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Effect of *Nostoc* (Cyanobacteria) inoculation on the structure and stability of clay soils

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Abstract The effect of *Nostoc* spp. (Cyanobacteria) inoculation on soil structure was studied in two clay soils (Calanco and Biancana) originating by erosion processes from Pliocenic marine sediments of central Tuscany (Italy). Two axenic *Nostoc* strains, AfS49 and KaS35, selected for their soil colonization and exopolysaccharide (EPS) production capacities, were inoculated in Petri dishes on the two clay soils sterilized by autoclaving. The soils, inoculated with an amount of cyanobacterial biomass corresponding to 1.0 g dry wt. m^{-2}, were incubated under continuous light at 27 °C for 3 months and periodically wetted using a pipette. The two strains showed different growth rates and EPS production on both soils: KaS35 produced more biomass, while AfS49 produced more EPS. This different behavior was also documented by scanning electron microscope (SEM) observations. The effect of cyanobacterial inoculation on soil structure resulted in the protection of soil porosity by reducing the damaging effect of water addition. Indeed, the incidence of transmission pores in the inoculated soils (about 30%) was higher with respect to the control soils (about 5%). Data also showed the beginning of a primary aggregation as a consequence of interaction between the secreted EPS and the morphological units of the fine soil fraction. However, no significant differences in water soil structure stability were measured between inoculated and non-inoculated soils. In this paper the interactions between the EPS produced by the two strains and the clay aggregates are discussed in order to understand the role of cyanobacterial inoculation in maintaining soil structure.

Key words Soil structure · Aggregate stability · Soil inoculation · Cyanobacteria · *Nostoc* spp. · Exopolysaccharides · Crusts · Infiltration

Dedicated to Professor J.C.G. Ottow on the occasion of his 60th birthday

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Introduction

Soil microbial polysaccharides are the most important factor influencing soil structure formation (Metting and Rayburn 1983; Metting 1988), In the past, most of the research on the potential use of bioconditioners has been focused on bacteria and fungi, regarded as the most important producers of polysaccharides (Baver 1963; Lynch and Bragg 1985; Metting 1986; 1987), even if some strains of green algae and cyanobacteria from soil have also been known to produce large amounts of extracellular polysaccharides (Barclay and Lewin 1985). Green algae of the genus *Chlamydomonas* have been used as inoculants for improving the stabilization of soil structure through the production of extracellular polymers (Metting 1986, 1987).

The practice of soil inoculation with cyanobacteria has been used for many years in tropical lowlands cropped to rice, due to their beneficial dinitrogen-fixing activity (De 1939). Nevertheless, the potential positive effects of cyanobacteria are not restricted to this biofertilizing activity but also to the positive effects on the soil structure. The soil-ameliorating effect of cyanobacteria has been known for a long time. Singh (1961) studied this extensively, and algal inoculation or stimulation of their growth has been performed to improve the soil structure of alkaline soils.

Crusts of cyanobacteria cause retention of silt and clay, thus producing effects on soil structure by the rearrangement of soil particles (Starks et al. 1981). Experiments on

the effects of cyanobacterial crusts (Roger and Reynaud 1982) showed that: (1) they did not slow the rate of water infiltration; (2) they had a very efficient protective effect against the erosive impact of rain; and (3) they increased the moisture content of the soil underneath the cyanobacterial stratum. In other experiments, Avnimelech et al. (1982) demonstrated the contribution of the cyanobacterium *Phormidium* in reducing clay dispersion into drains. Rao and Burns (1990) observed a significant improvement in soil aggregation in a brown earth silt-loam soil inoculated with *Nostoc muscorum*. Recently, it has been shown that the application of small quantities of cyanobacterial anionic exopolysaccharides (EPS) to selected fine-grained soils (Warrington et al. 1991) and clay soils (Falchini et al. 1992) leads to an improvement in soil and aggregate stability.

