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Spirulina, the Edible Microorganism

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INTRODUCTION

In 1940 the French phycologist Dangeard described in a communication to the Linnean Society of Bordeaux a sample received from Mr. Créach, pharmacist with the French Colonial troops stationed at Fort Lamy, at that time in French Equatorial Africa and now in the Republic of Chad (31). The sample was obtained from the market of Massakory, a small village located approximately 50 km east of Lake Chad. Dangeard reported that the material called Dihé (or dié) in the local language (Kanenbou), was eaten by the native population, and was obtained as follows: mats of microscopic algae, floating on the surface of small lakes or ponds around Lake Chad, were collected and sun dried on the sandy shores (Fig. 1). The hardened cakes were broken into small pieces and, without any further treatment, represented Dihé, object of some commerce in the local markets. According to Mr. Créach, Dihé was used to make sauces accompanying the standard millet meal. On studying the samples of Dihé, Dangeard concluded that it was "a true puree of a filamentous, spiral-shaped blue alga." The alga was *Arthrospira* (= *Spirulina*) *platensis*. A colleague of Dangeard, the abbot Frémy, informed him that the organism had already been identified by Rich as the main constituent of the phytoplankton in a number of lakes in the Rift Valley of East Africa (128). Rich had also reported that the organism represented the main food source for the population of lesser flamingoes (*Phoenicoptera*) inhabiting those lakes. Because of the war and, possibly, the limited circulation of the journal in which the communication was published, Dangeard's report went unnoticed. Almost 25 years later, J. Léonard (a botanist participating in the Belgian Trans-Saharan expedition), while looking for plant products in the native markets in and around Fort Lamy, was struck by a "curious substance green bluish, sold as dried biscuits" (91). Léonard had rediscovered Dihé and confirmed that it was composed almost exclusively of dried mats of *S. platensis* collected from the waters of the alkaline lakes in the subdesert Kanem area, northeast of Lake Chad. Léonard and his colleague Compére confirmed the report by Dangeard that Dihé was consumed by the local populations and performed a first group of chemical analyses that revealed a very high protein content, close to 50% of the dry weight. Although impressive, this figure was an underestimate since independent studies, carried out by the Institut Françaíse du Pétrole on laboratory-grown *S. platensis*, gave protein contents that ranged from 62 to 68% of the dry weight (28).

At the same time, the French group had begun to investigate also another species of *Spirulina*, *S. maxima* (= *S. geitleri*), that was growing abundantly in Lake Texcoco, near Mexico City (27, 40). Although there was no indication that *S. maxima* was used in Mexico as a food, a search of the historical literature revealed that, at the times of the Spanish conquest, *S. maxima* was harvested from the lake, dried, and sold for human consumption. The very attentive Spanish invaders duly recorded all animals, plants, and foods that they encountered in the newly conquered territories. (Indeed, for instance, Columbus himself noted that, during his first visit to Cuba in 1492, he saw a "type of grain like millet" that the natives called maize.) Bernal Diaz del Castillo, a member of Cortez' troops, described among the many astonishing items that he saw in the market of Tenochtitlan (today's Mexico City) " . . . small cakes made

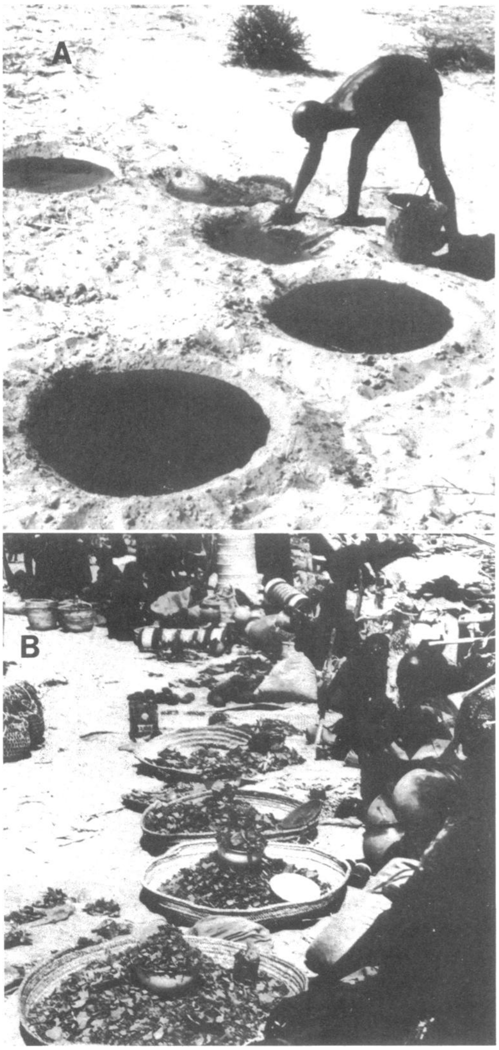

FIG. 1. Preparation and sale of Dihè. (A) Sun-drying of *S. platensis* mats on the shores of Lake Rombou (Republic of Chad), ca. 1967. (*Photo by A. Iltis.*) (B) Dihè on sale in the village market of Massakory (Republic of Chad), ca. 1967. (*Photo by A. Iltis.*)

from a sort of a ooze which they get out of the great lake, and from which they make a bread having a flavour something like cheese" (36). A few years later, a Franciscan friar, Bernardino da Sahagùn, described how fishermen " . . . with very fine nets in certain periods of the year collect a soft thing that is created on the waters of the lagoons of Mexico, and which curdles, and it is not grass nor earth, rather like hay . . . of clear blue color, from which they make bread, that they eat cooked" (34) (Fig. 2). The natives called it Tecuitlatl, literally "stone's excrement" since, as Farrar has pointed out (42), "breeding" of minerals was still a common belief in the 16th century. Tecuitlatl was mentioned by historians of the conquest or visiting naturalists up to the end of the 16th century. After that period, Tecuitlatl is not mentioned any longer, probably because the practice of making Tecuitlatl disappeared soon after the conquest. It is possible that the local population, decimated also by repeated outbreaks of contagious diseases, could satisfy its alimentary needs with more conventional foods. Further, it is possible that due to the profound social, political, and religious changes caused by the Spanish conquest many traditions were quickly lost (42).

Thus, over the ages, two populations, approximately 10,000 km apart, discovered independently and exploited the nutritional properties of *Spirulina*. Except perhaps for the Far East, this is the only record of traditional use of a microbial biomass as a food for human consumption. The aim of this review was to collate the information available in the case of the two species that appear to have been utilized as a food source, *S. platensis* and *S. maxima*. It is hoped that this information may encourage further investigation on the different aspects of the life and the possible exploitation of these organisms. This work may, in turn, be of some help in alleviating one of the most pressing problems now facing mankind.

OCCURRENCE AND ISOLATION

Spirulina is a ubiquitous organism. After the first isolation by Turpin in 1827 from a freshwater stream (159), species of *Spirulina* have been found in a variety of environments: soil, sand, marshes, brackish water, seawater, and freshwater. Species of *Spirulina* have been isolated, for instance, from tropical waters to the North Sea (68), thermal springs (6), salt pans (52), warm waters from power plants (49), fish ponds (122), etc. Thus, the organism appears to be capable of adaptation to very different habitats and colonizes certain environments in which life for other microorganisms is, if not impossible, very difficult. Typical is the population by alkalophylic *S. platensis* of certain alkaline lakes in Africa and by *S. maxima* of Lake Texcoco in Mexico. In some of these lakes *Spirulina* grows as a quasi-monoculture. In the case of the African lakes in the Chad region, Iltis has conducted an extensive survey of the phytoplankton of the alkaline lakes, permanent or temporary (75). These bodies of water have been classified into three groups according to their salt content, mostly carbonates and bicarbonates. The lakes with a salt concentration of <2.5 g/liter presented a very varied microbial population composed of *Chlorophyceae*, cyanobacteria, and diatoms. In the mesohaline lakes, characterized by salt concentrations ranging from 2.5 to 30 g/liter, the cyanobacterial population became predominant, although many species were present (*Synechocystis*, *Oscillatoria*, *Spirulina*, *Anabaenopsis*) (74). In the lakes containing salt concentrations >30 g/liter, the cyanobacterial population became practically monospecific and *Spirulina* was the only organism present in significant quantities (69, 70). Indeed, *S. platensis* was found in waters containing from 85 to 270 g of salt per liter, but growth seemed to be optimal at salt concentrations ranging from 20 to 70 g/liter, and it is possible that the population of *S. platensis* found at the highest salt concentrations, such as in temporary ponds just before drying, was that of the cyanobacterial biomass established when the concentration of salts was much lower (71). A detailed investigation of two lakes, Rombou and Bodou, both characterized by a very alkaline pH (10 to 10.3 for Rombou and 10.2 to 10.4 for Bodou) but different salt concentrations (13 to 26 g/liter for the former and 32 to 55 g/liter for the latter), seemed to confirm that salt concentration plays a direct role in the growth of *S. platensis*. In Lake Rombou, cyanobacteria represent <50% of the phytoplankton population, whereas in Lake Bodou they account for at least 80% of the total population. In addition, in the former, *S. platensis* represented major but not the only phytoplankton component with extensive quantitative seasonal variations, whereas in the latter *S. platensis* was practically the only cyanobacterium present. Indeed, with the exception of the months of November and December, when *S. platensis* accounted for 80% of the plankton, in all other months this species represented the totality of the biomass in Lake Bodou. The correlation existing between salt concentration and abundance of *Spirulina* was confirmed in a later study on a group of lakes characterized by a lower salt concentration (5 to 14 g/liter) (74). In the lakes of this group, characterized by the highest salt concentration, a variety of *Spirulina*, *S. platensis* var. *minor*, was practically the only cyanobacterium present, whereas in those with a lower salt concentration *Spirulina* was present but represented only a fraction of the microbial population. In addition, in the latter lakes wide fluctuations were observed in the relative abundance of *S. platensis* var. *minor* that accounted for, according to the season, 70 to 2% of the total biomass.

An analogous situation appears to exist in the alkaline lakes of the Rift Valley in East Africa. These lakes too are characterized by very high pH, reaching, in certain cases, values close to pH 11, and very high salt concentrations, particularly sodium carbonate originating from the sedimentary volcanic deposits. In some of these lakes, such as Nakuru, Elmenteita, and the Crater Lake, in which the pH ranges from 9.4 to 11, *S. platensis* and *S. platensis* var. *minor* are the predominant microorganisms present (79, 157). ("Two or three other members of *Myxophyceae* and a few Diatoms occur in the three lakes under consideration, but they do not constitute a conspicuous feature of the plankton" [128].) In a more recent survey of the photosynthetic rates in some of the lakes of this area, it was confirmed that in Lakes Nakuru (pH 10.5), Elmenteita (pH 9.4), Reshitani (pH 10.1), and Big Momela (pH 10.4), *S. platensis*, its varieties, and *S. laxissima* represent the most abundant, if not the only, constituents of the phytoplankton. In only one lake, Lake Nakuru, was another cyanobacterium, *Chroococcus minutus*, found

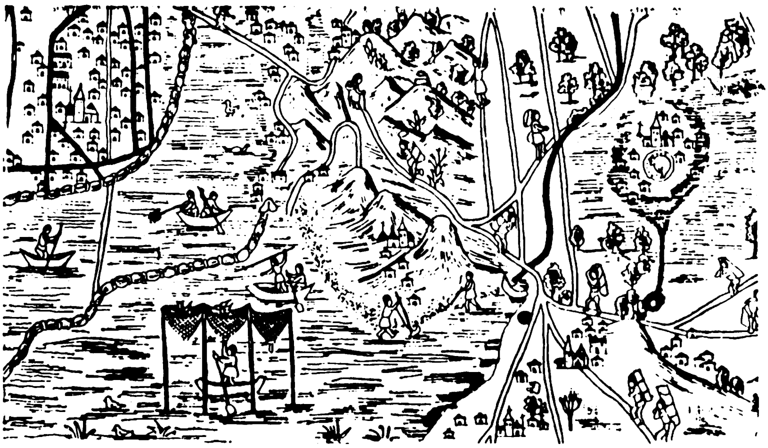

FIG. 2. Collection of *S. maxima* in Mexico. Anonymous Spanish map of the 16th century depicting the harvesting of *S. maxima* and, possibly, other algae from Lake Texcoco (reprinted, with permission from *Enciclopedia de México* [4]).

in considerable amounts. Yet, on a per-cell basis, even in this lake *S. platensis* was 5- to 10-fold more abundant than *C. minutus* (102). In another lake of the same area, Lake Simbi, *S. platensis* was the only organism that could be detected, reaching, in the first meters from the surface, a concentration of 200,000 cells per ml (each coil being composed of ca. 10 cells), which was responsible for the exceptionally high rates of photosynthesis (up to 13 g of O_2 produced/m^3 per h) (101). Similarly, two crater lakes in Ethiopia, Lakes Kilotes and Aranguadi, both characterized by a high salt content and an alkaline pH, support a dense population of *Spirulina* (150). In Lake Kilotes (pH 9.6), *S. platensis* is the predominant organism, although it is accompanied by an unidentified species of *Chroococcus*. In Lake Aranguadi, characterized by a more alkaline pH (10.3), *S. platensis* is the only microorganism present and its abundance is such that waters appear deep green (in Abyssinian, aranguadi means green). The high concentration of *S. platensis* was responsible for the extremely high photosynthetic rates (1.2 to 2.4 g of O_2 produced/m^2 per h).

