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Review Paper
Carbohydrate polymers in desert reclamation: the potential of microalgal biofertilizers

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The use of fast-growing, microscopic soil (edaphic) algae as 'green manure' seems to offer the only realistic hope of halting and reversing desert encroachment in the Sahel and other semi-arid regions. The capsular and sheath proteoglycans produced by green and blue-green edaphic algae are of central significance in soil neogenesis. Fundamental studies of their water-retaining and particle-aggregating properties, their ability to release phosphate and trace elements from insoluble minerals, and their ability to store nitrogen and release it slowly under field conditions are essential to the development of biofertilizer technology.

INTRODUCTION

Biofertilizer is a relatively new word, but it originates from traditional agricultural methods in Asia, where nitrogen-fixing blue-green algae (also called cyanobacteria) are allowed to grow in the rice paddies during the wet season (Watanabe, 1962; Venkataraman & Neelakantan, 1967; Venkataraman, 1972, 1979; Singh, 1979; Roger & Kulasooriya, 1980). During this period, they fix enough atmospheric nitrogen to meet the requirements of the rice plants, but at the same time they produce large amounts of exocellular proteoglycans as a slime or jelly (Painter, 1983*a*, *b*; Flaibani *et al.*, 1989). This immobilizes water in the surface layer of soil during the dry season. The water retained in this way is accessible to the rice plants, and results in a prolongation of the growing season, allowing more plants to reach maturity, and increasing the harvest substantially. The proteoglycans also have a particle-aggregating effect upon the topsoil, which resists 'wind erosion' during the dry season (Lynch, 1981; Metting & Rayburn, 1983; Barclay & Lewin, 1985; Lynch & Bragg, 1985).

During the past decade, improved scientific understanding has led to a more effective utilization of edaphic microalgae as biofertilizers. Especially in India, under the 'All India Co-ordinated Project on Algae', but also in Bangladesh, Burma, China, Nepal, Pakistan, the Philippines, Sri Lanka and Vietnam, rice paddies are now inoculated with mixtures of selected species of blue-green algae (Singh, 1961; Roger & Kulasooriya, 1980; Venkataraman, 1981). In the United States, production of nitrogen-fixing, filamentous blue-green algae and flagellated, unicellular green algae for use as biofertilizers is already an economically viable industry (Metting, 1981, 1987, 1988; Metting & Rayburn, 1983; Reynauld & Metting, 1988).

It is not hard to predict that further improvements in biofertilizers will occur in the near future. Genetic improvement through mutagenesis and strain selection, genetic transformation, viral transduction, and (in eucaryotic algae) hybridization could lead to the isolation of microalgal strains which fix more nitrogen, produce more exocellular proteoglycan, grow at a lower water potential on a wider range of soil types, and tolerate desiccation better (Metting, 1985, 1988; Lewin, 1977).

In this review it is suggested that biofertilizers offer the only realistic hope of halting and reversing desert encroachment in the Sahel and other semi-arid regions. Some of the special problems in the physics, chemistry and biochemistry of microalgal proteoglycans that must be solved in developing an appropriate technology are discussed.

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BLUE-GREEN ALGAE IN THE PRIMITIVE TERRESTRIAL ENVIRONMENT

The blue-green algae (Cyanophyta, or cyanobacteria) are among the world's oldest known living organisms, with fossil records dating back about 3×10^9 years (Schopf, 1970, 1978). Schopf has described the whole of the Proterozoic ($2{\cdot}5 \times 10^9$ until 570×10^6 years ago), which represents about two-thirds of the recorded history of life, as 'the age of blue-green algae'.

There was still considerable volcanic activity in this period, and the Earth must have been warmer than it is today, because there is no evidence of glacial activity until near the end of the period, 700×10^6 years ago (Sutton, 1979). The formation of sedimentary from volcanic rocks shows that there was heavy erosion, which may indicate extreme diurnal fluctuations in temperature. The oxygen tension in the atmosphere was initially very low, but is believed to have increased (due to photosynthesis), reaching ~10% of its present value at the end of the period (Cloud, 1976). The ozone shield would have been correspondingly thin, allowing more ultraviolet light to reach the Earth's surface (Brasier, 1979). Although the average salinity of the oceans is believed to have increased steadily since that time, there must have been numerous hypersaline, alkaline lagoons, because it is under these conditions that blue-green algal stromatolites (laminated, calcareous fossils) are formed today (Schopf & Walter, 1982).

Present-day species of blue-green algae are remarkably similar to the earliest fossils (Schopf & Walter, 1982), and it is generally agreed that there has been little evolution. This impression of the Proterozoic as a kind of primeval desert therefore helps to explain the ability of this phylum to colonize extreme environments today.