The present work considers the structural effects of soil inoculation with two strains of *Nostoc* spp., AfS49 and KaS35, isolated from dry soils and selected for their copious production of EPS and high soil colonization capability (Tomaselli et al. 1989). The two strains differ with regard to the organization and appearance of the produced EPS. In AfS49 the EPS forms a wide diffluent mass, loosely enveloping the trichomes and being easily released, whereas in the KaS35 the EPS is less wide, surrounds each trichome and is not easily released (unpublished data). Particular emphasis has been devoted to the analysis of pore size distribution for its implication in soil fertility (movement and availability of water and air). Laboratory experiments were carried out on two clay soils, representative of two physically degraded areas of central Italy. To emphasize the specific contribution of the inoculated cyanobacteria to the physical characteristics of the soils employed, the experiments were carried out under controlled sterile conditions, using axenic cultures of the two selected strains.

Materials and methods

Soil samples

The two examined poorly structured soils were sampled from the surface layer (0–20 cm) of two typical erosion forms of Pliocenic clay marine sediments, Calanco and Biancana, of central Tuscany (Italy). Their chemical and physical characteristics are reported in Table 1.

Table 1 Characteristics of the soils employed (*CEC* cation exchange capacity, *ESP* exchangeable sodium percentage)

	Biancana	Calanco
Sand (%)	13	47
Silt (%)	30	23
Clay (%)	57	30
pH	8.73	8.00
Total C (%)	0.39	0.62
Total N (%)	0.06	0.09
Total P (P_2O_5) (%)	0.16	0.11
Carbonates ($CaCO_3$) (%)	15.9	17.3
CEC [cmol(+)kg^{-1}]	17.7	20.5
ESP (%)	20.6	15.9

Cultures

Axenic cultures of the AfS49 and KaS35 strains were grown photoautotrophically in BG-11_o medium supplemented with A_5+Co micronutrient solution (Rippka et al. 1979). The cultures were incubated in a Gallenkamp orbital incubator with a shaking speed of 100 cycles min^{-1} at 27 °C in an atmosphere enriched with 5% (v/v) CO_2, under 50 µmol photons m^{-2} s^{-1} continuous photosynthetically active radiation (PAR).

Treatments

Soils were air dried, ground, sieved (from 1 to 2 mm), placed (100 g) in Petri dishes and sterilized by autoclaving (120 °C for 20 min). The Petri dishes were then inoculated with the two cyanobacterial strains. Actively growing cells, in the exponential growth phase, were harvested by centrifugation (14000 *g* for 15 min), washed and inoculated on the soil surface. The inoculum, applied in a concentrate suspension of fresh biomass in sterile distilled water to each Petri dish, corresponded to about 1.0 g m^{-2} dry wt.

Two treatments were used: (1) inoculated soils (AfS49 or KaS35 strain) and (2) non-inoculated control soils. Each treatment was done in triplicate. The Petri dishes, containing either the inoculated or the control soils, were incubated at 27 °C, under 18 µmol photons $m^{-2}s^{-1}$ continuous PAR, for 3 months. To maintain a moisture level near to field capacity, sterile distilled water was periodically added drop by drop to the Petri dishes using a pipette.

Analytical procedures

Biomass determination

Dry weight determination was used to estimate the cyanobacterial biomass of the culture suspensions used for the soil inoculation. Ten milliliters of culture suspension was filtered and washed through a membrane filter of 8 µm pore size, then dried at 70 °C, cooled in a desiccator and weighed. Cyanobacterial biomass in the soils was indirectly evaluated by chlorophyll *a* determination.

Chlorophyll a determination

In the culture suspensions the chlorophyll *a* cell content was determined spectrophotometrically, after 90% acetone extraction, using the extinction coefficient reported by Parson and Strickland (1963). In the soil samples the amount of chlorophyll *a* was determined according to Hoyt's procedure (1966) and was correlated to the cyanobacterial biomass dry weight on the basis of chlorophyll *a* mean contents of 0.87% and 1.04%, for AfS49 and KaS35, respectively.