It must be stressed that in many of these lakes dramatic changes in the population of *Spirulina* may result from fluctuations in the alkalinity and the salt concentration of the water. For instance, in the case of Lake Nakuru, the alkalinity (expressed as milliequivalents of HCO_3^- + CO_2^{2-} per liter) was 296 in 1929, 205 in 1931, 1,440 in 1961, and 122 in 1969 (102). A more recent investigation on the lakes studied by Rich (128) and Jenkin (79) has shown that the density of *Spirulina* not only undergoes seasonal variations but also may be reduced from the predominant, or sole, component of the phytoplankton to a minor component of the biomass. Thus, the contribution of *Spirulina* to the primary production may become almost negligible whereas that of other species (e.g., benthic diatoms) becomes predominant (157). These changes, at times fully reversible, may be of considerable importance for the animal communities associated with these lakes (see below). Of course the lakes whose physicochemical conditions do not vary may maintain a stable and abundant population of *Spirulina* as must be the case for some of the lakes in the Chad area and Lake Texcoco in Mexico. For these lakes it must be assumed that *Spirulina* has been the primary component of the biomass at least for centuries. In conclusion, some species of *Spirulina*, notably *S. platensis* and *S. maxima*, are capable of growing in waters whose chemical composition makes life for other microorganisms very difficult if not impossible. In these environments, *Spirulina* grows as a quasi-monoculture (Table 1). This does not mean, of course, that other microorganisms do not grow at all, as demonstrated, for instance, by the finding that all species of *Spirulina* so far isolated even from the most alkaline lakes are always contaminated by bacteria. The bacterial flora associated with the cultures of *Spirulina* is varied but with a preponderance of gram-negative rods (105). It is not known whether any mutualistic relations exist between these bacteria and the cyanobacterium, although in the laboratory axenic cultures of *S. platensis* grow as well, and perhaps even better, than nonaxenic ones (2, 105). In addition, although no comparison of the requirements (medium composition including trace elements, alkalinity, light, etc.) of axenic cultures and nonaxenic ones has been performed, all data so far available indicate that axenic cultures grow at least as well as nonaxenic cultures in the media and under the conditions developed for the latter isolates (2, 105, 106, 126, 160). The bacteria associated with the cyanobacterial trichomes may be easily isolated by plating trichomes on standard bacteriological media in which the pH has been adjusted to 9 to 9.2. If media with pH close to neutrality are used, very few bacterial colonies are found. It is possible to distinguish two main groups of bacterial contaminants, that of the organisms present mostly in the culture medium and loosely adhering to the trichomes and those, called epiphytic contaminants, bound or tightly adhering to the thin sheath enclosing the trichomes. Washing the trichomes with aqueous solutions removes the first group of bacteria without significantly affecting the second one. Fragments of trichomes containing no bacteria and hence amenable to the establishment of axenic cultures may be obtained by extensively washing the trichomes with sterile solutions followed by mechanical fragmentation to give single cells or short filaments containing two to four cells each. The fragments are then irradiated with UV light and, after further washings, used to inoculate test tubes of minimal medium in such a way as to give a concentration of ca. one cell per tube. After incubation in the light, a few tubes (3 to 5%) contain viable cultures of *S. platensis* that appear, even after repeated subculture, to be devoid of detectable bacteria (105, 127). Recently, a strain of *S. platensis* has been reported to have been rendered axenic simply by repeated streakings on agar plates (21). However, the possible presence of bacteria was assessed on standard microbiological media, that is, presumably on media whose pH is not suitable for growth of the bacteria associated with the trichomes. The availability of axenic cultures allowed it to be established that, although *S. platensis* cannot grow under heterotrophic conditions, mixotrophic cultures are possible, consistently giving yields higher than those of cul-

TABLE 1. *Spirulina* in lakes of Africa[a]

Lake	Country	Yr of observation	Area (km^2)	Maximum depth (m)	pH	Conductivity (μmhoms/cm) at 20°C	Alkalinity HCO$_3^-$ + CO$_2$ (meq/liter)	Species of *Spirulina* present	Relative abundance	Other phytoplankton components (in decreasing order of abundance)	Reference
Nakuru	Kenya	1971	34–42	3.3	10.5	10,010	122	*S. laxissima*, *S. platensis*	19,500,[b] 13,350[b]	*Chroococcus minutus*, *Anabaenopsis arnoldii*	102
		1979	49	4.5	10.5–11	14,000–26,000	5,000–90,000 mg/liter	*S. platensis* var. *minor*, *Spirulina* sp.	Predominant species (up to 98% of the biomass) in certain years. When *Spirulina* spp. population decreases, increase in unicellular cyanobacteria	*Synecocystis* spp., *Chroococcus minutus*, *Monoraphidium minutum*, diatoms	167
Elmenteita	Kenya	1971	18	1.9	9.4	11,700–25,000	107	*S. laxissima*	69,900[b]	*Chroococcus minutus*, *Chroococcus* sp., *Anabaenopsis arnoldii*	102
Bogoria	Kenya	1974–1976	33	8.5	9.8–10.3		480–800	*S. platensis*, *S. laxissima*	Extensive seasonal or yearly variations but always the predominant species	Diatoms	157
Simbi	Kenya	1973–1976	0.3–0.4	29	10.1–10.5	18,200	260–292	*S. platensis*	7,000[b]		102
Reshitani	Tanzania	1969	0.2	29	10.1	13,500–16,900[c]	164–233[c]	*S. laxissima*, *S. platensis*	35,300, 3,800[b]		101
Big Momella	Tanzania	1969	0.9	31	10.4	15,000–17,600[c]	168–239[c]	*S. platensis*	9,200[b]		102

TABLE 1—*Continued*

Lake	Country	Yr of observation	Area (km^2)	Maximum depth (m)	pH	Conductivity (μmhoms/cm) at 20°C	Alkalinity HCO_3^- + CO_2 (meq/liter)	Species of *Spirulina* present	Relative abundance	Other phytoplankton components (in decreasing order of abundance)	Reference
Tulusia	Tanzania	1974, 1976	0.5		10.1–10.4		189–287	*S. platensis*, *S. laxissima*	Extensive seasonal or yearly variations but always the predominant species	Diatoms	157
Dariba	Sudan	1965	3.7	11.6	9.9			*S. platensis*, *S. platensis* var. *minor*^{d}	Only species present		48
Aranguadi	Ethiopia	1964–1966	0.8	25	10.3		51–67	*S. platensis*	Only species present		150
Kilotes	Ethiopia	1964–1966	0.8	6.4	9.6		51–67	*Spirulina* sp.	Main component but showing quantitative seasonal variations	*Chroococcus* sp.	150
Bodou	Chad	1967–1968	0.75	1.5	10.2–10.4	34,400–47,800^c		*S. platensis*	From a minimum of 82% up to 100% of the biomass		70
Rombou	Chad	1966–1968	0.12	1	10–10.3	17,200–30,300^c		*S. platensis*	Predominant species (up to 87% of the biomass) only in certain months	*Cryptomonas* sp. *Oocystis* sp.	70
Djikare	Chad	1967–1968		2.5	9.7–10.2	13,000–16,000^c	146–217	*S. platensis* var. *minor*, *S. laxissima*	Predominant species (up to 93% of the biomass) all year	*Anabaenopsis arnoldii*	74

Mombolo	Chad	1967–1968		2	9.7–10.2	4,100–7,200[c]	45–67	*S. platensis* var. *minor*, *S. laxissima*	Predominant species only in certain months (2 to 70% of the biomass)	*Anabaenopsis arnoldii*, *Synecocystis* spp., *Chroococcidiopsis* sp.	74
Macu-Leyla	Chad	1967–1968	—[f]	—[f]	10–10.8	4,260–88,250[c]		*S. platensis*	Predominant species (up to 97% of the biomass) only in certain months	*Cryptomonas* sp., diatoms, *Volvocales*	71

[a] The values refer to the year(s) of observation. Due to the reduced size and depth, extensive variations in the volume and surface as well as in the physicochemical parameters have been recorded. Even for the lake with the greatest extension, Lake Nakuru, enormous fluctuations in size have been observed, including periods of complete dryness (e.g., 1944 to 1946 and 1961) (167). Similarly, Lake Huacachina in Peru was found in 1954 to support a very abundant population of *S. platensis* (153) but, in the following years, due to a decrease in the groundwater level, it underwent changes in physicochemical conditions that resulted in a steady decline of the *S. platensis* population, which in 1973 represented only a minor component of the phytoplankton (58). Since 1977 the lake has dried up (P. Heussler, personal communication).

[b] Filaments of three coils per milliliter.

[c] First value at water surface; second value at a depth of 10 m.

[d] Classified as *S. geitleri* var. *geitleri* and *S. geitleri* var. *minor* (48).

[e] Values determined at 23°C.

[f] —, Temporary lake, in general dry from November to June.

tures grown under photoautotrophic conditions (see below).

Due to the alkalinity of the growth medium, the microbial load of *S. platensis* cultures has been reported to be one order of magnitude lower than that of eucaryotic algae, such as *Scenedesmus acutus*, which grow in acid media (10). However, a quantitative study of the bacterial flora associated with open-pond cultures of *S. platensis* and *S. maxima* has shown that the contaminating bacteria may account for ca. 1% of the total biomass (100). If the trichomes are washed repeatedly with sterile physiological solution, the bacterial contribution to the total biomass becomes negligible ($<10^3$ bacterial cells per trichome) (127). Thus, harvesting of cultures by filtration or centrifugation followed by washing may result in biomasses that contain insignificant amounts of bacterial contaminants. Microbiological investigations of samples of Dihé obtained from local markets in the Chad area have indicated the presence of aerobic and anaerobic bacteria, and fecal streptococci have been isolated from *S. maxima* harvested from Lake Texcoco (76). As expected, the number of all viable bacteria, including those that may represent a danger to human health, decreases in the dried samples even in those processed by sun drying (10, 76).

MORPHOLOGY AND TAXONOMY

Spirulina is a multicellular, filamentous cyanobacterium. Under the microscope, *Spirulina* appears as blue-green filaments composed of cylindrical cells arranged in unbranched, helicoidal trichomes (Fig. 3). The filaments are motile, gliding along their axis. Heterocysts are absent.

The helical shape of the trichome is characteristic of the genus but the helical parameters (i.e., pitch length and helix dimensions) vary with the species, and even within the same species, differences have been observed in these parameters (98, 128) or may be induced by changing the environmental conditions such as growth temperature (162). The helical shape is maintained only in liquid media, and in solid media the filaments become true spirals (164). The transition from a helix to a flat spiral is a slow process depending on the water content of the agar surface, whereas the reverse occurs almost instantly when, for instance, a drop of water is deposited on the agar surface in contact with a spiral. The transition from helix to spiral is probably related to the necessity of reducing, in solid media, the surface area exposed to air. It is possible that these transitions are caused by hydration or dehydration of the oligopeptides in the peptidoglycan layer, resulting in changes in the rigidity of the cells (165).

