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World of Microalgae - Scope of Bioprospecting

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Microalgae and their Importance

Microalgae constitute a diverse group of prokaryotic (Blue green algae) and eukaryotic (Protists and unicellular algae) organisms which account for about 50% of global organic carbon fixation (Banares 2004). They are the chief producers and base of food chain in aquatic trophic structure. Recently these photosynthetic microbes have gained a great global consideration in various areas of R&D. Due to their high nutritive values and unique chemical composition, use of microalgae as food (e.g., Spirulina), feed and fertilizer is a years old practice. These autotrophs are important sources of proteins, PUFAs (poly unsaturated fatty acids), pigments (e.g., â Carotene) and vitamins. One of the latest tendencies in microalgal biotechnology is the production of bioactive compounds and their use in pharmaceutical, neutraceutical and cosmetic industries. Biofuels from microalgae are globally accepted as new generation biofuels. Microalgal phycoremediation has been proved to be the best technology of bioremediation in CO_2 sequestration and waste water treatments. Microalgal "green cell factories" are also succeeded in the field of transgenics. Present demand for microalgae for various applications has led to the large scale cultivation and thereby dramatic advancements in the microalgal industry. Their global distribution along with great adaptability to difficult agro-climatic conditions makes microalgae an appropriate commercially cultivable commodity.

Microalgae as food

Chemical composition: - Majority of microalgal applications are based on their chemical composition (Table1). The high protein content makes them an unconventional source of protein (Spolaore 2006). Microalgae can synthesize all amino acids hence provide all essential amino acids to the diets. The average lipid content of microalgae is about 1- 70% but can be 90% of dry weight under special conditions (Spolaore 2006). Microalgal fatty acids include all the essential fatty acids and are the rich sources of long chain poly unsaturated fatty acids (LC-PUFAs) like DHA (docosahexaenoic acid; 22:6(*n* “3)) and EPA (eicosapentaenoic acid; 20:5(*n* “ 3)) (Mansour 2005). Microalgal carbohydrates include glucose, starch, sugars and other polysaccharides. Presence of highly digestible algal carbohydrates make them suitable for human consumption as a whole (Becker 2004). Microalgae are also found rich in all essential Vitamins including A, B_1, B_2, B_6, B_{12}, C, E, Nicotinate, Biotin, Folic acid and Pantothenic acid (Spolaore 2006). The pigments in microalgae include chlorophyll (0.5-1.5% of dry weight), carotenoids (0.1- 0.2%) and Phycobiliproteins.

As food: Today in many parts of the world microalgae are sold as health food or food supplement in the form of tablets, capsules and liquids and they act as antioxidants, probiotics etc., there by positively affecting the health. *Spirulina (Arthrospira platensis* and *A. maxima)* is one among the most popular microalga as an SCP (single cell proteins) and a health food for humans and animals. Other important species like *Aphanizomenon flos-aquae* and *Chlorella* are also consumed as SCPs where as *Dunaliella* and *Haematococcus* are used as source of natural colorants like *â* Carotene and Astaxanthin respectively. Moreover microalgae have various possible health promoting effects (Table B). Incorporation of *Spirulina* in poultry diets showed high growth rates and low mortality. Algal diet showed an improvement in weight gain for pigs and similar positive effects were noticed for ruminants as well (Becker,W, 2004). More over no adverse symptoms and unwanted side effects were reported so far in relation with microalgal consumption.

Table A. General composition of human food sources and different microalgae (% of Dry matter) (Becker, 2004)

Commodity	**Protein**	**Carbohydrates**	**Lipids**
Baker's yeast	39	38	1
Meat	43	1	34
Milk	26	38	28
Rice	8	77	2
Soybean	37	30	20
Anabaena cylindrical	43-56	25-30	4- 7
Chlamydomonas reinhardii	48	17	21
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella salina	57	32	6
Porphyridium cruentum	28-39	40-57	9-14
Scenedesmus obliquus	50-56	10-17	12-14
Spirulina maxima	60-71	13-16	6-7
Synechococcus sp.	63	15	11

Table B: Various health promoting effects of commonly used microalgae in nutrition (P. Spolaore, 2006)