BLUE-GREEN ALGAE AS PRIMARY COLONIZERS OF EXTREME ENVIRONMENTS

Volcanoes and new volcanic islands

Volcanic ash is interesting because it is sterile and free from organic carbon and combined nitrogen. It is therefore an ideal substrate upon which to study *ab initio* soil genesis (Griggs, 1933). Following the gigantic explosion of the Krakatau volcano (Java) in 1883, which laid a carpet, 60 m thick, of ash and pumice fragments on Rakata and the neighbouring islands of Lang and Verlaten, Treub (1888) and Campbell (1909) studied the succession of species colonizing the new substrate. The first were blue-green algae, followed closely by fungi and green algae, and later by mosses and seed-bearing plants. A similar pattern was observed on the new volcanic island of Surtsey, which emerged from the North Atlantic ocean in 1963 (Brock, 1973). At the Kilauea volcano in Hawaii, which was less than ideal because of the close proximity of a forest, green algae established themselves simultaneously with the blue-green ones, and in greater numbers (Carson & Brown, 1978).

Waterless deserts

The Atacama desert in northern Chile is one of the driest places on Earth. One authority (Goodall, 1979) gives the average annual rainfall as zero. Another (Tricart, 1979) states that it does rain there, but only once in every 25–50 years. In spite of this, blue-green algae grow there (Forest & Weston, 1966; Friedmann & Galun, 1974). This is possible because the proteoglycans in the alga's extracellular sheath are so hygroscopic that they absorb moisture directly from the atmosphere. This allows a slow but finite rate of growth. The proximity of the Pacific Ocean, and the consequent high relative humidity of the air (*c.* 70%), lends credibility to this explanation.

Salt marshes and soda lakes

Most desert soils are alkaline and saline (Shields & Durrell, 1964; Skujins, 1984). Many blue-green algae thrive under these conditions. In salt marshes and soda lakes they are often the only living organisms that can be found. In Lake Magadi (Kenya) they grow in 0·1 M sodium carbonate-bicarbonate at pH 9·5–10·2 (Florenzano *et al.*, 1985). Tolerance of 0·5–1·0 M sodium chloride is common (Whitton & Potts, 1982), but halophiles that can grow in 4 M sodium chloride have been described (Walsby, 1982).

Acidic soils and hot springs

Few blue-green algae will grow below pH 5 (MacEntee *et al.*, 1972), but *Cyanidium caldarium* grows optimally at pH 2–3 (Ascione *et al.*, 1966). This species is eucaryotic and is considered by some authorities to represent a transition state between a blue-green and a red alga (Doemel & Brock, 1970). It is a typical inhabitant of hot springs, and will grow actively at up to 60°C (Doemel & Brock, 1970). It is also found in hot, acidic soils such as those at the Yellowstone National Park. Its limiting water potential for active photosynthesis is −26 bars. This corresponds to a water content of 5–25%, depending upon the kind of soil (Smith & Brock, 1973*a*, *b*).

SPECIAL CHARACTERISTICS OF EDAPHIC ALGAE

All algae will grow in liquid cultures, but many will fasten themselves to the walls of the culturing tank and

to any solid object immersed in the medium. Adhesion most often commences in the early stationary phase of growth, and is clearly correlated with the production of sheath or capsular proteoglycans (Flaibani *et al.*, 1989). It has an evident survival value for an edaphic alga, because it would tend to keep the cells near the surface of the soil, where light could reach them for photosynthesis.

An edaphic alga will, on average, grow at a lower water potential (ψ) than an aquatic one. This quantity is defined as $(RT/V_m)\ln a_w$, where V_m is the molecular volume of water (18 cm^3 mol^{-1}) and a_w is the activity of water. Since RT/V_m has the dimensions of pressure, and a_w has a value between zero and unity, ψ is usually expressed as $-$(bars) (Skujins, 1984; Lang, 1967). Tolerance of low ψ is evident also in aquatic algae that are adapted to saline conditions.

The minimum value of ψ that will support active growth is known as the limiting water potential (Smith & Brock, 1973*a*, *b*), and perhaps the most significant distinguishing feature of an edaphic alga is that its cells will remain viable when ψ falls below this value. In desert soils there may be total desiccation for long periods, with high temperatures in daytime.