The diameter of the cells ranges from 1 to 3 μm in the smaller species and from 3 to 12 μm in the larger ones. Comparison of an authentic isolate of *S. platensis* from Chad and of *S. maxima* from Mexico, grown in the laboratory under identical conditions, showed that *S. maxima* is characterized by a diameter of the helix of 50 to 60 μm and pitch of 80 μm; values >35 to 50 μm and 60 μm, respectively, were observed for *S. platensis*. On the other hand, cell dimensions were greater in *S. platensis* than in *S. maxima* (diameter, 6 to 8 μm in the former and 4 to 6 μm in the latter) (97). The cytoplasm of the smaller species appears homogeneous, with no gas vacuoles or inclusions and scarcely visible septa. On the contrary, the larger species such as *S. platensis* and *S. maxima* have a granular cytoplasm containing gas vacuoles and easily visible septa. As will be discussed later, the presence or absence of the septa has been one of the distinguishing characters used in the classification and generic assignment of these organisms. Trichomes are, in general, a few millimeters long, although under certain conditions trichomes of *S. platensis* as long as 20 mm have been observed (162).

Among the macromorphological variations occurring in *Spirulina*, the appearance of isolates with straight trichomes has been reported to occur spontaneously or after mutagenesis. This has induced Bourrelly (16) to consider *Spirulina* an *Oscillatoria*, deserving, at most, the status of a subgenus within the family *Oscillatoriaceae*. However, as discussed below, this opinion is not widely accepted.

Electron microscopy of ultrathin sections of *S. platensis* revealed that the cell wall is composed of possibly four layers (98, 160). The most external or outer membrane layer (L-IV) is composed of material arranged linearly in parallel with the trichome axis and is considered analogous to that present in the cell wall of gram-negative bacteria. Layer III is possibly composed of protein fibrils wound helically around the trichomes, whereas the peptidoglycan-containing layer (L-II) folds towards the inside of the filament, giving rise, together with a putative fibrillar inner L-I, to the septum separating the cells. However, if layers I and III were artifacts arising during the preparation of the samples for electron microscopy (38), the septum separating the cells would be composed of the peptidoglycan layer only. The septum appears as a thin disk, folded in part. This fold covers a portion of the septum surface, and its extent seems to be related to the pitch of the trichome; the larger the pitch, the smaller the folded area and vice versa. Indeed, whereas in *S. platensis* the fold covers ca. 5% of the total septum area, in *S. laxissima*, characterized by a much larger pitch, the fold covers ca. 3% of the septum area. Stretching of the filament results in the disappearance of the septal fold that, indeed, is apparently absent in nonhelical cyanobacteria (165).

The most prominent cytoplasmic structure is the system of thylakoids originating from the plasmalemma (57, 64, 97, 162) but quite distinct from the well-evident mesosomes (3, 154). At times, the thylakoids appear to be arranged in concentric whorls especially evident in adult cells. During cell division, the invagination of the plasmalemma, from the outer portion towards the cell center, is accompanied by breakage of the thylakoids that thus become distributed between the two daughter cells. Phycobilisomes, high-molecular-weight aggregates of phycocyanins, appear to be attached to the thylakoids (162), as expected on the basis of their function as light-harvesting antennae.

Cyanophycin granules, a reserve material composed in other cyanobacteria of copolymers of amino acids in general chains of poly-L-aspartic acid with arginine attached to the β-carboxyl groups, are present in *S. platensis*, their relative amount varying with the medium composition, cell age (163), and growth temperature (162). Polyglucan granules, cylindrical bodies, carboxysomes, and mesosomes have also been detected (3, 98, 154, 162, 163). Van Eykelenburg described these organelles in *S. platensis* and evaluated their occurrence in cultures grown at different temperatures and light conditions (162) and in media containing different concentrations of nitrate (163). At low temperatures, when the demand for amino acids is limited by the reduced growth rate, cyanophycin granules are the most abundant organelles, occupying up to 18% of the cell volume. On increasing the growth temperature, the content in these granules progressively decreases, and at 25 to 30°C they are practically undetectable. Polyglucan granules were most prominent at low temperatures (15 to 17°C), decreasing in concentration at higher temperatures. The relative concentration of the other cell organelles was not influenced significantly by light or temperature.

Carboxysomes, polyhedral bodies containing ribulose-1,5-bisphosphate carboxylase, were present only when *S. platensis* was grown at high light intensities and in media containing a high nitrate concentration. At low light intensities or at low nitrate concentrations, carboxysomes disappeared, thus supporting the view that these organelles may represent some sort of storage bodies for ribulose-1,5-bisphosphate carboxylase and, possibly, other proteins. Gas vesicles, in the shape of hollow cylinders with cone-shaped ends, with a diameter of ca. 65 nm and a length of up to 1 μm were easily detected. The vesicle membrane consists of coils of protein molecules, possibly all of the same type, arranged in ribs spaced 4 to 5 nm apart. The organelles are responsible for cell buoyancy and hence for distribution of the organism along the water column (174a). Unfortunately, no information is available on the relative abundance of these organelles in *Spirulina* or on their variation in relation to changes in the environmental conditions, especially light intensity and dissolved oxygen content.

A number of "unusual inclusions" have been reported in a cytological study of 60 different cyanobacteria, representing at least 30 species and including two isolates of *Spirulina* (82). The significance of these inclusions could not be assessed since many were present in a few of the cyanobacteria examined and, at times, were unique to one or a few species. The authors suggested that some, perhaps many, of these "inclusions" were due to infection by intracellular symbionts or viruses. This may be the case also of rhapidosomes, organelles of uncertain origin and function, perhaps components of a putative motility organelle or parts of an incomplete phage, which have been observed in an unidentified species of *Spirulina* (24) but not in *S. platensis* (162). Since rhapidosomes have been characterized in the flexibacterium *Saprospira grandis* (32), the occurrence in *Spirulina* would have strengthened the similarities between *Spirulina* and *Saprospira*, the latter being considered by some authors as an apochlorotic, nonphotosynthetic cyanobacterium (92).

The life cycle of *Spirulina* in laboratory culture is rather simple (Fig. 4). A mature trichome is broken in several pieces through the formation of specialized cells, necridia, that undergo lysis, giving rise to biconcave separation disks. The fragmentation of the trichome at the necridia produces gliding, short (two to four cells) chains of cells, the hormogonia, that move away from the parental filament to give rise to a new trichome. The cells in the hormogonium lose the attached portions of the necridial cells, becoming rounded at the distal ends with little or no thickening of the walls. During this process, the cytoplasm appears less granulated and the cells assume a pale blue-green color. The number of cells in hormogonia increases by cell fission while the cytoplasm becomes granulated, and the cells assume a brilliant blue-green color. By this process trichomes increase in length and assume the typical helicoidal shape. Random but rare spontaneous breakage of trichomes together with the formation of necridia assure growth and dispersal of the organism. Akinetes have not been reported.

Determination of the taxonomic position of *Spirulina* has proved to be rather difficult. The genus *Spirulina* was established in 1827 by Tur-

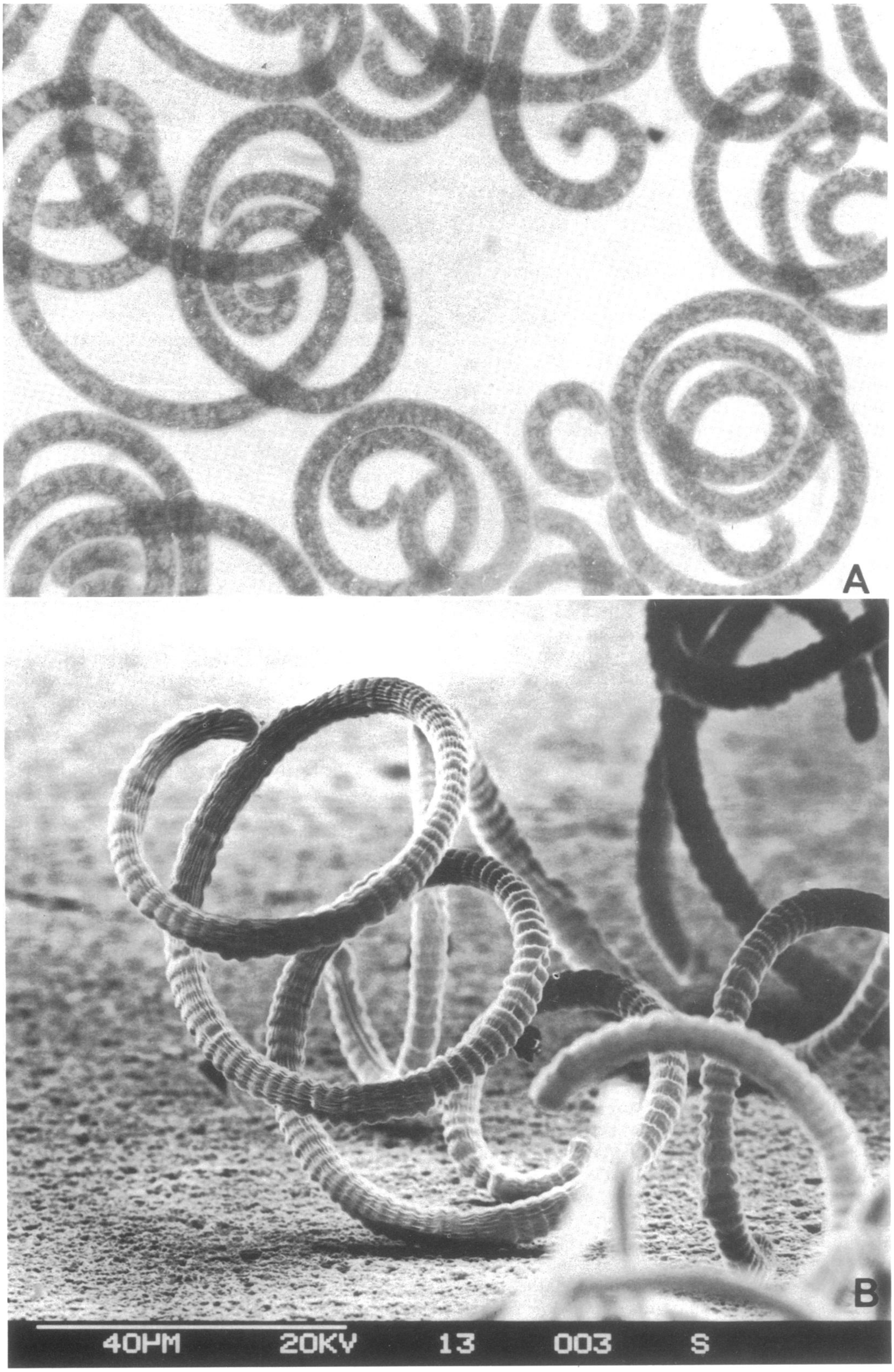

FIG. 3. Morphology of *Spirulina*. (A) Optical microscopy (×400) of axenic *S. platensis*. (*Photo by G. Caretta*.) (B) Scanning electron micrograph of a trichome of axenic *S. platensis*. (*Photo by R. Locci*.) (C) Scanning electron micrograph of a portion of a trichome of axenic *S. platensis*. (*Photo by R. Locci*.) (D) Scanning electron micrograph of nonaxenic trichomes of *S. maxima*. (*Photo by R. Locci*.)