Polysaccharide analysis

At the end of the experiment, to determine the amount of EPS produced in the soil by cyanobacterial growth, 10 g soil was extracted with 30 ml 0.5 *M* NaOH (Cheshire 1979; Swift 1991) and then hydrolyzed overnight by adding 12 *M* H_2SO_4 (Cheshire 1979). The samples were centrifuged (14000 *g*, for 10 min) and the extracted EPS were determined spectrophotometrically in the surnatant, using the phenol-sulfuric acid method (Dubois et al. 1956). Results were expressed as glucose equivalents.

Soil measurements

At the end of the experiment different soil samples were taken from the Petri dishes and treated as follows: (1) 0.5-cm-diameter cores of various thicknesses were oven dried at 40 °C to determine the total soil pore volume and pore size distribution and (2) small surface soil aggregates were dried as above for SEM observation and water stability measurements.

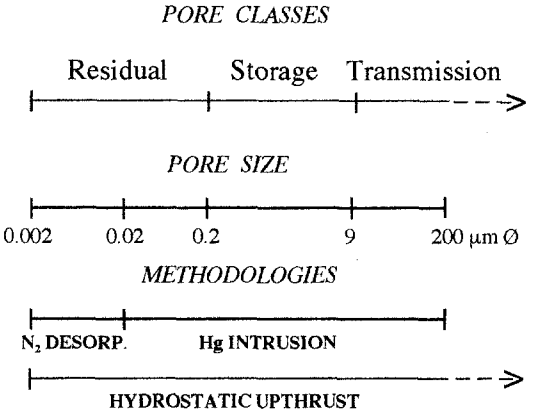

Fig. 1 Methodologies employed to describe total soil pore volume distribution in relation to pore size class and terminology

Total soil pore volume was determined according to Monnier et al. (1973) by measuring the hydrostatic upthrust in petroleum and calculated assuming a soil density of 2.65 g cm^{-3}. Using Greenland and Hayes's terminology (1981), this volume was subdivided into three different pore size classes: (1) residual pores (<0.2 μm in diameter); (2) storage pores (0.2–9 μm in diameter) and (3) transmission pores (>9 μm in diameter) as reported in Fig. 1. To evaluate the pore volume of these different pore size classes, we used the following methodologies, schematically shown in Fig. 1. Soil pore volume in the range from 0.02 to 200 μm in diameter was evaluated by Hg intrusion using a Carlo Erba 2000 Porosimeter, equipped with a Macropore Unit and automatic recording of the intruded Hg volume. The pore volume distribution was obtained from the derivative of the cumulative intruded pore volume (V) as a function of pore diameter. This latter parameter was determined using Washburn's equation (1) (1921), which correlates the intrusion pressure P (kg cm^{-2}) to the diameter d of the intruded pore (μm), supposing that it has a cylindrical shape. In Eq. 1, γ is the Hg surface tension (0.48 N m^{-1}) and θ (141.3°) is the contact angle between the Hg and the sample (Lawrence 1977).

$$d = \frac{4\gamma \cos \theta}{P} \cdot 10 \qquad (1)$$

The soil pore volume in the range from 0.002 to 0.02 μm pore diameter was determined from the isothermic N_2-desorption curves at −195 °C using a Carlo Erba 1800 Sorptomatic porosimeter (Ristori et al. 1983).

For SEM observation air-dried soil samples were covered with Au-Pd sputtering and analyzed using a Philips 405 electron microscope at 15 kV cathodic voltage. Soil water stability was determined spectrophotometrically according to Goldberg et al. (1991).

Results and discussion

Cyanobacterial growth and EPS production

The two inoculated *Nostoc* strains colonized the soil surface very quickly, showing a more luxuriant growth on Biancana than on Calanco. This behavior may be due to the higher total P content of the former soil although the amount of available P was not determined (Table 1). The beneficial effect of soil P content on cyanobacterial growth has been reported in other papers (Starks and Shubert 1982; Roger and Reynaud 1982; Roger et al. 1987). On both soils, the KaS35 strain showed a higher colonization capability and a more abundant growth than the AfS49 strain. The higher growth was evidenced by a large amount of biomass determined at the end of the experiment (Table 2). In contrast, the AfS49 strain produced a larger amount of EPS, especially on Calanco.