Predictably, it is the blue-green algae that tolerate these conditions best, but green algae also perform well (MacEntee *et al.*, 1972; Trainor, 1970). A dried specimen of *Nostoc commune* grew in culture after it had been kept in a herbarium for 87 years (Lipman, 1941). Bristol-Roach (1919, 1920) found two blue-green algae (*Nostoc muscorum* and *Nodularia harveyana*) in a sample of desiccated soil that had been stored for 70 years, and one green alga (*Chlorococcum humicolum*) in a sample stored for 59 years. Viable cells of blue-green and green algae have also been found in samples of soil heated for 24 h at 100°C, 2 h at 110°C, or 1 h at 140°C (Trainor, 1962; MacEntee *et al.*, 1972).

The sheath and capsular proteoglycans again have a survival value in this connection, not only because they retard the process of desiccation, but also because they dry out to a tough, horny material that protects the cells from mechanical damage (Dudman, 1977). This tolerance to desiccation, elevated temperatures, and mechanical handling makes it possible to think of biofertilizers as industrial products which could be manufactured, dried, packaged, transported and stored for long periods without loss of viability.

DESERT ALGAE — THE IMPORTANT GENERA

Friedmann and Galun (1974) have provided an excellent review of the microalgal floras of the world's major deserts. These are dominated by filamentous blue-green and coccoid green genera. A few yellow-green algae (Xanthophyta) and diatoms (Bacillariophyta) are also reported, but no red algae (Rhodophyta). The floras vary markedly from one desert to another, but the following blue-green algal genera are among the most widely distributed: *Anabaena, Anacystis, Calothrix, Lyngbya, Microcoleus, Nodularia, Nostoc, Oscillatoria, Phormidium, Plectonema, Schizothrix, Scytonema, Synechococcus* and *Tolypothrix*.

The following genera of green algae are also widely distributed in desert soils: *Bracteococcus, Chlamydomonas, Chlorococcum, Chlorella, Chlorosarcina, Chlorosarcinopsis, Cystococcus, Neochloris, Palmella, Palmogloea, Protococcus, Protosiphon, Radiosphaera, Scenedesmus, Spongiochloris* and *Tetraspora*.

Some of these genera are exclusively edaphic, but most of them include aquatic as well as edaphic species. Even within a single species, different strains may vary in their ability to survive and grow at low water potentials, so that some may be considered aquatic and others edaphic (Fritsch, 1922; Fritsch & Haynes, 1923).

Desert soils contain little combined nitrogen (Skujins, 1984), and the prominence of filamentous blue-green algae, many of which are nitrogen-fixers, is readily understood. Green algae, on the other hand, cannot fix nitrogen, and they have to obtain combined nitrogen from sources such as electrical storms or blue-green algae, or by forming a symbiotic relationship with a heterotrophic nitrogen-fixer such as *Azotobacter* (Lund, 1967; Sasson, 1972; Klubek & Skujins, 1980).

Green algae are generally less tolerant of extreme conditions than are blue-green algae, but they have a high photosynthetic efficiency and grow very rapidly. This is due in part to the fact that some genera are flagellated and phototactic.

THE SITUATION IN 'THE SAHEL' (SUB-SAHARAN AFRICA)

Low rainfall and soil erosion by water and wind

The average annual precipitation in the Sahel is 100–300 mm (Bartholomew, 1974; Toupet, 1979). This is only 10–30% of that in the world's most productive agricultural regions, but it is sufficient to support some agriculture and livestock.

Most of the rain falls in July, and the growing season lasts for 3–4 months, after which there is usually no more rain until the following year. This extreme inequality in the distribution of rainfall throughout the year is a more serious problem than the low average for the year as a whole. During the brief 'wet season', which may last no more than two weeks, flood conditions may prevail, and much of the water runs away. In so doing, it washes away fertile topsoil ('water erosion'), or it sinks through to the phreatic region.

Phreatic water may be recovered by digging wells, but in many areas its salinity is a problem if it is used

for irrigation, as it leads to a progressive salinization of the soil as water evaporates from the surface. Many ambitious irrigation projects have ended in disaster for this reason (Grainger, 1990).

During the long dry season, the topsoil dries out to a fine dust, which can be easily blown away by the wind ('wind erosion').

The soil's low content of organic matter

The low capacity of the topsoil to retain water is due mainly to its low content of organic matter. Soil erosion by both water and wind can also be traced to the same cause, because the organic matter in soil has a particle-aggregating effect, which converts dust into heavy clumps (Martin & Waksman, 1940; Harris *et al.*, 1966; Bailey *et al.*, 1973; Lynch, 1981; Lynch & Bragg, 1985; Barclay & Lewin, 1985). Even without irrigation, therefore, the existing level of rainfall could support more agriculture than it does at present, if a way could be found to increase the soil's content of organic matter.