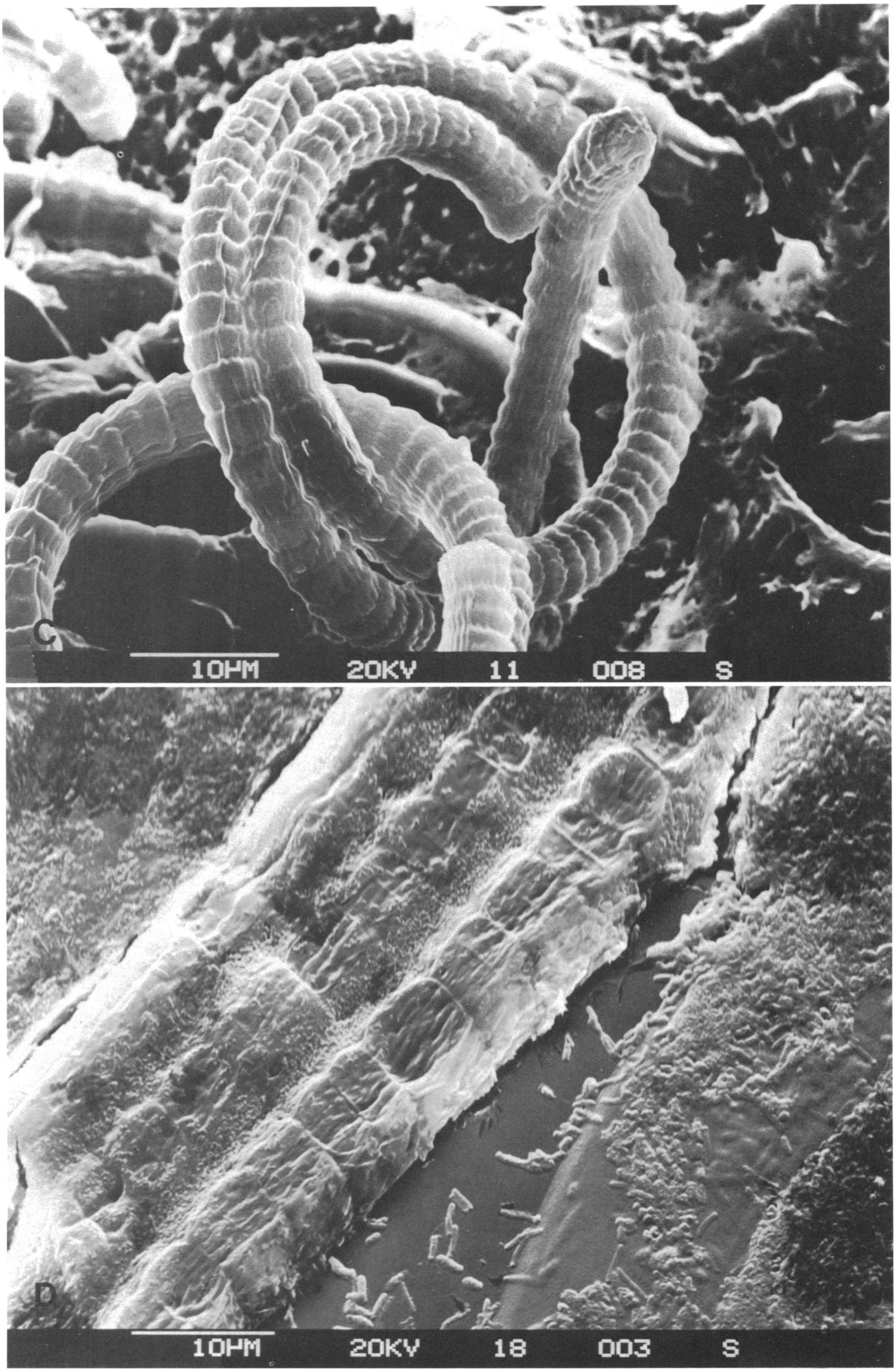

FIG. 3—*Continued*

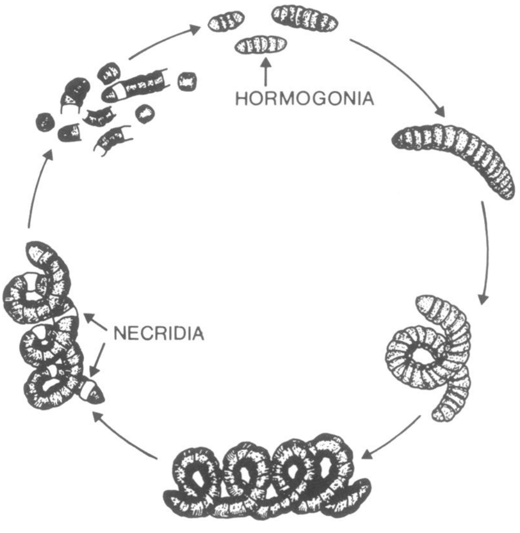

FIG. 4. Life cycle of *Spirulina*. For details, see text. (Redrawn from reference 8.)

pin for *S. oscillarioides* (159). Since Turpin did not mention the presence of septa in his isolate, in 1852 Stizenberger created the genus *Arthrospira* for the spiral cyanobacteria in which septa were clearly visible (149). This distinction, which also separates the larger forms from the smaller ones, was maintained for another 100 years. Thus, for instance, Gomont in 1892 (53) grouped in the genus *Arthrospira* Stizenberger the forms with visible septa and larger dimensions, such as *S. platensis*, whereas he reserved the genus *Spirulina* Turpin for the smaller forms in which septa were not visible and thus were characterized by "unicellular trichomes." In 1917, Gardner (49) questioned the validity of the presence of septa to distinguish between *Arthrospira* and *Spirulina* since he had "determined repeatedly that this distinction can no longer maintain." However, "for the sake of convenience," he suggested retaining the name *Arthrospira* for the forms "with conspicuous cross-walls" and that of *Spirulina* for those "with obscure cross-walls." Although a few years later Figini showed that, with appropriate stains, septa could be demonstrated in fresh and dry specimens of 13 different species of *Spirulina*, including the most minute ones such as *S. subtilissima* (47), Crow reported that, indeed, in certain *Spirulina* septa could be demonstrated but that it was impossible to do so in other isolates (30). The former, even if they had the typical small dimensions of a *Spirulina*, had to be grouped with *Arthrospira*, and the name *Spirulina* was to be reserved for the aseptate forms. This position was also that of Geitler (50), who revised the classification by grouping the two genera in a single one, *Spirulina*, subdived in section I, *Arthrospira*, for the "large forms with transverse wall which are seen in living algae" and section II, *Euspirulina*, for the smaller forms with invisible septa. The separation of *Spirulina* and *Arthrospira* was accepted as valid up to 1959 (35). Finally, in 1961 Welsh (176) clearly pointed out that "whether or not the transverse walls can be seen depends on whether the alga is dead or alive and on the microscopical technique used. With today's phase-contrast [microscopy] much can be seen which at the time of publication of Geitler's work was invisible." Thus he suggested that the generic name *Spirulina*, older than *Arthrospira*, be used regardless of the presence of visible septa that can, of course, be easily detected even in *Spirulina* by electron microscopy (64). Yet, as late as 1968, Drouet (39) still maintained the two genera, *Arthrospira* for the forms with apparent cross walls and *Spirulina* "for the forms appearing unicellular without easily demonstrable cross walls." In addition, much to the confusion of the nonspecialist, due to differences in the morphology of the outer wall of the terminal cells, both *S. maxima* and *S. platensis* are placed in the genus *Microcoleus*, as *M. lyngbyaceus*.

To make things worse, 2 years later, Bourrelly (16) concluded that "only the character of the helicoidal shape of the trichome separates *Spirulina* from *Oscillatoria*." Since certain *Oscillatoria* have portions of the trichome that appear helicoidal and the degree of spiralization of certain *Spirulina* may vary (72, 128) and, at times, even straight trichomes are evident (93, 98), Bourrelly suggested considering *Spirulina* a subgenus of *Oscillatoria*. Thus, *S. platensis* should be named *O. platensis* and *S. geitleri* (synonymous with *S. maxima*) should be named *O. pseudoplatensis* (the names of *O. maxima* and *O. geitleri* being used to designate other cyanobacteria) (16, 72). However, more recently Rippka and co-workers (134) emphasized that the helical shape of *Spirulina* "is a stable and constant property" of the genus that permits its differentiation from the other groups of filamentous, non-heterocystous cyanobacteria: *Oscillatoria, Pseudanabaena*, and the LPP (*Lyngbia, Phormidium*, and *Plectonema*) group (135). In addition, differences between *Spirulina* and *Oscillatoria* have been reported in the genome size (59), chemical composition (especially in the case of fatty acids [see later]), antigenicity (22), and ultrastructure (154). Thus, it does not seem unreasonable to maintain the genus *Spirulina* for at least the cyanobacteria characterized by highly spiralized trichomes living in freshwaters with high salt concentrations and alkaline pH; that is the object of this review. In addition, the names

S. platensis and *S. maxima* (= *S. geitleri*) are those utilized in practically all the recent literature dealing with these two cyanobacteria.

PHYSIOLOGY

Like most cyanobacteria, *Spirulina* is an obligate photoautotroph and cannot grow in the dark in media containing organic sources of carbon (85, 105). However, in the light it may utilize carbohydrates since, for instance, the addition of 0.1% glucose to the growth medium enhances growth rate and cell yield (106). Especially in dim light, mixotrophic growth results in cell yields that are two- to threefold higher than the corresponding yields obtained photoautotrophically. Peptone at the same concentration was less effective but glucose and peptone added together seemed to exert a synergistic effect (105). The utilization of glucose was verified by supplying cultures of *S. platensis* with [^{14}C]-glucose (106). Within less than 4 days of culture, all labeled glucose disappeared from the medium and almost 50% of the label was recovered with the cells, the rest being released either as CO_2 (34%) or as organic by-products excreted into the medium (19%). In these experiments an interesting phenomenon, the so-called mixotrophic lysis, was observed. If the size of the inoculum was kept small (i.e., cultures inoculated to give an initial optical density at 560 nm of <0.1 at 1-cm light path), after a brief period of growth, the cultures stopped growing and the cells underwent complete lysis. On the other hand, growth was normal if the culture was inoculated to an initial optical density of ca. 0.2. No explanation for the phenomenon of mixotrophic lysis has been offered but it has been observed that, before lysis, cells from a culture started with a "small" inoculum contained six to seven times more alkaline protease activity than cells from cultures with a "large" inoculum or cells from photoautotrophic cultures. Mixotrophic lysis could be prevented by depriving the medium of Mn ions. Both growth rate and cell yield may be increased under photoautotrophic conditions by increasing the amount of nitrogen supplied (up to 120 mM nitrate), especially when cultures were performed at temperatures (35 to 37°C) higher than the optimal ones (32 to 35°C) (163). In the laboratory, cultures of *S. platensis* show a wide pH optimum (8 to 11), but growth is evident also at pH values close to 7 and as high as 11.3 (54). The optimal light intensity was found to be between 20 and 30 klx (105). Under field conditions, optimal day temperature has been reported to be around 40°C and the night temperature is around 25°C (see below). Above 40°C the cultures do not grow. Laboratory cultures kept at 45°C for up to 24 h do not grow, but growth is resumed when the culture is brought back to 35°C. Above 45°C, massive breakage of the trichomes followed by cell lysis has been observed. Even a brief period (e.g., 10 min) of exposure at temperatures around 50°C results in death of the cultures (25). Compared with cells grown at suboptimal light concentrations, cells grown at ca. 20 klx have a higher content of carotenoids. A slight increase was also observed in the case of chlorophyll, whereas no difference was noticed in the case of phycocyanins (106).

With the view of obtaining massive cultures of *Spirulina* utilizing seawater, attempts have been made to cultivate *S. platensis* and *S. maxima* in media containing seawater in part or in toto. Although analogies exist between the salt composition of seawater and that of the alkaline lakes of Africa and Lake Texcoco (8), the low content in carbonates, phosphates, and combined nitrogen together with the high concentrations of Mg and Ca make seawater unsuitable for mass culture of *Spirulina*. Not only is growth of *Spirulina* inhibited by high concentrations of Mg (177), but also the addition of carbonates or phosphates or both causes precipitation of their Ca and Mg salts. A preliminary removal of these two cations allows addition of carbonates, phosphates, and nitrates (43). However, probably the pretreatment to remove Mg and Ca ions would render the process economically unacceptable.