Table 2 Biomass and EPS production in *Nostoc*-inoculated soils after a 3-month incubation period

	Biomass (mg g^{-1} dry wt.)		Polysaccharide[a] (mg g^{-1} dry wt.)	
	AfS49	KaS35	AfS49	KaS35
Biancana	0.48±0.08	0.79±0.08	0.39±0.06	0.31±0.05
Calanco	0.19±0.05	0.77±0.07	0.51±0.08	0.18±0.05

[a] Expressed as glucose equivalent

Table 3 Effect of the inoculated *Nostoc* strains on total soil pore volume and on structural soil stability

Soil inoculation	Total soil pore volume (cm³ g^{-1} dry wt.)		Structural soil stability (% transmittance at 420 nm)	
	Biancana	Calanco	Biancana	Calanco
None	0.161±0.003	0.192±0.002	61.6±1.8	83.5±2.0
AfS49	0.190±0.004	0.256±0.005	57.7±3.3	82.2±2.7
KaS35	0.233±0.006	0.283±0.004	68.4±1.1	87.5±2.2

Effects of cyanobacterial growth on soil structure

Porosimetric determinations

Data on the total pore volume of the soil samples (1.5-cm-thick cores) show that the highest values correspond to the inoculated soils (Table 3). A detailed analysis of the pore volume distribution shows in the latter samples (Fig. 2) a higher incidence of transmission pores (about 30%), particularly in those treated with the KaS35 strain, in respect to the control soils (about 5%). These data agree with the picture of the soil surface reported in Fig. 3b. By comparing this picture with that of the non-inoculated soil (Fig. 3a), we can observe the protective effect of cyanobacterial growth on soil surface structure against the break-up effect of periodic wetting.

Since the inoculated *Nostoc* strains are phototrophic microorganisms, their growth occurs on the soil surface and the possible effect on the soil microstructure may be limited to the surface layer (Metting 1988; Rao and Burns 1990). Thus, we analyzed the soil microporosity of the first 4–5 mm of the soil profiles in order to observe the interaction of the inoculated strains with the soil particles. The AfS49 strain caused a shifting in the pore size distribution in the porosity range between 0.02 and 2 μm, easily recognizable by the vertical line in Fig. 4. This is probably due to the beginning of primary aggregation between the secreted EPS and the fine soil fraction. This did not occur with the KaS35 strain.

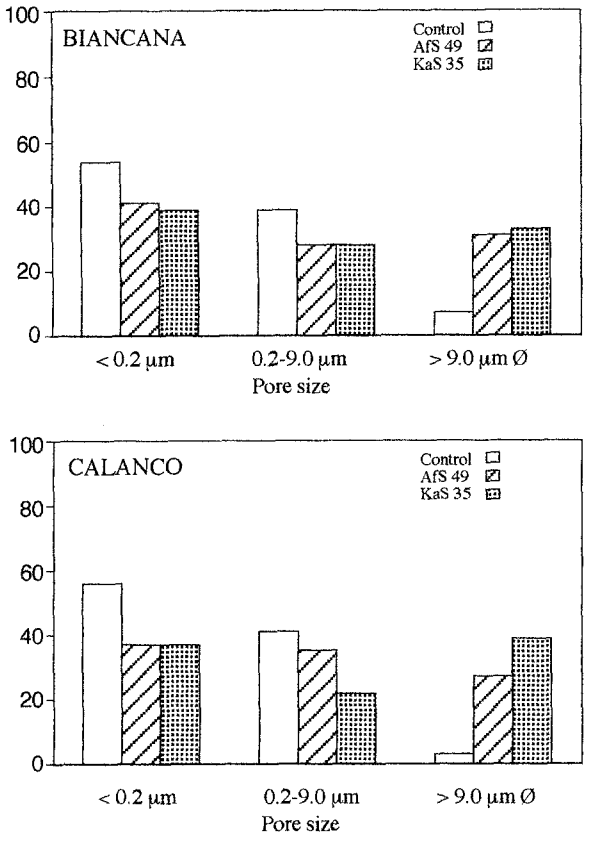

Fig. 2 Percentage pore volume distribution in different size classes of inoculated and control soils