Of all the different kinds of organic matter that occur in soil, none can compare, on a weight basis, with polysaccharides and proteoglycans for their capacity to bind water. Agar and xanthan, for example, can immobilize 200 times their own weight of water. Most proteoglycans also have adhesive properties which can fasten cells to solid surfaces, and aggregate soil particles (Flaibani *et al.*, 1989).

Although the mineral components of soil could, in principle, bind nitrogen (as ammonium ions, for example), there is in practice a close correlation between organic matter and nitrogen in desert soil. For 7 out of 8 samples of soils from the Arizona, Chihuahua, Great Basin (Utah), Mojave (Nevada) and Sahara deserts, a plot of organic C against total N gave a straight line with a slope (C/N ratio) of 9 (Skujins, 1984). Hence, a low content of organic matter implies a low content of total combined nitrogen.

In-situ photosynthesis

Apart from special situations where it may be economical to use organic fertilizers, in-situ photosynthesis is the only way of increasing the soil's content of organic matter. In temperate climates, this is done by ploughing legumes such as clover or lucerne back into the soil. In wet, tropical regions, the 'green manure' may be the foliage of the leguminous shrub *Leucaena*, or the nitrogen-fixing fern *Azolla*. A suitable crop for semi-arid regions must, however, meet the following requirements:

(1) The plants must be able to grow, when necessary, on soil that has no organic matter content to start with, and which therefore dries out quickly. Higher plants will not do this unless they are constantly watered, but algae produce their own 'water reservoir' in the form of a proteoglycan capsule or sheath.

(2) They must be able to grow without artificial fertilization. Heterocystous blue-green algae can fix more than enough atmospheric nitrogen to meet their own needs. Since they grow on rocks and inside cavities in rocks (Friedmann & Galun, 1974), they must also be able to derive phosphate, essential metal cations and other trace elements from insoluble minerals.

Algae and lichens are believed to break down minerals in two ways. The first involves the adhesive properties of the exocellular proteoglycans, and consists in the tearing away of lamella-like particles from the surfaces of rocks and stones as the slime contracts due to desiccation (Kononova, 1961). The second involves the cation-binding properties of the proteoglycans, and consists of the dissolution of insoluble phosphates, carbonates, silicates and aluminosilicates by sequestration of Mg^{2+}, Ca^{2+}, Al^{3+} and heavy-metal cations (Zunino & Martin, 1977). This action is similar to that of humic acids in the formation of podsols (Kononova, 1961; Schnitzer, 1978). The sheath proteoglycan of *Nostoc calcicola* has a higher affinity for Ca^{2+} than does soluble humic acid from peat (Smidsrød & Painter, 1984; Painter, unpublished).

(3) They must grow very quickly, preferably within a few weeks of the first heavy rain. The photosynthetic efficiency of microscopic algae is much higher than that of seed-bearing plants. In liquid cultures it is easy to obtain 1·5–2·0 doublings per day. The growth rate on soil is harder to measure accurately. The most direct method, based upon radiometric assay of assimilated $^{14}CO_2$, has indicated a peak productivity of 3·6 g (dry weight) m^{-2} day^{-1} (Shimmel & Darley, 1985). The average productivity of *Chlamydomonas mexicana* for a complete growing season was estimated to be 1·5 g m^{-2} day^{-1} (Metting, 1986). On desert soils, application of the same alga at the rate of 0·1–0·5 g m^{-2} resulted in the production of 5–20 g m^{-2} of biomass in 3–4 weeks (Lewin, 1977, p. 31).

(4) The most important soil-conditioning components of the new biomass must persist in the soil long enough to have an effect. The viscosity and gel-forming properties of the pectic component of higher plants are of little use when fungal and bacterial pectinases can eliminate them in a few minutes. In contrast, the proteoglycans of certain blue-green and green algae have been found to persist in soil for many months (Verma & Martin, 1976). In the longer term, a kind of humus is produced (Shields & Durrell, 1964), possibly with the participation of fungal symbionts (Skujins, 1984).

(5) The physical characteristics of the biomass

must be such that the quantity produced has a significant effect. The main cellulosic component of higher plants has a density of *c.* 1·5 g cm^{-3}, and it entraps little water. In contrast, algal biomass is highly hydrated. After growth has proceeded well into the stationary phase, when exocellular proteoglycans comprise *c.* 70% of the total biomass (Barclay & Lewin, 1985; Flaibani *et al.*, 1989), a 0·2% (w/v) suspension of *Nostoc* trichomes or *Chlamydomonas* palmelloids has roughly the consistency of marmalade.

