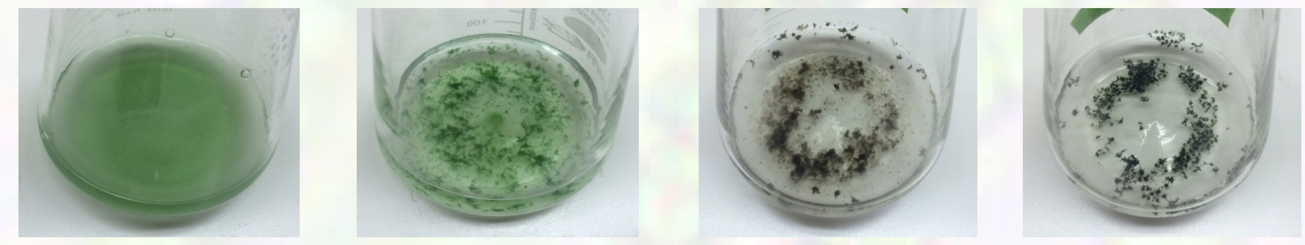

Figure 4. Five selected N_2-fixing cyanobacterial candidates cultured with N-free liquid medium in 125ml flasks, showing distinct morphological types.

Criterion 2: High stability after application

Nostoc spp. and DG enrichments were resistant to flooding treatments while *Anabaena* spp. were not. *Nostoc muscorum* and DG emrichment also showed rapid growth response following the application.

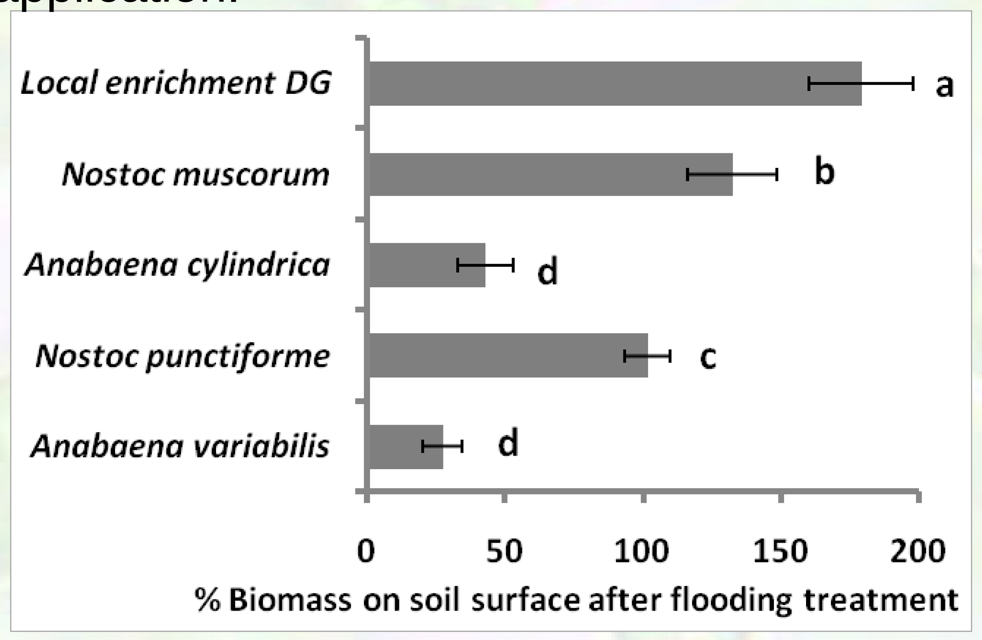

Figure 5. Biomass stabilities of freshly applied cyanobacteria on sand surfaces after the 24 - hour flooding treatment. Error bars are standard deviations. Different letters show the result of Tukey's multiple comparison tests. ($p<0.05$).

Criterion 3: Robust growth on soil surface

All cyanobacteria reached steady states after one month cultivation in soil microcosms. *Anabaena variabilis* and DG enrichment had significantly higher biomass densities on soil surface at steady state.

