Identification of Cyanobacterial Isolates From Desert Soil Crusts

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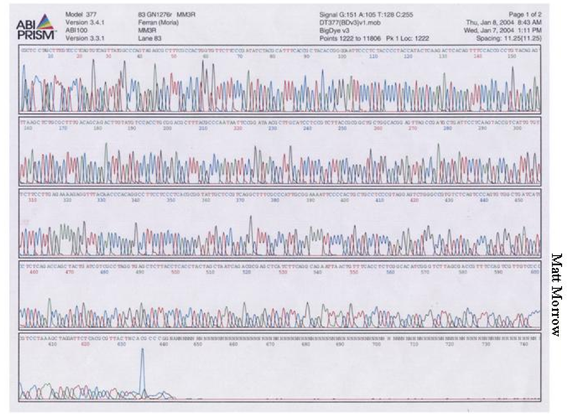
Bates Well soil crust, Organ Pipe National Monument



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Desert Soil Crusts

Biological desert soil crusts are dark patches in the desert floor where intricate systems of microscopic organisms live. The darker the area the more mature the ecosystem. Numerous bacteria and cyanobacteria live in these areas and have adapted to to the hot and arid conditions. These desert soil crusts hold the soil together and prevent wind and water erosion. Furthermore, some species within the soil crust fix nitrogen to use or be used by other organisms. Desert soil crusts can take hundreds of years to mature and be destroyed within a few seconds.

Objective

Which cyanobacterial species live in the desert soil crusts of southern Arizona?

This experiment will attempt to classify the cyanobacteria harvested from a soil crust by comparing morphological characteristics and to molecular characteristics (using the 16s gene of the cyanobacteria). Six isolates have been harvested from a soil crust near Bates Well, Organ Pipe National Monument, Ajo, Arizona.

Methods

The cyanobateria were grown on BG11 noble agar and allowed to grow under a fluorescent light, using photosynthesis. When sufficient biomass existed, the genetic material was harvested and then extracted using the MoBio Plant DNA extraction kit. Afterwards, the amount of DNA was increased through Polymerase Chain Reactions in a thermocyclyer. During PCR, DNA unwinds and splits into two single strands. Forward and reverse primers bind to the strands then nucleic acids bind to the primers to make a new DNA strand. The process repeats with a result of the duplication of approximately 700 base pairs of the 16s gene of the original template. Gel Electrophoresis was done using all of the product in a 1% agarose gel and the DNA bands were cut for purification. Next, the DNA was purified using the MoBio DNA purification kit. Finally, the DNA was sequenced in the Arizona State University DNA sequencing lab allowing for the order of the nucleic acids to be recorded. The sequence was obtained and analyzed then compared to other cyanobacterial sequences. Also, morphological characteristics of the cyanobacteria were observed and recorded. Then the cyanobacteria were classified using this data.

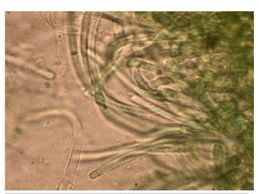
Conclusions

In conclusion, neither method worked decisively better than the other. In order to be considered the correct species match by molecular means, the resulting proximity must be greater than or equal to 98%. Only one species obtained this match. Furthermore, many of the sequences did not have a species identity attached to them. However, with the morphological methods a genus was assigned but with no degree of certainty. Also, by molecular means, some isolets were assigned genera which were completely morphologically incorrect. Another error of the molecular method was possible contamination of harvested species. Isolate 8 was a contaminant of isolate 6 so the results of isolate 6 could have been affected by this. In short, none of the isolets could be definitely matched to a genus and species. Further research of desert crust cyanobacteria is necessary in order to achieve this.

Data





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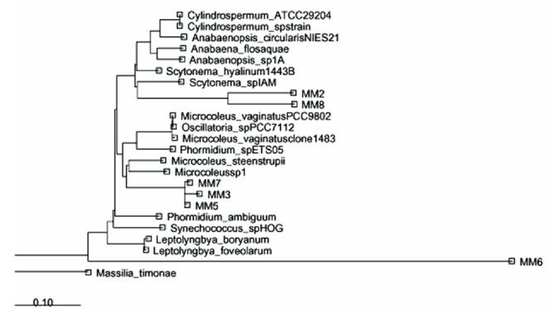
	Number	2	3	5	6	7	8
	Observations						
Filament	Width	14µ	4µ	5µ	14µ	7µ	3µ
Filament	Color	light green	light green	light green	light green	light green	light green
Filament	Sheath	closed, clear, rigid, not straight	open, clear, rigid, straight	open, clear, rigid, straight	closed, clear, rigid, not straight	open, clear, rigid, straight	not common, open
Filament	Sheath Layers	1	1	1	1	1	1
Filament	# of trichomes	1	1	1	1	1	1
Trichome	Width	11µ	3µ	3µ	12µ	4µ	2µ
Trichome	Length	>50µ	>50µ	>50µ	>50µ	>50µ	>50µ
Trichome	Branching	occasional single false	no	no	occasional false branching	no	no
Trichome	Tapering	slight, last 5 cells	slight, last 10 cells	slight, last 7 cells	slight,last 5 cells	slight, 10-20 cells	no
Trichome	General Shape	bent, mostly uniform	straight, uniform	straight, uniform	bent, mostly uniform	straight, uniform	curved, looped
Trichome	Motility	none	waving and gliding	no	no	no	no
Trichome	Hormogonia	none	yes, slight wiggling	yes, wiggling	no	yes, twitching	yes, wiggling
Trichome	Necridia	yes	no	yes	yes	no	yes
Trichome	Heterocysts	yes	no	no	yes	no	no
Cell	Length	7µ	2µ	2µ	8µ	2µ	1µ
Cell	Typical Dimensions	wider than long	wider than long	wider than long	wider than long	wider than long	wider than long
Cell	End Cell	rounded	not different	thinner	rounded and shorter	not different	not different
	Results						
	Morphological	Scytonema tolypothrichoides	Microcoleus steenstrupii	Microcoleus steenstrupii	Scytonema tolypothrichoides	Microcoleus steenstrupii	Leptolygnbya purperea
	#1 Molecular	Uncultured	Uncultured	Uncultured	Leptolyngbia crispata	Leptolyngbia sp.	Leptolyngbia sp.
	%	96%	95%	96%	93%	98%	96%
	#2 Molecular	Scytonema hyalinum	Synechococcus sp.	Synechococcus sp.	Leptolyngbia sp.	Uncultured	Leptolyngbia crispata
	%	93%	91%	93%	93%	96%	93%



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