THE CHIHUAHUA DESERT (MEXICO): AN HISTORIC EXPERIMENT

Microscopic algae have already been used to improve desert soils. In the mid-1970s, 500 000 ha of Chihuahua desert were sprayed with a suspension of living cells of a wild strain of *Chlamydomonas mexicana* which Lewin (1957) had found in the same desert. The crops were potatoes and cotton. Compared to the controls, a 5–15% increase in harvest was obtained with a 35–40% saving in water (Lewin, 1977, p. 31; Barclay & Lewin, 1985). There are therefore grounds for optimism that a doubling in harvest could be achieved for the same amount of water consumed.

Despite this encouraging result, the project failed as a business venture because of certain mistakes (Barclay & Lewin, 1985). It is useful to identify some of these:

(1) A wild, native strain of *Chlamydomonas* was used. This choice was probably right at the time, but it emphasizes that the genetic refinement of edaphic algae has only just begun. This is in contrast to all of the world's major food and forage crops, which have been improved by selective breeding for centuries. By mutagenesis and hybridization, Lewin (1977, p. 31) has now isolated many new strains of *Chlamydomonas* that could be tested. The possible advantages of using exotic species should also be considered. It is worth recalling that the potato plant originated in the Andes mountains, and the wheat plant in Mesopotamia (Iraq). Exotic species often do well because they have no natural enemies in their new habitat.

(2) The large area under cultivation included different types of soil, and it would have been appropriate to use different strains or species for each. The pH and salinity are especially important when choosing between green and blue-green species. The microbial floras of the soils should also have been investigated, to check for the presence of natural enemies of the algae. These would include heterotrophic bacteria and fungi capable of breaking down the protective proteoglycan capsules, and soil protozoa (Class Rhizopodea, Order Acrasida) which ingest whole cells of coccoid green algae. Certain nematodes, rotifers and numerous arthropods also feed upon algae. Pest control is as important for algal crops as it is for others, and the selection of resistant strains is likewise a relevant strategy.

(3) *Chlamydomonas* does not fix atmospheric nitrogen, and it was therefore necessary to use inorganic nitrate as a fertilizer. This could have been avoided by using blue-green algae or a mixture of green and blue-green algae.

PROTEOGLYCANS OF BLUE-GREEN ALGAE

The sparse literature on blue-green algal mucilages has been reviewed (Painter, 1983*a*, *b*; Bertocchi *et al.*, 1990). The earliest workers removed firmly bound protein by degradative isolation procedures, such as treatment with cuprammonium hydroxide followed by washing with ~1 M ethanolic hydrogen chloride (Hough *et al.*, 1952), or boiling with 1 M sodium hydroxide for 6 h (Bishop *et al.*, 1954).

Purposeful removal of the polypeptide moiety may be an essential step in investigating the carbohydrate part. Analysis is not then complicated by a Maillard reaction between reducing sugars and amino acids during acid hydrolysis (Gottschalk, 1966; Olsson *et al.*, 1977, 1978). It may also allow the separation by physical methods of differently constituted glycan chains which were originally attached to a common protein core.

It is important, however, to study the effect of the polypeptide moiety upon the properties of the macromolecule as a whole, and its functional significance and biological fate in soil. Preliminary observations indicate that it is implicated in the adhesiveness, sliminess and gel-forming properties of the proteoglycans (Flaibani *et al.*, 1989), and in their affinity for multivalent metal cations (Painter, unpublished).

Although the proteoglycans have an extended lifetime in soil (Verma & Martin, 1976), they are ultimately broken down and their nitrogen released. The polypeptide moiety is therefore also significant as a reservoir of organic nitrogen. Its role may parallel that of humic acids from higher plants in releasing nitrogen slowly in aerated soil (Kononova, 1961).

Table 1 shows the sugar compositions and polypeptide contents of blue-green algal proteoglycans isolated by mild procedures. The figures do not add up to 100% because of the Maillard reaction and other difficulties in securing complete release of monosaccharides. The use of methanolysis instead of acid hydrolysis in aqueous solution seems, however, to minimize losses due to the Maillard reaction.