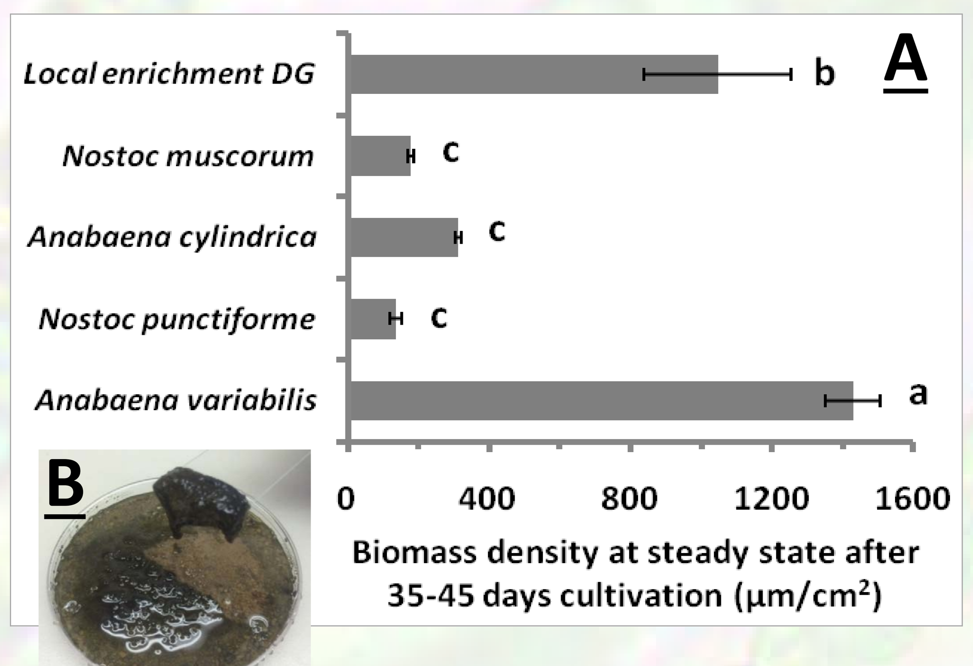

Figure 6. (A) Biomass density of cyanobacteria at steady state in soil microcosms. Error bars are standard deviations. Different letters show the result of Tukey's multiple comparisons ($p<0.05$). (B) Stable crust of local enrichment DG on soil surface after 40 day cultivation.

Summary

Commercial cyanobacterial strains with distinct morphological types had different growth potentials and stabilities on soil surface. The local enrichment DG performed better than pure, commercially available cultures and had high potential for agricultural application.

Table 2. Summary of distinct cyanobacteria based on three criteria

Cyanobacteria	N_2 fixation	High stability after application	Robust growth on soil surface
Anabaena variabilis	X		X
Anabaena cylindrica	X		X
Nostoc muscorum	X	X	
Nostoc punctiforme	X	X	
Local enrichment DG	X	X	X

Future Work

Metagenomic characterization and toxicity analysis of the DG enrichment are in process.

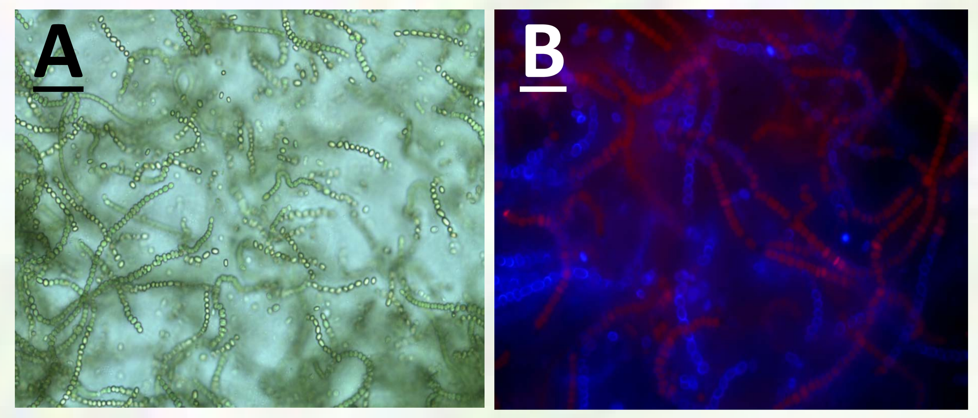

Figure 7. Microscopic view of local enrichment DG (400x magnification). (A) Under white light. (B) Fluorescent view at different wavelengths. Blue and red colors indicate at least two cyanobacterial strains.

Table 3. Blast analysis on preliminary 16SrRNA gene sequencing of the local enrichment DG showed it has equal similarity to different Nostocaceae strains.

Description	Max score	Total score	Query cover	E value	Ident
Uncultured bacterium clone JFR0702_jaa51e01 16S ribosomal RNA gene, partial sequence	2603	2603	99%	0.0	99%
Anabaena flos-aquae strain UTEX LB2557 16S ribosomal RNA gene, partial sequence	2603	2603	99%	0.0	99%
Anabaena flos-aquae strain UTEX LB2338 16S ribosomal RNA gene, partial sequence	2591	2591	99%	0.0	99%
Nostoc sp. SAG 34.92 16S ribosomal RNA gene, partial sequence	2588	2588	98%	0.0	99%
Nostoc sp. 8964:3 partial 16S rRNA gene, strain 8964:3	2588	2588	98%	0.0	99%
Nostoc sp. PCC 9231 16S small subunit ribosomal RNA gene, partial sequence	2588	2588	99%	0.0	99%
Nostoc entophytum IAM M-267 gene for 16S ribosomal RNA, partial sequence	2582	2582	98%	0.0	99%

Acknowledgements

We thank Dr. Don Bryant, Penn State Dept of Biochemistry and Molecular Biology, for the gifts of *A. variabilis* and *N. punctiforme;* Mark Sign, Penn State Shared Fermentation Facility, for advice on photo-bioreactors; and Dr. Richard Macur, Center for Biofilm Engineering at Montana State University, for his insightful discussions. We also thank all members from Dr. Bruns' lab for their great support. This research was funded by the Research Applications for Innovation (RAIN) grant from the Penn State College of Agricultural Sciences.

References

[1]Belnap, J., Lange. L. E. (eds) 2001. *Biological Soil Crusts: Structure, Function, and Management*. Springer: New York, U.S.A.