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Potential antimicrobial properties of the cyanobacterium *Microcoleus vaginatus* in relationship to the moss *Bryum argenteum*

Biological soil crusts play important ecological roles in arid desert regions. These crusts cycle nutrients, prevent wind/water erosion, and form the basis of food chains and soil formation in desert communities. Primary components of these structures include two desert moss species *Bryum argenteum* and *Syntrichia caninervis*, and *Microcoleus vaginatus,* a cyanobacterium. Our Phase I experiment strongly suggests that in an environment of intense light, a condition of stress to *Syntrichia caninervis*, there is an increase in shoot regeneration when cyanobacteria are present compared to when they are absent. *Microcoleus* is a highly motile species and our lab observations of fewer deleterious bacteria, algae, and fungi in cultures containing the cyanobacterium led us to hypothesize that the cyanobacterium may be deterring the development of bacteria/algae/fungi that can slow moss growth. The current experiment seeks to determine whether a benefit of *Microcoleus* to the mosses lies in its antimicrobial activity.

Two microbial candidates (a fungus and a bacterium) were selected from early lab cultures and determined to impede the growth of these moss species. These microbes were then cultured individually and in combination with the moss only, with the cyanobacterium only, and with both moss and cyanobacterium together. Each treatment was allowed to incubate under simulated natural conditions of light and moisture for a period of eight weeks. Final results will be determined through biomass weights and area measurements.

Potential antimicrobial properties of the cyanobacterium *Microcoleus vaginatus* in relation to the moss *Bryum argenteum*

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Abstract

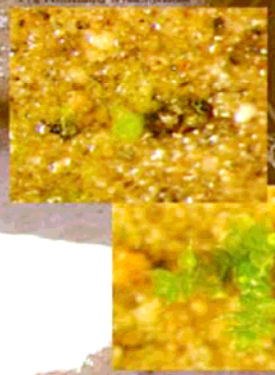
Biological soil crusts play important ecological roles in arid desert regions. These crusts cycle nutrients, prevent wind water erosion, form the basis of food chains and soil formation in desert communities, and appear to contribute to the growth of surrounding plants living in or near these crusted soils. A key factor in the stability of soil crusts is the interconnected relationships of the various organisms which comprise the crust ecosystem. These diminutive soil organisms can potentially hinder or enhance the establishment and regeneration of coexisting organisms through various means (States and Christensen 2001).

Two species that contribute to this soil structure, the desert moss *Bryum argenteum* and the cyanobacterium *Microcoleus vaginatus* coexist together in nature within desert crust soils. *Microcoleus* is a highly motile species and our lab observations of a selection of deleterious bacteria, algae, and fungi in cultures containing the cyanobacterium led us to hypothesize that the cyanobacteria may be deterring the development of bacteria/algae/fungi that can slow moss growth.

Two microbial candidates (a fungus and a bacterium) were selected from early lab cultures and determined to impede the growth of some desert moss species found in areas of soil crusts. These microbes were then cultured individually and in combination with the moss only, with the cyanobacterium only, and with both moss and cyanobacterium together. Each treatment will incubate under simulated natural conditions of light and moisture for a minimum period of eight weeks. Final results will be determined through biomass weights and area measurements.

Hypothesis

Microcoleus is a highly motile species and our lab observations of a selection of deleterious bacteria, algae, and fungi in cultures containing the cyanobacterium lead us to hypothesize that the cyanobacteria may be deterring the development of bacteria/algae/fungi that can slow moss growth.



Introduction

Biological soil crusts are a unique, intricate composite of organic and inorganic components that together create a living ground cover. Important in harsh regions with arid climates and sparse plant cover, these aggregate soils provide significant diversity of organisms and ecological processes that contribute to the health and maintenance of their respective ecosystems. Threatened by a multitude of disturbance and ecological change, these fragile communities take on a larger role as key components in the future of environmental stability.