SEM observations

SEM observations of the inoculated soil samples indicate a different morphological development of the strains. The cells of the KaS35 strain are smaller than those of the AfS49 strain and the trichomes appear completely surrounded by the secreted polysaccharide; conversely the cells and the trichomes of the AfS49 strain do not often appear enveloped by the EPS in spite of a substantial secretion (Table 2, Figs. 5, 6). This different development caused a different type of interaction between the two strains and soil. By observing the surface layer of the inoculated soils, it may be noted that the KaS35 strain caused a reorganization of the scattered fine superficial soil particles, recognizable for their dimensions and appearance as clay particles (kaolinite). Indeed, in Figs. 7 and 8, we can easily observe the perpendicular orientation of the clay particles, towards the secreted polysaccharide. This is probably due to the interaction between the positively charged edges of the clay particles (Baver 1963) and the negatively charged cyanobacterial polysaccharides, due to the presence of COO^- groups (Vincenzini et al. 1990). Moreover, it is possible to see that the cyanobacterial biomass and the large amount of EPS produced coated the walls of surface pores larger than 10 µm in size (Fig. 8). We believe that such a coating could have reduced the shrinkage effect and could explain the higher values of the total pore volume and transmission pores in the soils inoculated with the strain. The uniform coating produced by both the cyanobacterial biomass and the secreted polysaccharide also allowed the binding of some aggregates (Fig. 9).

As far as it concerns the AfS49 strain, in Fig. 10 the close interaction of the EPS with the single clay particles is shown. We suppose that the repeated addition of distilled water could have allowed the release of the secreted EPS, which in this strain is diffluent, and its penetration into the soil pore space, where it closely interacts with the single clay particles, as shown in Fig. 10. It is possible to see that the diameter (about 1 µm) of the pore shown in Fig. 10 is quite close to that of the most representative

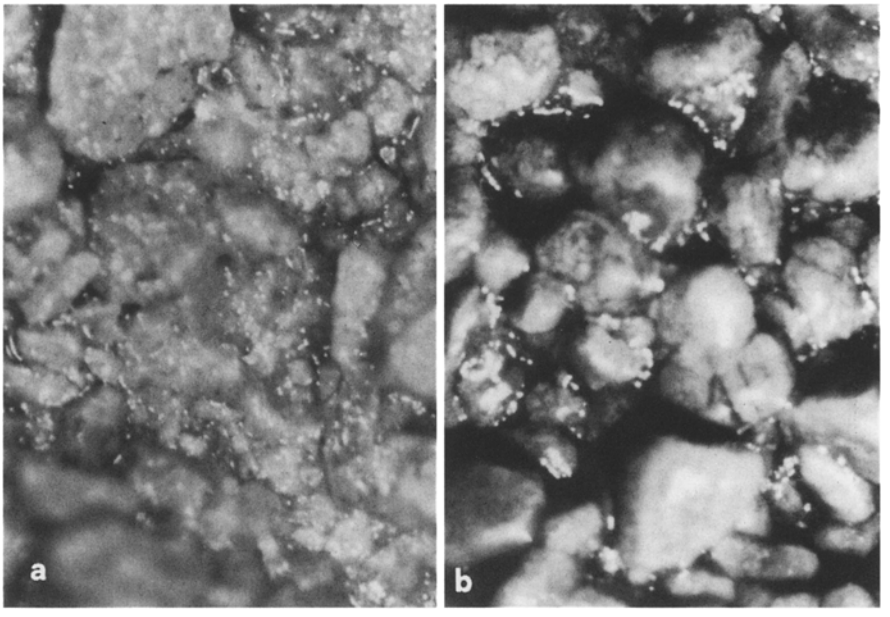

Fig. 3a,b Soil surface after 3 months of incubation and periodic wetting: **a** control soil, **b** inoculated soil, x13