BIOCHEMISTRY

The phycocyanins, biliproteins involved in the light-harvesting reactions, have been resolved by gel electrophoresis in *S. platensis* and *S. maxima* (29) and isolated from the former (17). Both c-phycocyanin and allophycocyanin appear to be oligomeric complexes composed of at least two different subunits that may be resolved by electrophoresis under denaturing conditions. The α- and β-subunits of c-phycocyanins showed mobilities corresponding to molecular weights of 20,500 and 23,500, respectively, resulting in an oligomer with a minimum molecular weight of ca. 44,000. Allophycocyanin was found to be composed of subunits with molecular weights of ca. 18,000 and 20,000 to give an oligomer with a minimum molecular weight of ca. 38,000. Absorption and fluorescence spectra were similar to those reported for c-phycocyanins and allophycocyanins isolated from other cyanobacteria. A study of the denaturation and renaturation of c-phycocyanin indicated the possibility that more than one chromophore exists in this biliprotein (143).

Phycocyanins may serve also as a storage material since it has been found that the phycocyanin concentration was highest when *S. platensis* was cultivated under favorable nitrogen concentrations (18). If the level of available nitrogen in the medium decreased, or the cultures were completely deprived of nitrogen, a corresponding decrease in the phycocyanin content was observed. No other nitrogen-containing compounds decreased under these conditions, and the decrease of phycocyanin concentration was associated with an increase in the activity of a protease acting on purified c-phycocyanin. If, under these conditions as well as after inhibition of protein synthesis, the cellular concentration of phycocyanins decreased, severe inhibition of photosynthesis and growth was observed. Ribulose-1,5-bisphosphate carboxylase, accounting for ca. 12% of the soluble protein of *S. platensis* and *S. maxima*, was purified and partially characterized (138). By gel electrophoresis the molecular weight of the enzyme was estimated to be ca. 500,000, a value similar to that reported for the enzyme isolated from chloroplasts of higher plants and unicellular algae as well as some cyanobacteria (148). Electrophoresis under denaturing conditions of the enzyme from *S. maxima* revealed the presence of two types of subunits, a larger one with an apparent molecular weight of ca. 55,000 and a smaller one of ca. 12,000. Thus, it is quite likely that the holoenzyme is composed of eight large subunits arranged around a "core" of eight small subunits. The amino acid composition of ribulose-1,5-bisphosphate carboxylase purified from *S. maxima* was found to be very similar to that reported for the enzyme isolated from higher plants and unicellular algae. Thus, just like the enzyme from higher plants such as tobacco (41), ribulose-1,5-bisphosphate carboxylase from *Spirulina* also may be considered as a promising source of high-quality protein for human nutrition.

Cytochrome c_{554}, a cytochrome with high redox potential that links photosystems I and II, has been purified from *S. platensis* (178) and *S. maxima* (63). The molecular weight of the protein was found to be ca. 10,000, like that of cytochrome c_{554} from other cyanobacteria, unicellular algae, and higher plants. Another cytochrome involved in photosynthesis, cytochrome *f*, was purified from *S. maxima* (5, 62, 63) and *S. platensis* (139). The molecular weight was ca. 38,000, a value close to that of cytochrome *f* isolated from spinach chloroplasts. Similarly, striking similarities were observed in the amino acid composition (62).

Ferredoxin, one of the electron carriers of photosynthesis, was purified from *S. maxima* (55) and sequenced from *S. maxima* (151) and *S. platensis* (174). As the protein isolated from higher plants, *S. maxima* ferredoxin contained two atoms of Fe and two atoms of sulfur per mole. Optical adsorption, other spectral characteristics, and oxygen evolution assays confirmed the close similarity existing between the ferredoxin isolated from the cyanobacterium and that isolated from higher plants or algal chloroplasts. The similarity between the ferredoxin from *Spirulina* and that from chloroplasts was substantiated by immunological tests (152). The stability, especially at room temperature, of the ferredoxin isolated from *S. maxima* was much higher than that of the protein isolated from higher plants and unicellular algae. This characteristic, together with the ease of isolation from dried cells and even spray-dried commercial preparations (55), render *Spirulina* a promising source of ferredoxin. Ferredoxin II, another ferredoxin characterized by a different redox potential and present in smaller amounts, was isolated in a subsequent investigation (20, 63). The presence of two different ferredoxins may be ascribed to the other biochemical activities associated with these electron carriers (donor of electrons to nitrite reductase, sulfite reductase, glutamate synthase, electron acceptor in the phosphoclastic cleavage of pyruvate, etc.) for which each type of ferredoxin may be more suited. The amino acid sequences of the last 23 amino acids from the amino-terminal position of the ferredoxins (not specified, but probably type I) from *S. platensis* and *S. maxima* appear to be almost identical, differing in only one amino acid (144). A coupling factor complex, linking phosphorylation to electron transport, was also partially purified from *S. platensis* (109).

A preliminary characterization of a cyanide-insensitive superoxide dismutase from *S. platensis* indicated the presence of Fe, as in some of the bacterial and cyanobacterial dismutases, rather than Cu or Zn as found in the enzymes from chloroplasts (94, 95).

The only data on the transport of inorganic nutrients in *Spirulina* concern sulfur. *S. platensis* appears to possess an active, energy-dependent transport system for sulfate. Under photoautotrophic conditions, probably two sulfate permeases are present. One permease is constitutive, as found for the only other cyanobacterium studied, *Anacystis nidulans*, whereas the other is inducible like that of heterotrophic bacteria, fungi, and some higher plants (103).

A number of mutants of *S. platensis* resistant to two analogs of phenylalanine (β-thienylalanine and *p*-fluorophenylalanine), methionine (ethionine), proline (azetidin-2-carboxylic acid), and tryptophan (5-fluorotryptophan) were recently isolated and partially characterized (126). A few mutants appear to be resistant to one analog only, whereas the majority seem to have become resistant simultaneously to the analogs of phenylalanine, methionine, and proline but not of tryptophan. All of the cross-resistant mutants analyzed appear to overproduce the respective parental amino acids and thus become resistant to the analogs by reducing their uptake into the cells and their incorporation into protein (124). It appears likely that in these strains a mutation in one enzyme at the beginning of a metabolic branch point results in an alteration in the mechanisms regulating its activity. This may lead to overproduction of groups of amino acids. Such a pattern of metabolic control, the so-called endo-oriented control because of being most sensitive to endogenous products, is typical of cyanobacteria in contrast to the "exo-oriented" control (most sensitive to exogenous products) that is typical of many heterotrophic bacteria (82). Indeed, obligate photoautotrophs, like most cyanobacteria, are barely capable of utilizing exogenous amino acids, for which some of them appear even to lack an active transport system (56). The majority of the mutants of *S. platensis* so far isolated are presumably deregulated at a proximal metabolic branch point rather than at the terminal branchlet, resulting in the production of more than one end product and, as a consequence, resistance to analogs of different amino acids. However, a mutant resistant to azetidin-2-carboxylic acid was found to be resistant to this analog only and to overproduce only proline (124). A mutant resistant to ethionine did not overproduce any amino acid and had an altered methionyl-tRNA synthetase (125). The mutant's enzyme, unlike that from the parental strain, had a reduced affinity for the analog so that its incorporation into protein, in place of methionine, was practically reduced to nil. Finally, the only mutant resistant to 5-fluorotryptophan seemed to possess an altered tryptophanyl-tRNA synthetase. Thus, in *S. platensis*, mutations conferring resistance to amino acid analogs may involve the regulation of amino acid biosynthesis or mechanisms for the uptake and incorporation of amino acids into protein. Although in a wild-type strain the cellular pool of free amino acids has been reported to be very small (177), many of the overproducing mutants excrete during growth only a portion of the amino acid(s) overproduced and >50% is released in the medium at cell lysis (126). This finding renders attractive the utilization of some of these mutants for mass cultures. Since, at least under laboratory conditions, most of the mutants grow at the same rate and attain the same cell concentration as the wild-type strain, it is conceivable to produce *S. platensis* cells with different qualitative-quantitative levels of selected amino acids and, possibly, other metabolites. It would be possible, for instance, to improve the nutritional value of *S. platensis* by compensating for the low content in methionine (see below) through the use of mutants that have higher intracellular pools of this amino acid. Since in higher plants and unicellular organisms overproduction of proline is one of the mechanisms responsible for stress resistance, including the presence of high salt concentrations, the mutants of *S. platensis* overproducing proline were analyzed for the capacity to grow in media containing high concentrations of sodium chloride (123). All mutants overproducing proline grew in media containing NaCl concentrations that inhibited growth of the parental strain. In addition, a positive correlation was found between the amount of proline overproduced and the degree of osmotolerance, suggesting the possibility that these mutants may be utilized for cultures in brackish waters unsuitable for the strains of *S. platensis* so far utilized.

Little is known concerning the mechanisms for genetic recombination in cyanobacteria, especially in the case of the filamentous species. Indeed, whereas there are a few reports demonstrating the occurrence of transformation in the unicellular forms (90), for the filamentous cyanobacteria there is only one report giving indirect evidence for the presence of transformation in *Nostoc muscorum* (155). As far as I am aware, no information is available for *Spirulina* or for any of the *Oscillatoriaceae*, yet it is likely that a mechanism for genetic recombination may exist also in these cyanobacteria. Spheroplasts, which may now be prepared efficiently in *S. platensis* (136), may be of considerable help in attempting heterologous fusions, a technique successfully used for other procaryotes, such as streptomycetes, recalcitrant to conventional genetic recombination.

So far no phage infecting *Spirulina* or other *Oscillatoriaceae* has been isolated (110, 137) nor have plasmids been found in *S. maxima* and *S. platensis* (G. Riccardi, unpublished data). However, one may hope that phages or plasmids may be found eventually or that plasmids with selectable markers now constructed for other cyanobacteria (e.g., *A. nidulans* [89]) and also capable of transforming bacteria may be utilized in the case of *Spirulina*.

The genome sizes of 128 strains representing all major taxonomic groups of cyanobacteria have been determined by renaturation kinetics analysis (59). The genome sizes could be grouped into four classes that could correspond to a progressive duplication of an "ancestral genome" of ca. 1.2×10^9 daltons to give genomes two, three, four, and six times this ancestral genome. The value of 2.53×10^9 daltons found for an unidentified species of *Spirulina*, and close to the values reported for many bacteria such as *Escherichia coli* would correspond to two copies of the putative ancestral genome. The DNA base compositions of the only two isolates of *Spirulina* analyzed have been reported to be 44 and 52 mol% guanosine plus cytosine (60). The lower value is close to that found for 10 isolates of *Oscillatoria*, the higher one clearly differentiating this isolate from the other *Spirulina* as well as from *Oscillatoria*. However, for the moment at least, Rippka et al. (134) consider both isolates as *Spirulina* even if they show gross morphological differences, one being characterized by thick filaments with many gas vacuoles and the other having much thinner filaments and no observed vacuoles. In addition, the habitats were also different, one being a marine isolate and the other growing in brackish water but not in seawater.

CHEMICAL COMPOSITION

Already the first analyses performed on Dihé indicated a high protein content: 45% of the dry weight in the samples analyzed by Léonard and Compère (91) and 62% in laboratory-grown *S. platensis* (28). More recent analysis confirmed that protein represents more than 60% and, in certain samples, even 70% of the dry weight (Table 2). The protein content of *Spirulina* appears to be high also when compared with that of unicellular algae and other cyanobacteria. For 10 species of eucaryotic algae, protein accounted for 10 to 46% of the dry weight, whereas four cyanobacteria gave values between 42 and 51% (19). However, values close to 60% have often been reported for the strains of *Chlorella* and *Scenedesmus* that have been extensively studied as possible sources of alimentary protein (1, 175).

An exhaustive study was performed by Paoletti et al. (115) of the chemical composition of *S. platensis* and *S. maxima* grown in the laboratory or in open ponds (Table 3). For both species, cells grown in the laboratory contained more protein than cells grown in open ponds; the latter, on the other hand, contained a higher percentage of carbohydrates and ash. In the same investigation, a comparison was made of the chemical composition of both species grown either in open ponds or in polyethylene tubes (see below). Cultures from open ponds contained more protein and less carbohydrate than those grown in the closed system.