Existing in a wide range of ecosystems across the globe, biological soil crusts are composed of a variety of organisms ranging from an assortment of bacteria, microorganisms and fungi to lichens and mosses. The interconnected communities of organisms that make up the biota of crust systems contribute to an intricate arrangement of interdependence that often extends to surrounding native plant communities and which contributes to various important ecological functions. These functions include carbon and nitrogen fixation, improved soil fertility and decomposition rates, aiding to the maintenance of nutrient levels in surrounding plants growing on these crusted soils and aiding in the promotion or inhibition of seed germination (Rosfield et al. 2002). Biological crusts play especially important ecological roles in arid desert regions, improving soil cohesion and water retention, thus reducing the incidence of wind and water erosion which can be particularly damaging to desert ecosystems.

Cyanobacteria comprise some the most diverse organisms in nature. This diversity enables these creatures to exist in a variety of habitats and conditions. In arid and semi-arid lands, species of *Microcoleus* comprise a large volume of photosynthetic cyanobacteria biomass in these open spaces (Belnap et al. 2001). Investigations into the biological activities of various species of cyanobacteria indicate a vast resource potential for biotechnical applications within areas such as mariculture, food, feed, fuel, fertilizer, medicine, industry and in combating pollution (Thajuddin and Subramanian 2005). An evaluation of the antimicrobial potential of *Microcoleus vaginatus* is valuable in further understanding the relationship structure of cyanobacteria and desert moss species.

Experimental Design and Procedure

Experiment installed in two stages:
- Stage 1 moss shoulets (asexual fragments) installed
- Stage 2 treatments including cyanobacteria and microbes under study

Treatments designated as follows:

Moss only	Cyano only	Microbe 1 only	Microbe 2 only
Moss + Cyano	Cyano + Microbe 1		
Moss + Microbe 1	Cyano + Microbe 2		
Moss + Microbe 2			
Moss + Cyano + Microbe 1			
Moss + Cyano + Microbe 2			

Replicates: 8 + 10 + 11 treatments x 10 reps = 98 dishes

- Treatments cultured in individual sterile Petri plates containing autoclaved soils and hydrated with sterile water
- Treatments placed in growth chamber under conditions of a 12 hour photoperiod (20C lighted, 8C darkened). (Fig 4)
- Observations of treatments containing *Bryum* specimens made daily on day 2 through day 10, day 14, day 17, then continuing weekly for an initial period of 56 days; observations include:
  a) First emergence of early stage regenerative structures
  b) First emergence of secondary regenerative structures
  c) Number of shoots
  d) Shoot expansion
  e) Percent coverage of biomass by quadrat

- Growth chamber racks rotated daily first 14 days, then once weekly
- Treatment plates are randomly rotated per rack once weekly
- Treatment plates hydrated when necessary with sterile water
- 50% Hoagland's nutrient solution added once weekly from day 21 forward
- Final data results to be measured are included in fig 5

Bryum argenteum (silver moss) Preparation

- Six (3 male, 3 female) gametophytes collected at shoulet stage
- Gametophytes pre-sterilized dish number and treatment group

Microcoleus (Cyanobacteria) Fig. 6

- Populations initially cultured in nature soil collected from Frenchman Flat at the U.S. Department of Energy's Nevada Test Site
- Individual strains subsequently isolated and cultured on autoclaved soils and hydrated with sterile water, allowing proliferation over a period of 30 days.
- Single strain selected for study, filaments isolated and cut to similar length

Deleterious microbes (white filamentous fungus and undetermined cyanobacteria) Fig 9 and Fig 10

- Populations cultured from samples collected in previous lab studies
- Identifications to be determined

Prediction

Measurements taken of final biomass and area coverage for each treatment at the end of the study period will aid in the determination of the potential antimicrobial effects of *Microcoleus* on selected deleterious microbes. Decreased growth or reduced spread of microbe biomass when cyanobacteria are present will indicate a possible antimicrobial component. This experiment will also indicate whether the moss *Bryum* has antimicrobial properties (Sabovljevic et al. 2006).

Future Direction

Ongoing and prospective studies of the antimicrobial properties of a variety of cyanobacteria and the potential applications of these results within numerous fields suggest a role for research into the function of *Microcoleus* in furthering an understanding of the ecological benefits of soil crusts for use in restoration of desert ecosystems and inhibition of invasive species through enhanced growth and regeneration of desert moss species.

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