Microalgal species	**Health promoting effects**
Arthrospira sp.	Alleviation of hyperlipidemia; Suppression of hyper tension;Protection against renal failure; Growth promotion of intestinal *Lactobacillus*; Suppression of elevated serum glucose
Chlorella vulgaris	Immune-stimulation; Free radical scavenging; Reduces blood lipids; Efficacy on gastric ulcers, wounds, and constipation; Prevention of Atherosclerosis and hypercholesterolemia; Antitumer action
Dunaliella salina	Ingredient of dietary supplements and functional foods; source of antioxidant and provitamin A
Aphanizomenon flos-aquae	Promotes good overall health

Microalgae for aquaculture

Microalgae are utilized in aquaculture as live feeds for all growth stages of bivalve molluscs (eg. oysters, scallops, clams and mussels), for the larval/early juvenile stages of abalone, crustaceans and some other fish species, and for zooplankton used in aquaculture food chains. Main application of microalgae in aquaculture is nutrition as they play a vital role in rearing of aquatic animals. PUFAs derived from microalgae, i.e. docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) are known to be essential for various larvae. For salmonids microalgae impart colour to the flesh along with other nutritional benefits. *Chaetoceros calcitrans, Skeletonema costatum, Tetraselmis sp., Isochrysis galbana, Pavlova lutheri, Chlorella sp*. etc. are some of the commonly used microalgae in aquaculture. In rearing aquatic animals the “Green water” technique not only improves water quality by oxygen production, pH stabilization etc., but also regulates bacterial population, probiotic effects and stimulates immunity of the reared animals. Microalgae could also be used in the fish feed formulations as growth promoter. The high production cost of microalgae remains a main constraint to the end users viz., fish and shellfish hatcheries. However the selection of appropriate species, use of mixed cultures and uptake of new technologies (like specialised mass-culture) may help to overcome the limitations to some extent (Brown 2002).

Biofuels from microalgae

Microalgae can be a suitable alternative feedstock for next generation biofuels because certain species contain high amounts of oil, which could be extracted, processed and refined into transportation fuels, using currently available technology (Gouveia and Oliveira 2009). Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass; biodiesel derived from microalgal oil and photobiologically produced biohydrogen (Yusuf Chisti 2007). Microalgae have fast growth rate, permit the use of non-arable land and non-potable water, use far less water and do not displace food crops cultures. Rather their production is not seasonal and they can be harvested daily. Microalgae can couple CO_2-neutral fuel production by CO_2 sequestration and produce non-toxic and highly biodegradable biofuels (Schenk 2008). Furthermore the microalgal residue produced can be utilized either as manure or for the production of BTL (biomass to liquid), bio-ethanol and bio-methanol. (Benemann 2000). However microalgal biodiesel needs to be technically feasible and economically competitive, with reference to petrodiesel, microalgal production, harvesting and extraction and biotechnology(Gouveia and Oliveira 2009).

Table C. Comparison of crop-dependent biodiesel production efficiencies from plant oils (Modified from Schenk 2008)

Plant source	Biodiesel (L/ha/year)	Area to produce global oil demand (hectares × 106)	Area required as percent global land mass	Area as percent global arable land	
Cotton	325	15,002	100.7	756.9	
Rapeseed/canola	1,190	4,097	27.5	206.7	
Jatropha	1,892	2,577	17.3	130	
Oil palm	5,950	819	5.5	41.3	
Algae (10 g m”2 day”1 at 30% TAG)		12,000	406	2.7	20.5
Algae (50 g m”2 day”1 at 50% TAG)		98,500	49	0.3	2.5

Microalgae as biofertilizers

Cyanobacteria, the largest prokaryotic group of microalgae play a major role among the N2-fixing microorganisms in rice fields. They are ecologically fit and economically feasible than the chemical fertilizers (Mishra and Pabbi 2004). Free living forms have a modest potential of about 30kgNha^{-1} crop cycle^{-1}, which may translate to a yield increase of 300-450 kg ha^{-1} (Roger, P.A, 2004). The symbiotic association of *Anabaena azollae* with the fresh water fern Azolla is of great agronomic significance, as this has been used as green manure in rice fields for centuries, in Vietnam and China. Other important species are *Nostoc*, *Aulosira* and *Tolypothrix*.