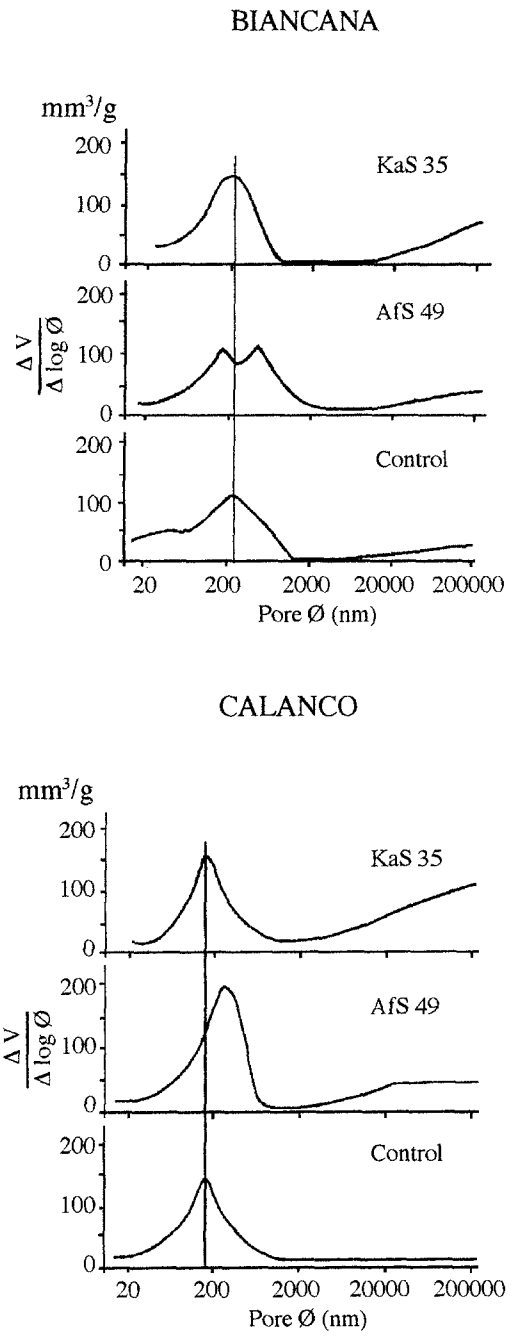

Fig. 4 Pore volume distribution (Hg porosimetry) in the surface layer (0–5 mm after 3 months of incubation. The derivative of the cumulative pore volume (V) is shown on the y-axis. Vertical line is the reference for the peak shift in the pore volume distribution following *Nostoc* spp. inoculation

pores in the surface layer of the soils inoculated with this strain (Fig. 4). Therefore, the porosimetric determinations are corroborated by SEM observations.

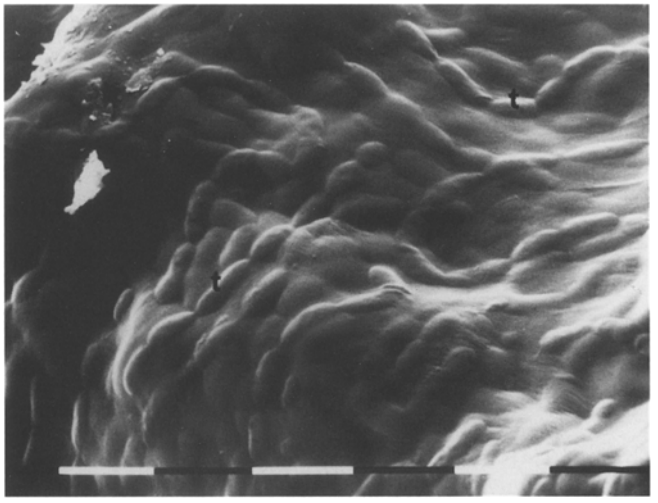

Fig. 5 Soil covered by the development of KaS35 strain *t* trichomes. *Bar* is 10 μm

