In search of cyanobacteria strains with bioconditioning and biofertilization effects for degraded soils in the semi arid and arid areas of South Africa

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Abstract
The ability of cyanobacteria to fix N_2 and produce exopolysacharides varies widely among different strains. The objective of this study was to identify and isolate cyanobacteria strains with ability to fix N_2 and produce exopolysaccharides with a view to evaluate them for their biofertilization and conditioning potential of degraded soils in arid and semi arid areas of the Eastern Cape Province, South Africa. Cyanobacteria were isolated from four sites by culture and plating techniques using growth media BG11 for all strains and BG110 for atmospheric N2-fixing strains. The numbers of strains isolated were 2, 28, 35, and 32 for Guquka, Hertzog, Fort Cox College, and Qunu soils, respectively. Only three of the 97 strains had bioconditioning and biofertilization potential emphasizing the importance of screening in order to identify cyanobacteria strains suitable for improving the physical and chemical fertility of soils. Other isolated cyanobacteria strains produced no exopolysaccharide and exhibited negligible nitrogenase activity under the assay conditions used. The results suggested that soils in arid and semi arid areas of South Africa have cyanobacteria strains with potential to improve the physical and chemical fertility of degraded soils.

Key Words
Degraded soils, cyanobacteria strains, exopolysaccharide production, nitrogen fixation, South Africa.

Introduction
Cyanobacteria are known to improve the contents of nitrogen (N) by N2-fixing activity and carbon (C) by photosynthesis, and activities of microbial communities. They also contribute to the stabilization of soil aggregates through the production of OM (Barclay and Lewin 1985) and exopolysaccharides (EPS). Their ability to increase N, a major limiting factor in the productivity of many South African soils, is shared by many species belonging to the genera *Nostoc, Anabaena, Fischerella* and *Scytonema* (Steppe *et al.* 1996). Some cyanobacteria strains exhibit both bioconditioning and biofertilization effects but others exhibit only one (Klubek and Skujinš 1980). Therefore, as a first step in exploring the possibilities of exploiting the positive benefits of indigenous soil cyanobacteria in South Africa this study sought to isolate, characterize and select cyanobacteria strains capable of fixing atmospheric N_2 and producing EPS.

Material and methodology
Soils from Hertzog, Guquka, and Qunu villages in Seymour, Alice and Mthatha, respectively, and from Fort Cox College in Middledrift, characterised by susceptibility to form crusts and low crops yields were characterised and used. All cyanobacteria strains were isolated and purified in the growth medium BG11 while medium $BG11_0$ was used to isolate strains that can fix atmospheric N_2 (Rippka et al. 1979). Cyanobacteria strains with one kind of colony morphology were grown separately and labelled following the guidelines by Stanley and Krieg (1984) to separate them from other strains. Quantitative determinations were done by estimating the number of cyanobacteria per 100 mL of samples using the Most Probable Numbers (MPN) method. Nitrogenase activity was determined by the acetylene reduction assay, whilst a mixture of India ink and the liquid culture observed under a light microscope were used for EPS content.

Results and discussion
Soil characterization
All the selected soils were characterised by low C and N contents that could be the result of continuous cropping with low or no use of organic and/or inorganic fertilizers, and high silt plus fine sand contents that could make soil structural units unstable upon wetting or physical disturbances like ploughing or animal movement.

Cyanobacteria enumeration, isolation and growth

A greater proportion of the cyanobacteria strains isolated from Qunu soil (22 strains) was from virgin soil, followed by fallow soil (10 strains), with no strains isolated from the cultivated soil. A similar trend for Hertzog soil was observed (Table 1). Virgin soils could have had higher proportions of cyanobacteria strains because they had no nutrient uptake by cultivated plants. The higher number of cyanobacteria strains isolated from the cultivated Fort Cox College soil (22 strains) (Table 1), was possibly because of higher use of fertilizers and irrigation. However, none of the strains isolated from this soil could fix N_2 and neither did they produce detectable EPS. These findings evidenced how the use and the soil conditions affect the number and characteristics of the populations of cyanobacteria.

Table 1. Quantities of cyanobacteria strains isolated and purified from soils sampled from cultivated, fallow, and virgin land use systems at different sites.

Site	Number of different cyanobacteria strains			Total
	Cultivated	Fallow	Virgin	
Guquka	1	-	1	2
Hertzog	8	9	11	28
Fort Cox College	22	8	5	35
Qunu	0	10	22	32
Total	31	27	39	97

Nitrogenase activity and EPS production of isolated cyanobacteria

Cynanobacteria strains 3v, 3g, 8a and 22b exhibited good growth in liquid $BG11_0$ growing medium (Table 2). Mass production of cyanobacteria can be done more easily in liquid than solid growing medium, hence the need to select for strains that not only fix high amounts of N_2 and/or produce large quantities of EPS, but are also able to grow well in liquid culture. Strains 3v, 3g and 7e produced EPS.