Table 2 shows the amino acid compositions of the polypeptide moieties of three proteoglycan fractions from *Nostoc calcicola*. To minimize losses due to the Maillard reaction, most of the carbohydrate was first

Table 1. Compositions (%) of exocellular proteoglycans from blue-green algae

Species and extraction conditions	Ara	Rha	Fuc	Xyl	Man	Gal	Glc	GalA	GlcA	Protein
Nostoc calcicola[a]										
Culture medium	1·9	2·1	6·1	11·8	3·9	8·9	13·9	7·6	7·1	7·9
0·1 M EDTA, 20°C	—	4·2	10·7	17·4	5·6	6·4	14·3	9·5	11·2	5·0
Water, 80°C	4·3	3·2	4·2	8·9	6·1	9·4	20·5	4·4	8·1	15·0
Nostoc commune[b]										
0·1 M EDTA, 80°C	2·8	2·0	3·3	23·0	2·9	14·1	4·4	9·3	15·6	16·7
Phormidium foveolarum[b]										
Water, 100°C	2·9	8·0	6·4	4·4	7·0	14·0	28·0	←— 9 —→		13
Cyanospira capsulata[b]										
Water, 50°C	7·2	—	7·8	—	9·3	—	9·3	19·7	—	9·4

[a]Strain 79WA01 isolated by Metting (1981) from an agricultural soil in Washington State (USA), and now used as a biofertilizer in the same area.
[b]Aquatic strain included for comparison.

Table 2. Amino acids (mol %) in some extracellular algal proteoglycans[a]

Amino acid	*Nostoc calcicola*[b]			*Chlamydomonas*		
	CM[c]	EDTA[d]	HW[e]	*mexicana*[f]	*peterfii*[g]	*sajao*[h]
Asp	7·8	11·0	21·2	11·2	9·5	9·3
Thr	4·3	5·7	4·2	8·9	5·2	9·3
Ser	10·3	10·0	4·6	11·9	13·7	11·0
Glu	12·9	11·3	7·6	13·7	11·6	15·7
Gly	12·8	11·5	5·9	8·4	13·4	9·4
Ala	7·1	9·2	6·0	11·5	9·6	11·8
Val	5·8	6·3	6·9	4·2	5·9	7·7
Met	9·9	1·0	0·3	—	—	—
Ile	2·5	3·9	3·3	2·7	2·1	2·6
Leu	4·9	6·7	6·0	7·1	4·7	9·3
Tyr	—	2·3	1·1	2·0	—	—
Phe	4·1	4·0	3·9	5·8	3·5	4·1
Orn	3·6	2·8	0·3	2·3	6·2	0·6
Lys	1·8	2·6	2·8	1·9	2·5	0·9
His	0·9	1·3	—	—	1·9	0·9
Arg	6·9	4·6	12·6	1·0	5·6	2·0
NH_3	4·2	5·8	12·4	7·3	4·5	5·5

[a]Not corrected for selective losses occurring during acid hydrolysis; for experimental details, see Flaibani *et al.*, 1989.
[b]Strain 79WA01, isolated by Metting (1981).
[c]Soluble in the culture medium.
[d]Extracted with 0·1 M EDTA at 20°C.
[e]Extracted with hot (80°C) water.
[f]Wild type isolated by Lewin (1957) from desert soil in Chihuahua (Madera), Mexico.
[g]Wild type isolated by Lewin from sandy soil from the Anza-Borrego Desert, California.
[h]Wild type found by Lewin (1984) in duckweed near Guangzhou (Canton), China.

removed by mild acid hydrolysis, before the polypeptide was hydrolysed with 6 N hydrochloric acid.

None of the fractions contained hydroxyproline (Hyp) or hydroxylysine, two of the amino acids that are known to be implicated in carbohydrate–peptide bonding (Lindahl & Rodén, 1972; Sharon, 1975). *O*-Glycosidic linkages to Ser and/or Thr are possible, but they are very labile to alkali, and therefore unlikely in the exocellular proteoglycans of an alga that will grow at pH 10. Consistent with this, it was found that mild alkaline treatments had no effect on the proteoglycans, but boiling for several hours with 1 M sodium hydroxide cleaved them into almost pure glycan and polypeptide fragments. Sodium borohydride (5% (w/v))

was included in the reaction mixtures to prevent alkaline peeling of the liberated glycan chains. These conditions are typical of those required to cleave *N*-glycosidic linkages to the amido group of Asn (Neuberger *et al.*, 1972; Lindahl & Rodén, 1972; Sharon, 1975).

A structural study of a hot-water extract of an aquatic strain of *Phormidium foveolarum* has been reported (Matulewicz *et al.*, 1984). The proteoglycan had the composition shown in Table 1. Gel-permeation and anion-exchange chromatography showed a very broad composition distribution, but no discrete components. Periodate oxidation, partial acid hydrolysis, and methylation analysis revealed a highly ramified structure in which all of the neutral sugar residues were present both as end-groups and as intrachain units. 3-Linked glucose and 4-linked galactose residues were the most prominent features.