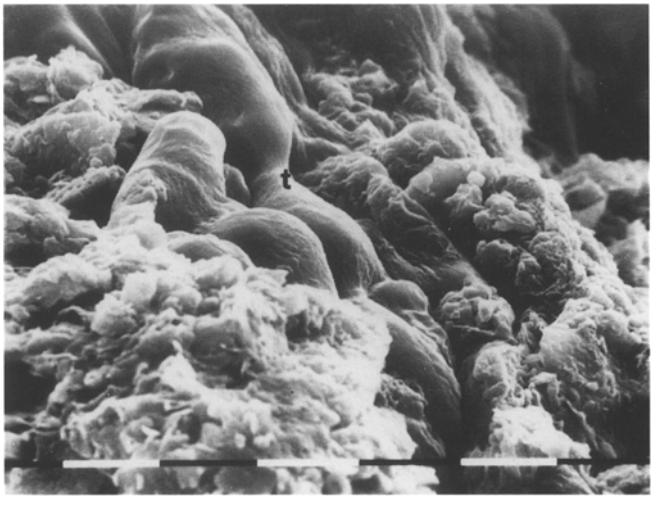

Fig. 6 Soil covered by the development of AfS49 strain *t* trichomes. *Bar* is 10 μm

Effect of cyanobacterial growth on structural soil stability

The results obtained from the analysis of structural soil stability show that the two strains had a modest but opposite effect on both soils (Table 3). Indeed, the dispersion of the fine soil particles was reduced in the soils inoculated with the KaS35 strain, whereas it was increased in those inoculated with the AfS49 strain, as shown by the transmittance values at 420 nm. The effect of the KaS35 strain in stabilizing the soil structure seems to be correlated to the wide surface coating of soil particles by the cyanobacterial development, as shown by SEM observations (Fig. 7). This probably reduced the break-up of the soil aggregates due to wetting, partially preventing their dispersion (Tan 1993).

In the case of the AfS49 strain, the non-uniform coating (Fig. 6) favored a slight soil aggregate break-up.

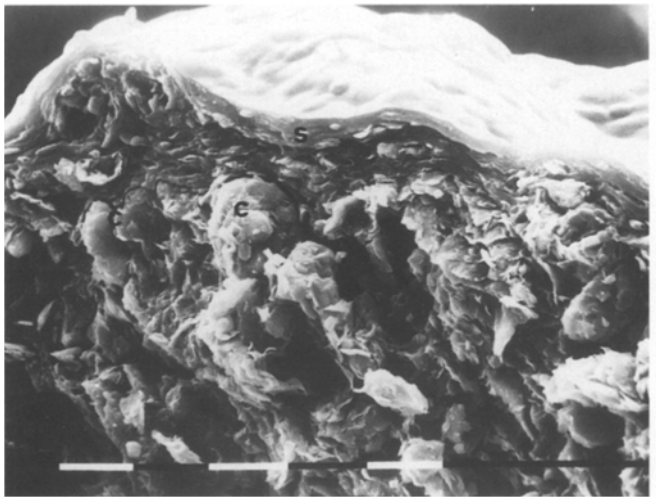

Fig. 7 Cross-section of soil surface layer inoculated with KaS35 strain. *s* cyanobacterial stratum, *c* oriented clay particle clusters. *Bar* is 10 μm