TABLE 2. Approximate composition of *S. platensis*, *S. maxima*, and soybean meal

Sample	% of dry wt					
	Water	Ash	Crude lipids	Crude fiber	Crude carbohydrates	Crude protein
S. platensis[a]	6–10	4–5	9–14	3–8	10–18	56–77
S. maxima[b]	4–7	6–9	4	1	8–13	60–71
Soybean meal	7–10	4	16–20	3–5	19–35	34–40

[a] Laboratory and pond grown, lyophilized or drum dried. Data from reference 115.

[b] From Lake Texcoco, spray dried. Data from reference 40.

TABLE 3. Approximate composition of *S. platensis* and *S. maxima* grown in the laboratory and in open ponds

Component	% of dry wt[a]			
	S. platensis		*S. maxima*	
	Laboratory	Pond	Laboratory	Pond
Crude protein	64–74	61	68–77	60
Crude lipids	9–14	12	9–14	15
Crude carbohydrates	12–20	19	10–16	16
Ash	4–6	8	4–6	9

[a] Data from reference 115.

The amino acid spectrum of *Spirulina* protein is similar to that of other microorganisms (83, 175) and, in comparison to standard alimentary proteins such as those of eggs or milk, it is somewhat deficient in methionine, cysteine, and lysine (1, 10, 28). The amino acid compositions of *S. platensis* and *S. maxima* grown in open ponds or in polyethylene tubes have been evaluated (46, 115). Compared with the protein standard elaborated by the Food and Agriculture Organization or egg albumin, variations were reported in the content of a number of amino acids, with significant differences in the case of the above-reported amino acids. However, the various authors conclude that, as a protein source, *Spirulina*, albeit inferior to standard alimentary protein such as meat or milk, is superior to all plant protein including that from legumes. Thus, it appears that the high concentration of protein together with its amino acid composition make *Spirulina* a source of nonconventional protein of considerable interest.

Because uric acid is produced in humans and other mammals in the metabolism of purines and high levels of this metabolite may cause pathological conditions such as gout, a constant worry in the utilization of microbial cells as food or feed has been their high nucleic acid content. In *S. maxima* and *S. platensis*, RNA has been reported to represent 2.2 to 3.5% of the dry weight, whereas DNA represents 0.6 to 1% (10, 27, 140). The total nucleic acid content, therefore, is <5% of the dry weight, a value close to that reported for unicellular algae, such as *Chlorella* and *Scenedesmus* (4 to 6%), but definitely lower than that of bacteria or yeasts (4 to 10% but up to 9 to 22% when the microorganisms are cultivated at the high growth rates required for industrial production) (99). Thus, whereas a typical single-cell protein (SCP), such as yeast grown on alkanes, contains at least 1 g of nucleic acids per every 10 g of protein (65), *S. maxima* and *S. platensis* grown in the laboratory or in open ponds contain 0.6 to 0.7 g per 10 g of protein (115).

Considerable variations have been reported in the fatty acid content. Although differences have been demonstrated in the lipid content of *S. platensis* and *S. maxima* or in that of laboratory- and pond-grown cultures (115), the wide variations reported in the literature (1.5 to 12% of the dry weight) (44, 66, 113, 118, 140) indicate that there may have been striking differences in the procedures for the extraction or the estimation, or both, of the lipid content. In *S. platensis* and *S. maxima* free fatty acids account for 70 to 80% of the total lipids, the remaining being chiefly mono- and digalactosyl glycerides and phosphatidyl glycerol (66). Interesting variations have been observed in the degree of unsaturation of octadecatrienoic acid (86), and attempts have been made to utilize the presence of the different isomers of this acid as a taxonomic criterion. The presence of high concentrations of γ-linolenic acid, synthesized in *S. platensis* by direct desaturation of linoleic acid, seemed to be characteristic of *Spirulina*, the α-isomer being predominant in the other cyanobacteria and leaf lipids (104). The high content of these two essential fatty acids of possible considerable importance for human nutrition (components of the so-called vitamin F), even in commercial preparations of *S. maxima* (66), may be of some interest for the utilization of *Spirulina* as a food. More recently, γ-linolenic acid was reported to be present in other cyanobacteria, e.g., in strains of unicellular *Synechococcus*, *Aphanocapsa*, and *Microcystis* (84) as well as in filamentous ones such as *Oscillatoria* (85). Further, the latter investigators reported that, of the three isolates of *Spirulina* examined, one had a predominance of α-linolenic acid. Therefore, for the moment at least, the presence of γ-linolenic acid in all isolates of *Spirulina* is in doubt, thus rendering its presence of uncertain importance for taxonomic purposes. However, the fairly high concentration of these two fatty acids in *S. platensis* and *S. maxima* remains of considerable nutritional interest. Among the hydrocarbons, *n*-heptadecane is the most abundant (65 to 70%) in *S. platensis* and in *S. maxima* (44, 51, 158), as in the majority of the other cyanobacteria so far examined (51). Cholesterol and β-sitosterol are the main sterols present in *S. maxima* and *S. platensis* (96, 114, 140). The presence of these and other sterols was reported to be somewhat related to an antimicrobial activity of *S. maxima* (96) which, however, has never been characterized (76).

Poly-β-hydroxybutyrate, a reserve of carbon and energy in many bacteria, has been isolated from *S. platensis* (21). The compound accumulates during exponential growth, reaching, at the beginning of the stationary phase, a concentration corresponding to 6% of the dry weight.

Among the pigments, the most abundant is chlorophyll *a*, the only chlorophyll present, which accounts for 0.8 to 1.5% of the dry weight in *S. maxima* and *S. platensis* (115). Mixoxanthophyll and β-carotene are the major carotenoids (61, 111, 112), their content representing approximately 0.2 to 0.4% of the dry weight (111, 112, 115, 140). The fairly high content of some of these pigments, possibly responsible for the color of the feathers of certain species of flamingos (see below), has stimulated the use of *Spirulina* also as a source of pigments for fish, chickens, and eggs.

Carbohydrates, accounting for 15 to 20% of the dry weight (23, 140), are represented in *S. platensis* essentially by a branched polysaccharide, composed of only glucose and structurally similar to glycogen (23). Another glucose-containing polysaccharide, representing ca. 1% of the dry weight, was also isolated and characterized (161), whereas the presence of a rhamnan, reported to be the main polysaccharide of *S. platensis* (120), has not been confirmed (23). Finally, all vitamins have been found in *S. platensis* and *S. maxima* and their concentrations have been evaluated (27, 115, 140). Cyanocobalamin appears to be rather abundant, reaching a concentration of up to 11 mg per kg of dried cells (27, 140).

PRODUCTION

The only plant for large-scale production of *Spirulina* operating at the moment is that of Lake Texcoco, in the Valley of Mexico. The lake is located 2,200 m above sea level but in a semitropical climate (average yearly temperature, 18°C). As already mentioned, *S. maxima* grows naturally in the lake, and the firm that operates a plant to extract soda from the lake is now recovering and commercializing the cyanobacterial biomass. *S. maxima* is harvested from the most external portion of a giant solar evaporator of spiral shape (hence the name caracol, meaning snail in Spanish) with a diameter of 3 km and a surface area of 900 ha (40) (Fig. 5). The biomass is recovered by filtration and, after homogenization and pasteurization, spray dried. Daily production of the plant has been reported to approach 2 tons (dry weight), with a yield of 28 tons of protein/ha per year (140). Although the long-term goal is that of a protein source for human consumption, so far it appears that *S. maxima* biomass is commercialized mostly as a feed for animals (including some fancy uses such

FIG. 5. Plant for the production of *S. maxima* on Lake Texcoco. *S. maxima* is harvested from the external portion of the 900-ha solar evaporator (*caracol*) built on Lake Texcoco, Mexico. (*Photo courtesy of Sosa Texcoco S.A.*)

as to enhance the color of certain Japanese ornamental fish) or as a health food. No data have been published on the other microorganisms present in the waters of Lake Texcoco, how the culture is accomplished, or which nutrient is added to enhance growth. It seems likely that a source of nitrogen (probably nitrate) and phosphorus might be added to achieve rapid growth unless these nutrients are supplied by seepage of effluents into the lake. The latter possibility would explain the high bacterial counts, including the presence of fecal streptococci, reported for samples of *S. maxima* from Lake Texcoco (76) and would justify the pasteurization step involved in the production of *S. maxima* biomass.

Extensive investigations have been conducted in Israel on the possibility of large-scale cultivation of *S. platensis*, utilizing brackish water unsuitable for human consumption or agricultural use. In the Negev desert, the cultures are grown in shallow (0.2-m), black plastic-lined channels 50 to 300 m long. The channels run back and forth to make, eventually, ponds of 5 to 10 ha. To ensure mixing of the culture, the channels are dug with a constant slope that provides a flow of ca. 30 ml/min. At the end of the channel maze, the culture is pumped back to the highest (starting) point (130). Turbulence has been found to be essential to increase growth rate, especially at high population density, since it increases the proportion of cells receiving light. At the high cell concentrations necessary for industrial production, if the culture is not agitated only the upper 3 cm of the pond, containing about 20% of the cell population, receives light. In addition to its effects on photosynthetic efficiency, the occurrence of a "light/dark" cycle seems to be beneficial for growth. Thus, at any cell density agitation increases output rate, and at optimal ratios of turbulence/population density it may double production (132). Temperature may severely limit growth in winter months: in open ponds in the Negev desert, practically no *S. platensis* was produced from December to February (131). The arrest of growth during the winter season was found to be due to insufficient temperature during the day (average temperature of 18°C compared to values close to 40°C in summer) rather than to the cold temperature of the nights (5°C and, at times, even 0°C). If the day temperature was artificially raised from 18 to 25°C, the growth rate was similar to that obtained during the summer months even when the night temperature approached 0°C. On the other hand, raising the night temperature to ca. 10°C did not enhance growth if the day temperature was not increased. During the summer months, the limiting factor was found to be solar irradiance, and peak productivity was reached when light level was highest. In spring and fall both light and temperature appeared to limit growth. Examination of productivity in open ponds over a 2-year period demonstrated that growth increased proportionally with an increase in temperature and solar irradiance. Indeed, at any given light intensity, growth increased on increasing the temperature and similarly, for any temperature range, an increase in light resulted in growth increase (172). Low temperatures, such as those common in winter, play a role also in the maintenance of culture purity in open ponds. In winter months, when growth of *S. platensis* was severely reduced, a population of *Chlorella vulgaris* appeared in the ponds reaching up to 50% of the biomass. The increase of the *C. vulgaris* population, as well as of its grazers and their predators, could be controlled by raising the pond temperature. Simply covering the ponds with polyethylene sheets raised the temperature 5 to 7°C, and this led to a threefold increase in the *S. platensis* population with a concomitant decrease in that of *C. vulgaris* (from 35 to 40% of the total cell volume to <5% [173]). Laboratory experiments revealed that another factor, namely, the concentration of bicarbonate and gaseous CO_2, may play a major role in the relative proportion of *C. vulgaris* and *S. platensis* in a mixed culture. High concentrations of bicarbonate (0.1 M upward) favored growth of *S. platensis*, whereas at low bicarbonate concentrations and in the presence of CO_2 *C. vulgaris* was favored. The effects were specific and not due to differences in the osmotic pressure or the pH of the medium resulting from the removal of bicarbonate or CO_2, respectively. Thus, it appeared possible to maintain a culture of *S. platensis* without significant contamination by the faster-growing *C. vulgaris* simply by maintaining in the medium a high concentration of carbonates and a low concentration of CO_2 (129). Alternatively, it was possible, at least in a chemostat, to control the contamination of an *S. maxima* culture by an unidentified species of *Chlorella* by recycling part of the biomass recovered after filtration through a nylon screen that retains only the cyanobacterial filaments (175a).