Table 2. Growth on solid and liquid medium $BG11_0$ and EPS production capability of some isolated strains of cyanobacteria isolated.

Site	Land use system	Strain number	Growth on BG 11_0		EPS observed
			-----Solid	--------Liquid-----	
Guquka	Cultivated	22b	++	+++	-
Hertzog	Cultivated	1af	++	+	-
	Fallow	2i	++	+	-
	Virgin	3v	++	+++	++
		3g	+++	++	+
Qunu	Virgin	7e	++	+	+
	Fallow	8a	++	++	-

For growth on BG 11_0: +++ is very good growth, ++ is good growth, + is weak growth
For EPS production: ++, +, - means thick, thin and no EPS zones observed respectively.

On the other hand, although the growth of strains 7e was relatively weaker in liquid medium, it could still be considered adequate for the mass production of this strain. Only the heterocyst-forming strains 3v, 3g and 7e showed appreciable nitrogenase activity, but only strain 7e exhibited levels comparable to those of the reference strain (Figure 1).

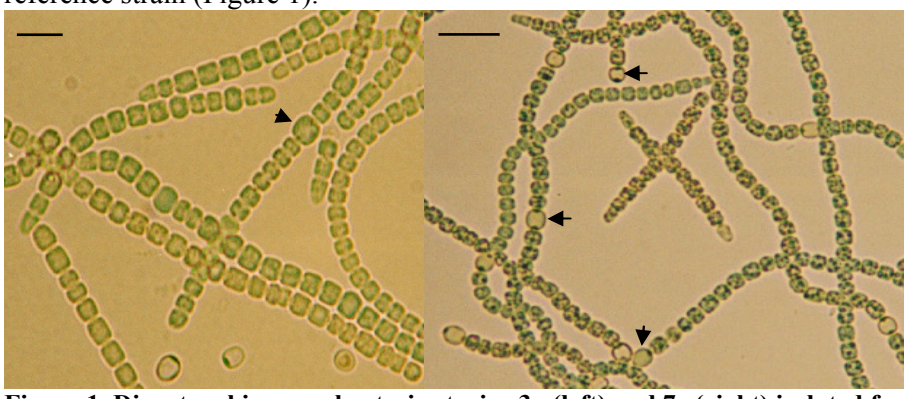

Figure 1. Diazotrophic cyanobacteria strains 3g (left) and 7e (right) isolated from Hertzog and Qunu soils, respectively, in the Eastern Cape, South Africa. Arrowheads point to some heterocysts. Bars, 10 µm.

The higher nitrogenase activity of strain 7e with regard to strains 3v and 3g could be due to a higher percentage of heterocysts (Figure 2), which are cells devoted to N_2 fixation. The fact that cyanobacteria strains 1af, 2i, 8a and 22b exhibit apparent diazotrophic growth (Table 3) but no detectable nitrogenase activity could be explained if these cyanobacteria are non heterocyst formers, for which the nitrogenase assay used here could not be suitable.

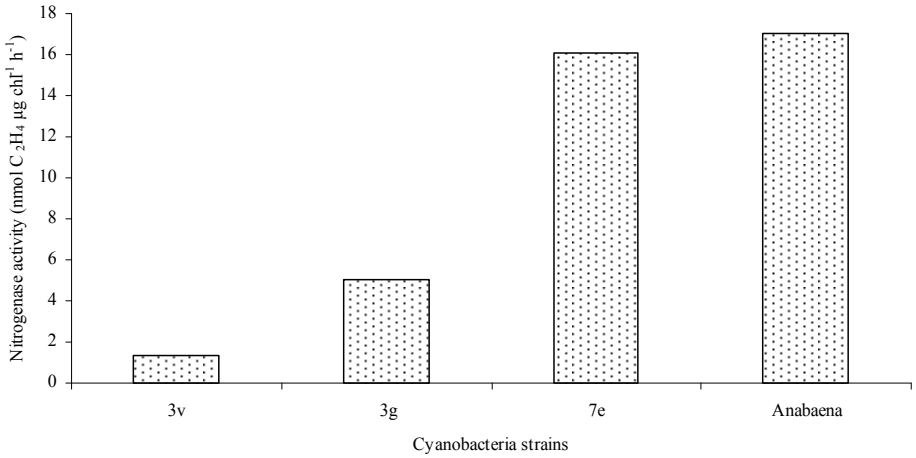

Figure 2. Nitrogenase activity of strains 3v, 3g, 7e, and a reference strain, PCC 7120 (values are means of duplicate analyses).

Conclusion

Of the 97 cyanobacteria strains isolated from degraded soils only three had soil bioconditioning and biofertilization potential emphasizing the importance of screening in order to identify cyanobacteria strains suitable for improving the physical and chemical fertility of soils.

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