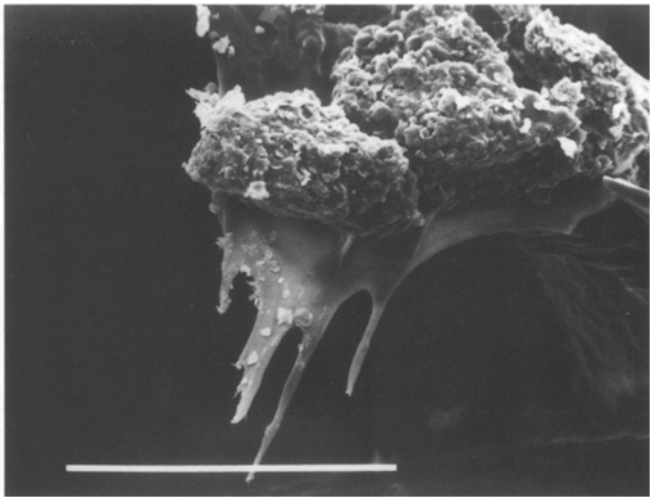

Fig. 9 Small soil aggregates surrounded by EPS from the KaS35 strain. *Bar* is 100 μm

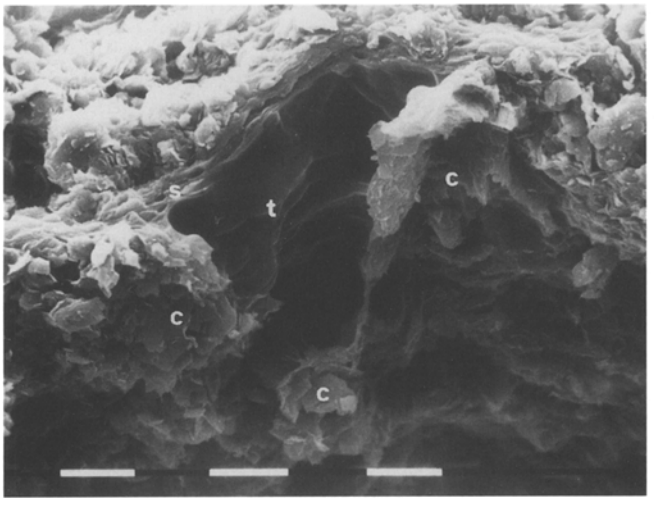

Fig. 8 Soil pore with an inner coating of cyanobacterial biomass and EPS in a soil inoculated with KaS35 strain. *t* EPS-surrounded trichomes, *s* cyanobacterial stratum, *c* oriented clay particles. *Bar* is 10 μm

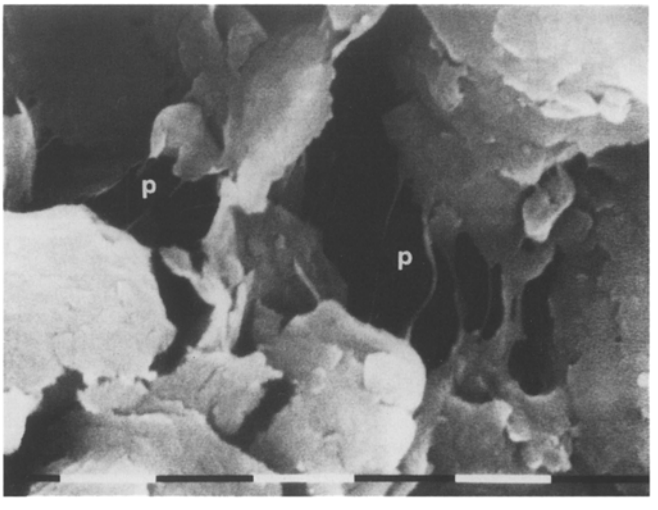

Fig. 10 Interaction between clay particles and polysaccharide secreted by AfS49 strain. *p* polysaccharide bundles. *Bar* is 1 μm

Moreover, we suppose that partial detachment of the polysaccharide macromolecules from the clay particles edges, during the water stability test, increased the clay negative charge, promoting a slightly higher dispersion (Oades 1984).

In conclusion, the use of these microorganisms as soil conditioners may represent a valid alternative to algae, especially in alkaline soils, where algal growth is prevented (Metting 1990). To be successful, the inoculation with cyanobacteria requires a preliminary strain selection in relation to the soil type (Tomaselli et al. 1990). Moreover, compared to the algae, cyanobacteria belonging to the genus *Nostoc* offer another advantage: the improvement of total-N soil content, owing to the capability to fix atmospheric nitrogen.

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