For the Negev area, daily productions of 40 and 10 g (dry weight)/m^2 for the summer and winter months, respectively, have been obtained to give a yearly production exceeding 62 tons (dry weight)/ha. In warmer climates, such as at Arava in the Rift Valley of southern Israel, where year-round operation of the plants seems feasible, yearly production may reach 74 tons (dry weight)/ha (11, 130). In Florence, in the central part of Italy, experiments performed in small (up to 100 m^2), open ponds have given daily yields, in the peak summer months, of 14 g (dry weight)/m^2. However, due to the fairly continental weather, production lasts only 5 to 6 months, giving a yearly yield of *S. platensis* or *S. maxima* not exceeding 18 to 22 tons (dry weight)/ha (100). The authors have calculated that in the southern part of the country, up to 300 days of production per year could be obtained, increasing productivity to ca. 30 tons (dry weight)/ha. An interesting alternative is that of growing *S. platensis* and *S. maxima* in polyethylene tubes 0.3 cm thick and with a diameter of 14 cm (37). The tubes are arranged in a "raceway" fashion and the culture is pumped through the tubes. The advantage of the system is that the tubes function as solar collectors, thus increasing the culture temperature in the seasons when the atmospheric temperature is too low to allow growth of *Spirulina* in open ponds. Daily productivity in tubes has reached 15 g (dry weight)/m^2 in summer and 10 g (dry weight)/m^2 in winter, to give a yearly production estimated, perhaps a bit optimistically, to be ca. 40 to 50 tons/ha. Other advantages of the tubular system are the considerable reduction of water loss by evaporation, the possibility of utilizing sloping (up to 10%) terrains, and the screening from external contaminants (biological or otherwise). In the peak summer season, however, temperatures may exceed the limits (40 to 45°C) tolerated by *Spirulina* and the tubes must either be shaded or cooled with water. In addition, as already mentioned above, the quality of the biomass produced in tubes, in terms of protein content, seems to be lower than that of the biomass produced in open ponds.

A species of *Spirulina*, probably *S. platensis*, is being considered for mass culture in Taiwan, possibly as a substitute for the too expensive cultures of *Chlorella* (147). Good results have been reported in preliminary trials on the utilization of the biomass as food for fish and shrimp. However, the reported economic difficulties encountered in the production of *Chlorella*, which was mostly sold as a health food in Japan, raise serious doubts about the financial viability of the production of *Spirulina* as a feed.

Small trials for the production of *Spirulina* to be used as a feed or food have been performed or are under way in different parts of the world (10, 40, 168).

An attractive possibility is the growth of *Spirulina* on wastewaters to couple protein production to recycling of nutrients, removal of organic and inorganic pollutants, and disposal of wastes. Laboratory or small-scale experiments have been performed on the growth of *S. maxima* and *S. platensis* on city wastewaters (67, 142), cow manure (108, 145), or swine wastes (26). In addition, to reduce the costs of the nutrients to be added to the medium, attempts are under way to grow *S. platensis* by utilizing a variety of cheap and easily available (especially at the village level) substrates such as manure, bonemeal, animal blood, and other wastes (10, 121, 146, 149). An interesting possibility is the production, in rural areas, of carbon dioxide-enriched air for growth of *Spirulina* by dung composting ("aerobic biogas") (169). All authors report encouraging results, but so far no information about large plants or practical applications have been reported. It is quite likely that in media containing organic sources the yields may greatly surpass those obtained in simple, inorganic media (111) but the economics of the process, requiring sterilization of the biomass before its use even for animal consumption, may still be unfavorable. Nevertheless, it is fairly obvious that, in the long run, accomplishment of two goals, recycling of industrial or urban wastewaters plus production of protein biomass, makes such processes very attractive. One can envisage cultures of *Spirulina* in "clean" waters with chemically defined media to produce biomasses for alimentary consumption and cultures in "dirty" waters to give biomasses to be used as a feed or as a starting material for the extraction of chemicals.

NUTRITION AND TOXICOLOGY

Several investigations have been performed on the possible utilization of *S. platensis* or *S. maxima* as a food source for human or animal consumption. Such investigations were stimulated by the discovery of the high protein content of *Spirulina* biomasses and by reports indicating its use as an aliment by some populations of the Chad area and, possibly, by the inhabitants of Mexico before the Spanish conquest. Further, the blooms of *Spirulina* have always represented

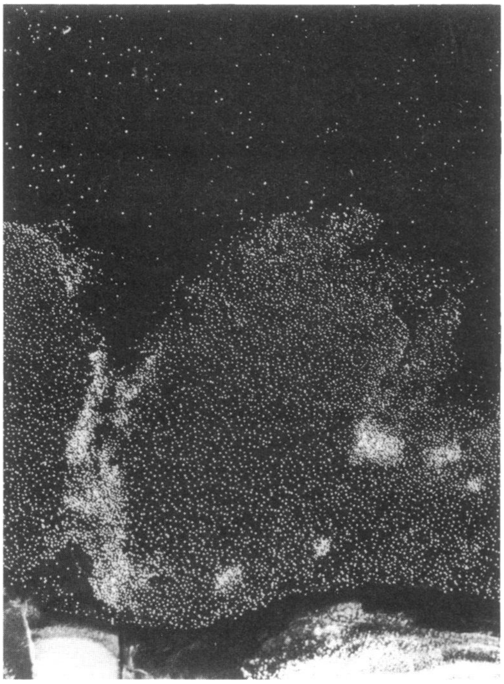

FIG. 6. Lesser flamingos feeding on *S. platensis*. Aerial photo of a flock of lesser flamingos (*P. minor*) feeding on *S. platensis* along the north shore of Lake Nakuru (Kenya) in 1973. Over 90% of the birds in the flock congregate close to shoreline where wind induces the formation of high-density mats of *S. platensis*. (*Photo by E. Vareschi; reprinted from reference 166 with permission of the publisher.*)

a major, if not the only, source of food for birds in the areas where *Spirulina* is the predominant component of the phytoplankton. Indeed, all observers, from the Spanish chroniclers of the middle ages (34) to today's ecologists (79, 156, 166), have recorded the abundance of avifauna in lakes containing *Spirulina*, especially in the periods in which the cyanobacterium is more abundant (Fig. 6). Analysis of the stomach content of the lesser flamingos (*Phoeniconaias minor*), the most numerous birds of the Rift Valley lakes, showed that, at least during the periods in which the collections were made, the birds were feeding entirely on *Spirulina* (128, 133). For some lakes of the same area, Tuite correlated the presence of lesser flamingos with that of high-density blooms of the cyanobacterium; when the *Spirulina* blooms were abundant, the birds fed on the lakes and stayed in the area to breed (156). If, however, the population of *Spirulina* decreased, as has happened in some of the Rift Valley lakes after a change in the concentration of chemicals in the waters, then the birds were compelled to rely on other cyanobacteria or on benthic diatoms. Since the lesser flamingos have apparently evolved a filter-feeding apparatus (80) that is very efficient for *Spirulina* and much less so for the unicellular forms (Fig. 7), the disappearance or the reduction of the *Spirulina* population causes dispersing of the bird flocks to other bodies of water in eastern and southern Africa. Thus, when, in 1973 to 1974, a dramatic decrease in the *Spirulina* population occurred in Lakes Nakuru and Bogoria, the flamingo population almost disappeared from these lakes. When, 5 years later, due to heavy rainfall, the concentration of chemicals changed again in these two lakes, the population of *Spirulina* rose rapidly at least in Lake Bogoria, resulting in an almost immediate return of a dense and stable population of flamingos. For Lake Nakuru, Vareschi has recorded the quantitative variations of the cyanobacterial and algal populations for almost 10 years (1972 to 1980) and the associated variations in the flamingo flocks (166, 167). *S. platensis*, which accounted for almost 100% of the biomass at the beginning of 1973, began to decline so that, at the end of that year, it represented <1% of the phytoplankton. Up to 1980 this cyanobacterium was present but did not constitute a significant proportion of the phytoplankton. Concomitant with the decline in *S. platensis* population, another unclassified species of *Spirulina* became the most abundant species in the years 1974 and 1975. In turn, this species too declined in abundance, and by 1977 the lake presented a mixed population of *Anabaenopsis*, single-cell cyanobacteria, green algae, and diatoms. These changes were probably brought about by an increase in the water salinity which favored growth of other organisms while reducing that of *Spirulina* and that of the zooplankton population responsible for most of the nutrient recycling. These factors, possibly associated with others such as a postulated appearance of cyanophages specific for *Spirulina* or its selective photooxidation, were responsible for the practical disappearance of *Spirulina* and the associated population of lesser flamingos that decreased from ca. 10^6 in 1973 to just several thousand.

Evaluation of the nutritional characteristics in vitro confirmed that drum-dried *S. platensis* or *S. maxima* represents a valuable source of alimentary protein (93). Although, as already mentioned, the content of methionine+cysteine and lysine of *Spirulina* protein is somewhat lower than that of reference protein sources such as lactalbumin or the Food and Agricultural Organization protein pattern, in vitro studies indicated that the cyanobacterial protein is nutritionally superior to legume protein, although inferior to meat protein. A number of nutritional studies have been performed on different animals (mice, rats, pigs, chicken, calves) fed diets in which

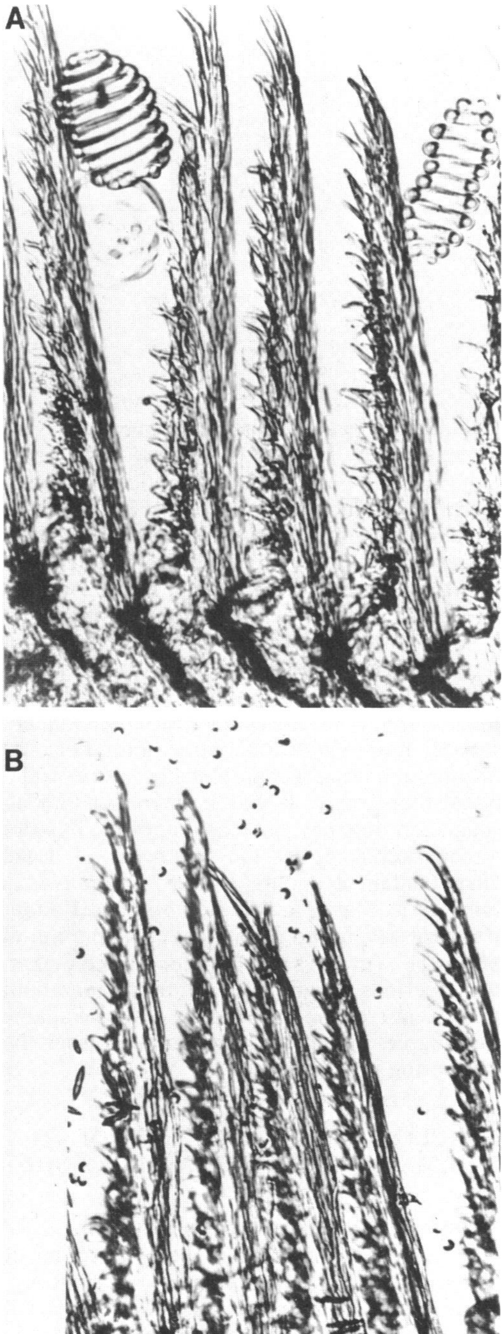

FIG. 7. Filter-feeding apparatus of lesser flamingos. The platelets of the filter-feeding device in the bill of lesser flamingos (*P. minor*) appear to be well adapted to trap filaments of *S. platensis* (A) rather than unicellular cyanobacteria (B). (*Photo by E. Vareschi; reprinted from reference 166 with permission of the publisher.*)

Spirulina was substituted totally or in part for the protein requirement. By and large, *S. maxima* or *S. platensis*, either drum or spray dried, was well accepted by animals, giving, in general, weight increases and nitrogen deposition in the body comparable to, if not better than, those obtained with most other plant protein sources (10, 12–14, 26, 45, 170). No toxic effect or abnormality on postmortem observation was reported in these experiments or in long-term (18-month) feeding trials (15) or short-term massive feeding trials in which up to 800 mg/kg of body weight was administered orally for 12 days (88). In addition, negative results were reported in a multigeneration study in mice (121) and in mutagenicity tests with *Salmonella typhimurium* and *Schizosaccharomyces pombe* performed on urines of animals fed *Spirulina* for 4 months (12). Careful evaluation in rats of several parameters such as increase in body weight, total body nitrogen, and levels of serum total protein and albumin led to the conclusion that *S. platensis* and *S. maxima* represent protein sources as good as legumes, including soybeans, but inferior to the best protein source, lactalbumin (78). A similar conclusion was reached by assaying protein synthesis in vitro by ribosomes isolated from skeletal muscles of rats fed with different protein sources (107). The assay, which is positively correlated with the quality of alimentary protein and hence is a measure of the nutritional value of a protein source, revealed that the nutritional quality of *S. platensis* was acceptable although lower than that of casein plus methionine. If methionine was added to the *Spirulina* diet, its nutritional quality improved, although it never reached that of casein and methionine. Similar conclusions were reached for different preparations of *Saccharomyces cerevisiae*, the most typical source of SCP. In hens, administration of *Spirulina* slightly stimulated egg production but not the eggs' size, whereas it greatly increased yolk color (10, 13). In young shrimps, prawns, and fries of different fish of commercial importance, *Spirulina* sustained increases in body weight and length as well, if not better, than standard diets. In addition, for a number of species, sexual maturity was reached earlier, thus allowing shorter breeding cycles. Finally, *Spirulina*-fed fish acquired a pink-yellow pigmentation of the meat which appears to be of certain importance from a commercial point of view.