The impression of extreme complexity and irregularity in the structures of blue-green algal proteoglycans is, however, relieved by that of *Cyanospira capsulata*. A purified fraction of this material (Navarini *et al.*, 1990) had the composition shown in Table 1, which corresponds closely to the molar ratios 1:1:1:1:2 for Ara, Fuc, Man, Glc and GalA respectively. The macromolecule had an intrinsic viscosity (extrapolated to zero rate of shear) of 20 dl g^{-1}, and displayed strong pseudoplasticity. A partial structure, based upon the assumption of a hexasaccharide repeating unit, has been proposed (Cesàro *et al.*, 1990).

All the known methods of mutagenesis have been successfully applied to blue-green algae, and nitrosamines appear to be particularly effective (Herdman, 1982; Craig *et al.*, 1988). Among the many viable mutants that have been isolated may be some that produce 'core' proteoglycans of simpler structure, which could provide an insight into the structures of the more intractable, wild-type proteoglycans.

CAPSULAR PROTEOGLYCANS OF *CHLAMYDOMONAS* SPECIES

This genus comprises more than 400 different species, and is the only group of unicellular green algae that has so far been used to condition soil on a commercial scale. The vegetative cells are biflagellated and phototactic, and their reproductive cycle passes successively through haploid (male and female) and diploid stages. The cell walls contain a glycoprotein ('volvin') similar to extensin, in which oligosaccharides containing Ara_f, Gal_f, Gal_p, Glc_p, Man_p and Xyl_p residues are linked *O*-glycosidically to Hyp residues in the polypeptide moiety; the flagella contain a similar glycoprotein which appears to be involved in sexual recognition (Roberts *et al.*, 1985).

Apparently in response to environmental stress, which may consist of nutrient depletion or possibly any decrease in water potential, the vegetative cells lose their flagella and surround themselves with a gelatinous capsule, occupying many times their own volume. These cells are said to be in the 'palmelloid' state. This is not identical with a 'resting' state, because the cells can continue to grow slowly in this condition, when supplied with sufficient nutrients (Olsen *et al.*, 1983). The slimy, capsular material is usually considered to be the main contributor to the soil-conditioning (water-retaining and particle-aggregating) effect, but it should be emphasized that vegetative cells also liberate large amounts of water-soluble polysaccharides or proteoglycans into the medium during the logarithmic phase of growth (Lewin, 1956).

The essentially polysaccharidic nature of the soluble exopolymer and the capsular material was first recognized by Lewin (1956). The materials would be more correctly described as proteoglycans, however, because firmly bound polypeptide is always present. In fractions that are soluble in water as their sodium salts, the proportion of this is 3–12%, but it is higher in other fractions that disperse in water as microgel particles (Flaibani *et al.*, 1989). Increasing proportions of polypeptide are associated not only with a decrease in solubility but also with an increase in sliminess (viscoelasticity) and adhesiveness. This correlation appears to be simple and direct, because variations in the sugar compositions of the different fractions are comparatively minor.

Table 2 shows the amino acid compositions of water-soluble proteoglycan fractions from three different *Chlamydomonas* species. Hydroxyproline is absent, showing that there is no relationship to the cell-wall and flagellar glycoprotein, volvin. Preliminary observations on the sensitivity of the carbohydrate–peptide linkages to alkali indicate that some of the glycan chains are linked *O*-glycosidically to Ser and/or Thr, while others are linked *N*-glycosidically to Asn (Painter, unpublished).

Table 3 shows the sugar compositions of fractions that were soluble in water after conversion into their sodium salts by dialysis against aqueous EDTA. Within the limits of experimental error, Fuc, Xyl, Gal, Glc and GlcA show a marked tendency to exist in stoichiometric proportions, whereas Ara, Man and GalA are harder to accommodate as components of regular, oligosaccharidic repeating units. The comparative simplicity of the chromatographically purified fractions from the mutants, *C. peterfii* strain C2 and *C. sajao* strain M18, is especially noteworthy. Further work on the characterization of these materials is in progress.

CONCLUSIONS

Specialists in all aspects of the chemistry and biochemistry of polysaccharides and proteoglycans can

help to solve the problem of the world's expanding deserts. This would also address the problems posed by the 'greenhouse effect', because desert reclamation implies the conversion of atmospheric carbon dioxide into soil organic matter and standing vegetation.

Research is needed on all of the physical properties pertaining to the immobilization of water by microalgal proteoglycans, including rheology, gel formation, and the kinetics and thermodynamics of their dehydration and rehydration. The affinity of the proteoglycans for multivalent metal cations is especially relevant to the release of soluble nutrients from minerals, and special studies are also required of their adhesiveness in relation to the aggregation of soil particles and the erosion of minerals.

The mean lifetime of the proteoglycans in soil is of critical significance, and hence their biodegradation by bacterial, fungal, viral and other enzymes secreted by soil microorganisms must be thoroughly investigated. Because of the intense solar radiation, the possibility of photochemically induced depolymerization must also be considered. In this connection, it should be noted that the sheath materials of some blue-green algae contain pigments that are presumed to protect the cells from ultraviolet light (Whitton & Potts, 1982). It has been found that a proteoglycan fraction from *Nostoc calcicola* contained a firmly bound chromophore which absorbed intensely at 258 nm (Flaibani *et al.*, 1989).

On the biological side, there is an urgent need for research on the physiological, metabolic and environmental regulation of exocellular proteoglycan production. It is a popular generalization that this is activated simply by nitrogen depletion. This notion seems to be based upon the misconception that the exopolymers are pure polysaccharides that do not contain nitrogen. If it were true, it would be impossible to grow crops at the same time as the algae are synthesizing proteoglycan, and 'algalization', in the form in which it is practised in much of south-eastern Asia, would be of little value. Fortunately, it is disproved by definitive studies on a few individual species (Mehta & Vaidya, 1978; Kroen, 1984; Kroen & Rayburn, 1984; Sili *et al.*, 1985; Flaibani *et al.*, 1989), but a systematic approach is lacking.

Green algae are seldom parasitic and never pathogenic in animals, and toxin production by some blue-green species may be the only feature requiring special caution in experiments with recombinant DNA technology. DNA transformants have been successfully isolated from blue-green algae, including the nitrogen-fixing genera *Anabaena* and *Nostoc*, and also from coccoid green algae, including *Chlamydomonas reinhardtii* (Craig *et al.*, 1988). A wide variety of genetic markers has been used in these experiments, but these have not so far included any structural feature or property of the exocellular proteoglycans. This is evidently because too little is known about the structure and biosynthesis of these materials, and hence research is urgently needed on these aspects also.

Finally, it should be noted that genetic transformation is not the only way of getting genes from different organisms to cooperate: symbiosis does this too. Algae are remarkably adept at forming symbiotic relationships with other organisms. The lichens are well-known examples, but there are many others. For example, the gelatinous sheaths of filamentous, blue-green algae are so readily colonized by heterotrophic bacteria that it is hard to prepare axenic (pure) cultures

Table 3. Compositions of capsular proteoglycans from *Chlamydomonas* species[a]

Species	Strain	Water-soluble material[b] (%)	Molar ratios								Protein[c] (%)
			Ara	Fuc	Xyl	Man	Gal	Glc	GalA	GlcA	
C. mexicana											
Male (−) gametes	WT[d]	40[f]	0·2	1·9	—	0·2	0·1	1·0	0·5	0·6	8·1
Female (+) gametes	WT[d]	60[f]	0·2	2·0	—	0·1	0·1	1·0	0·5	0·5	6·9
C. peterfii	WT[d]	100	0·6	1·1	0·4	0·5	1·1	1·0	0·1	0·3	10·0
	C2[e]	61[f]	0·1	—	—	—	2·0	—	—	1·0	7·5
C. humicola	WT[d]	52[f]	0·2	—	3·0	—	0·1	1·0	—	0·7	5·6
	WT[d]	22[f]	2·7	—	1·8	—	1·1	1·0	—	1·1	6·9
C. gymnogama	WT[d]	100	0·5	—	—	—	2·7	—	—	1·0	11·9
C. sajao	WT[d]	100	0·4	—	—	—	2·6	0·1	—	1·0	9·4
	M18[e]	100	0·5	—	—	—	2·3	0·1	—	1·0	7·5
	M18[e]	57[f]	0·1	—	—	—	2·1	0·1	—	1·0	10·6
	M24[e]	100	0·5	—	—	—	2·7	0·2	—	1·0	10·6

[a]Data from Flaibani *et al.*, 1989 and Flaibani & Painter, unpublished.
[b]Percentage of the total amount of water-soluble capsular material (Na^+ salt).
[c]N (%) × 6·25.
[d]Wild type.
[e]Mutant isolated in Lewin's laboratory.
[f]Material eluted as a single peak by chromatography on DEAE-Sepharose CL-6B.

of the former, and almost impossible to keep them axenic under conditions of large-scale cultivation. This is a problem for the researcher, but it implies that the properties of the exocellular polysaccharides of heterotrophic bacteria are also relevant in desert reclamation.

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