Becker (9) has summarized the standard parameters used to evaluate the nutritional quality of a protein source. The values reported for *S. platensis* are lower than those of a standard protein, casein, but similar to those found for *Scenedesmus acutus* and superior to those reported for two other algae under test (Table 4).

TABLE 4. Nutritive value of *S. platensis* and various algae[a]

Algae	Protein efficiency ratio[b]	Net protein utilization[c]	Biological value[d]	Digestibility coefficient[e]
Spirulina platensis	1.80	62.0	75.0	83.0
Scenedesmus acutus	1.93	65.8	80.8	81.4
Uronema sp.	1.43	46.0	55.0	82.0
Coelastrum sp.	1.68	57.1	76.0	75.1
Casein (standard)	2.50	83.4	87.8	95.1

[a] Data from reference 9.
[b] Calculated from the gain in body weight and protein consumption.
[c] Percentage of N consumed which is retained in the body.
[d] Ratio of N adsorbed to total N intake.
[e] Proportion of food N absorbed.

There are few and incomplete reports of experiments on humans. Pirie (119) quotes experiments performed in Mexico in which 20 to 40 g of dried *S. maxima* was given daily to athletes for periods ranging from 30 to 45 days "with good results." Similarly, reports of favorable results obtained in the case of Mexican children or infants suffering from severe malnutrition have been cited (27, 40). In a more detailed study, diets in which up to 50% of the protein came from *S. maxima* were fed through a plastic tube to five undernourished adults for periods varying from 4 to 5 days (141). A significant weight gain and a positive nitrogen balance were observed and no side effects were reported. Of the other biological parameters evaluated, only a modest increase in the serum, but not in urinary, uric acid was noticed.

A careful evaluation of the consumption of *S. platensis* in the Chad area was reported in 1976 (33). It appears that the area in which Dihé is eaten regularly is restricted to a fairly limited region, east and northeast of Lake Chad, with a total population of ca. 300,000. Of this population only the quantitatively most important ethnic group, the Kanenbou, consumes Dihé regularly, whereas its consumption is nil among the fishermen living around the lake and the nomads north of it. Among the Kanenbou, Dihé is eaten frequently; depending on the season, Dihé is present in 7 of 10 meals. Direct consumption of the Dihé biscuits takes place only for superstitious reasons among pregnant women because of the belief that its dark color will screen the unborn baby from the eyes of sorcerers. In general, Dihé is eaten as a constituent of a number of sauces that always accompany the standard millet meal. The dry Dihé is pounded in a mortar and the powder is suspended in water. Salt, pimento, tomatoes, and, if available, beans, meat, or fish are added to complete the sauce. In a meal, a person eats approximately 10 to 12 g of Dihé which satisfies at most 8% of the caloric need and little more than 10% of the protein requirement. That Dihé may represent an "emergency" sort of food may be inferred from the finding that its consumption decreases when the economic conditions, or the local availability, allow consumption of meat or fish. However, during periods of severe famine, Dihé is still consumed extensively although one expects that these periods may often be the result of severe droughts that may cause drying of many temporary lakes, thus reducing the supply of *Spirulina*. No information is available to indicate if, in the past, Dihé represented a quantitatively more important source of food or if its consumption was geographically more widespread than today. It was pointed out that although certain lakes, such as that of the oasis of Ouniangakébir, 1,000 km northeast of Lake Chad, contain a population of *S. platensis* as abundant as that of Lakes Rombou and Bodou, no record was found for a local consumption of Dihé (91). Yet, it seems rather unlikely that, during periods of famine, the information about the presence in the lakes of an easily available food source should not have arrived from the neighboring populations.

CONCLUSIONS AND PROSPECTS OF THE UTILIZATION OF *SPIRULINA* AS A FOOD SOURCE

First, one may ask whether there is any sense today in looking for nonconventional sources of protein such as SCP for animal and, possibly, human nutrition after the failure in the last 2 decades in developing its production, at least in western countries. Second, but perhaps even preliminary, is the question: does the so-called protein gap in human nutrition that polarized the attention of nutritionists, food scientists, and agronomists on the production of protein-rich foodstuff still exist? If the answer to these two questions is affirmative, one may then ask whether *Spirulina* has characteristics both intrinsic (e.g., chemical composition, toxicity) and extrinsic (e.g., production technology, yield) which justify singling out this organism among all those recognized as possible sources of food or feed.

Concerning the first question, it must be considered that even today starvation and malnutrition are still widespread in vast portions of the world and that, notwithstanding the success of the various "green revolutions," present-day agriculture seems incapable of satisfying the most basic human need, adequate nutrition. The production of microorganisms to be used as food or feed has evident advantages. It does not compete with conventional agriculture for land, it is less dependent on favorable weather conditions, and its yields, in terms of surface or time, are much higher than those of agriculture. The negative outcome of the attempts at SCP production (mostly yeasts on hydrocarbons) in the 1960s and 1970s in western countries was the result of the increase in petroleum costs and of the negative reaction of the potential consumers afraid, rightly or wrongly, of the possible detrimental effects on health associated with the direct consumption of SCP or even of meats from animals fed these biomasses. Yet production and use of SCP in the form of yeasts grown on petroleum derivatives are increasing in other countries. Indeed, for instance, in the U.S.S.R. its annual production was approaching 5×10^5 tons already in 1977 (90a). (Ironically, it is possible that some of the countries that have banned the use as a feed of petroleum-grown SCP regularly import meat from animals fed this type of SCP.) Thus, production of microbial biomasses other than those grown on petroleum derivatives is still conceivable, as demonstrated, for instance, by the British development in the 1980s of the mass production on methanol of the bacterium *Methylophilus methylotrophus*.

Even if the protein gap is now a bit passé (117, 179), there is no doubt that one of the deleterious effects of undernutrition is due to insufficient protein ingestion. It appears that protein deficiency, especially in very early life including that before birth, results in serious and irreversible damages to body development and mental health. Yet, the typical crops of the world areas where starvation or malnutrition occur regularly are energy rich but protein insufficient (rice, cassava, wheat, etc.) (77). Thus, there appear to be regions of the world in which calorie intake may be sufficient but physical and mental development, especially of children, is impaired by lack of an adequate protein supply. In many areas of Asia, for instance, over the last 2 decades rice and wheat production have increased significantly but production of legumes, a source of protein complementary to that of cereals, has fallen drastically (67). Therefore, it still appears desirable to produce protein to be used, directly or indirectly, for human consumption in a way that complements, rather than competes with, traditional agriculture.

In this context, therefore, the possible exploitation of *Spirulina* as a source of protein must be looked at in the light of the following considerations.

(i) Natural alkaline lakes, in which *S. platensis* and *S. maxima* grow abundantly if not exclusively, are usually found in arid areas of the tropics and subtropics where malnutrition is often endemic.

(ii) The requirements of a very alkaline pH of the growth medium ensures that carbon dioxide is retained in the waters in contrast to the rapid loss observed at the acid pH required by unicellular algae such as *Chlorella*, *Scenedesmus*, and *Euglena*. The alkalinity of the medium drastically reduces growth of the majority of other microorganisms, including those pathogenic to humans and other animals.

(iii) The spiral shape of the trichome and the presence of gas vacuoles result in the formation of floating mats that may be easily harvested by gravity filtration, thus reducing considerably the energy requirements associated with the recovery of single-cell microorganisms.

(iv) *S. platensis* and *S. maxima* have an extremely high protein content (up to 70% of the dry weight), thus representing one of the richest protein sources of plant origin. The protein quality is among the best in the plant world and any amino acid unbalance may be easily corrected.

(v) Like all other microbial cells, *Spirulina* contains vitamins and growth factors, but its γ-linolenic acid content, a candidate growth factor for humans, is the highest after milk and the oil of the evening primrose (*Oenothera biennis*).

(vi) The concentration of nucleic acids is among the lowest recorded for microbial cells considered for use as food or feed.

(vii) *Spirulina* cells are enclosed by a thin trichome sheath and by a murein-containing cell wall which make *Spirulina* protein more easily digested by animals than that in yeasts and unicellular algae (7).

(viii) Yields per unit area are, at least in the laboratory and small pilot plants, spectacular, and these figures become even more impressive if expressed in terms of protein yield (Table 5). Thus, it has been calculated that the amount of land necessary to satisfy the yearly protein requirement of a human is ca. 5 ha for meat from cattle on grassland, slightly less than 1 ha in the case of wheat, and approximately 10 m^2 in the case of *S. platensis* (171). Although the production figures have not been tested in large-scale industrial plants, it must be remembered that

TABLE 5. Yields of traditional crops[a] and of cultures of *Spirulina*

Crop	Total yield (tons/ha per yr)	Protein content (%)	Protein yield (tons/ha per yr)
Wheat	6.7	9.5	0.64
Maize	14	7.4	1.04
Rice (hulled)	8	7.1	0.57
Soybeans	4	35	1.4
S. platensis[b]	60–70	65	39–45
S. maxima[c]	40	70	28

[a] Data from reference 11.

[b] Estimated for production plants in Israel (11, 130).

[c] Calculated for Lake Texcoco (40).

nothing so far has been done to improve the strains or the culture conditions in terms of medium, plant design, or operation. In addition, cultures may be performed on marginal land unsuitable for conventional agriculture and utilizing industrial surpluses such as CO_2 from fuel combustion, warm waters from cooling plants, etc.

(ix) As for all photosynthetic organisms, the growth requirements of *Spirulina* are minimal. However, compared with traditional crops, cultures in open systems of *Spirulina* and of other microorganisms as well have a very high water requirement. Especially in tropical or subtropical climates, evaporation causes a significant loss of water that may be only in part compensated by rainfall. Thus, it has been calculated that microalgae, and certainly also *Spirulina*, grown in open ponds consume more water per unit area (25,000 m^3 of water per ha) than even rice (17,000 m^3 of water per ha) (116). Yet, if water consumption is calculated in terms of the protein yield, microalgae have water requirements (1,000 m^3 of water per ton of protein) that are lower than those of traditional crops, including soybeans (7,000 m^3 of water per ton of protein).

(x) Extensive nutritional and toxicological tests in a variety of animals indicate that *Spirulina* is a valuable and safe source of protein. From a nutritional point of view, although inferior to the best protein of animal origin, *Spirulina* protein ranks as one of the best protein sources of plant origin.

(xi) Records exist that, since time immemorial, *Spirulina* has been a component of the everyday diet of certain human populations and that it is still the main source of food of some species of birds.

Development of mass production of *Spirulina* depends on the availability of reliable data on the economics of the process, at least at the pilot plant scale, possibly on year-round operation. It would be equally important to evaluate the performance in terms of growth velocity, yield, chemical composition, and susceptibility to contamination by other microorganisms of the strains so far available when utilized on continuous or semicontinuous processes. On a laboratory scale, it may be especially fruitful to search for other strains or for the isolation of mutants endowed with more favorable characteristics (faster growth, better yields in terms of total biomass, or of specific cell constituents, capacity to grow at temperatures above or below the optimal ones for the existing isolates, etc.). Finally, any extensive genetic alteration of the presently available strains depends on the unraveling of the mechanism for genetic recombination, if it exists, or the development of other means to manipulate *Spirulina* genetically.

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