Microalgal high value compounds and other bioactive products

a. Fatty acids

Fatty acids play important roles in membrane transport, energy storage and chemical precursors in many materials (Masaki ota, 2009) and poly unsaturated fatty acids (PUFAs) have many neutraceutical and pharmaceutical applications (V.Patil et. al., 2006). Eicosapentaenoic acid (EPA, C20:5 ù-3), docosahexaenoic acid (DHA, C22:6 ù-3) arachidonic acid (AA, C20:4 ù-6) and ã linolenic acid (GLA, C18:3 ù-6) are the most important among them. Recent studies indicate that DHA is essential for the development of nervous system and retina (A.Sukenik, 1999) and EPA to be effective in coronary disease and in certain types of cancer (Alonso and Castillo, 1999). Fish and microalgae are the primary sources of these PUFAs. Due to the negative aspects and drawbacks of fish oil (T.Tonon et al., 2002; V.Patil et. al., 2006), and as fishes obtain these PUFAs from microalgae through food chain, microalgae themselves are found to be an alternative source of good quality PUFAs (Table D). PUFA content in microalgae can also be modified by environmental factors like, light, nutrients, temperature etc. and by genetic approach (Alonso and Castillo, 1999) and this kind of strain improvement required to make PUFA production economically feasible for industrial production.

Table D. Particularly interested microalgal PUFAs, (modified from Pauline Spolaore, 2006)

PUFA	Potential Application	Microalga producer
GLA	Infant formulas for full term infants Nutritional suppliments	*Arthrospira*
AA	Infant formulas for full term/pre-term infants Nutritional suppliments	*Porphyridium*
EPA	Nutritional suppliments Aquaculture	*Nannoccloropsis, Phaeodactylum, Nitzschia, Porphyridium, Monodus*
DHA	Infant formulas for full term/pre-term infants Nutritional suppliments Aquaculture	*Crypthecodinium, Schizochytrium, Isochrysis, Pavlova*

b. Pigments

Carotenoids: Microalgal carotenoids include â-carotene, astaxanthin, lutein, violaxanthin, lycopene, zeaxanthin etc. Among them â-carotene (from Dunaliella) and astaxanthin (from Haematococcus) are the most important commercially used ones. They are natural colourants with antioxidant property, hence used as food colorants, and in cosmetics. â-carotene act as provitamin A and have anti-inflammatory properties. The green halophilic flagellate D. salina can produce â-carotene up to 14% of its dry weight (Spolaore, 2006). Dunliella products like powder or extract for human use and for feed use are available @ US$ 300-3000/kg. Astaxanthin is produced from *H. pluvialis* (up to 1.5 – 3% of dry wt.) and priced US$ 2500/kg. Salmon feed industry is the major consumer of astaxanthin, but human neutraceuticals and cosmetics industry have also show shown interest (Spolaore, 2006).

Phycobiliproteins: Phycobiliproteins (present in cyanobacteria, red algae and cryptomonads) are natural dyes with health promoting properties and pharmaceutical and cosmetic applications. They are highly sensitive fluorescent reagents, hence can serve as labels for antibodies, receptors etc., and are used in immunolabelling experiments and fluorescent microscopy or diagnostics. The cyanobacteium *Arthrospira* and the rhodophyte *Porphyridium* are the main commercial producers of phycobiliproteins, i.e., phycocyanin and phycoerythrin (Spolaore, 2006).

c. Others

Polysaccharides: Sulphated polysaccharides from red microalgae (Porphyridium, Rodella and Rhodosorus) are used as gelling agents, stabilizers, thickeners and emulsifiers in food stuffs, paints, photographic films, pharmaceuticals etc. These polysaccharides have also applications in human and animal health as dietary fibres and antiviral agents (S. Arad, 1999).

Polyhydroxyalkanoates (PHAs) (Bioplastics): PHAs are thermoplastic polymers produced by many organisms including microalgae. High molecular weight PHAs can be produced and accumulated by Cyanobacteria in the form of cytoplasmic amorphous granules with material properties similar to conventional synthetic plastics. Their biodegradability and high moisture resistance makes PHAs superior to other cheaper verities of materials used in place of synthetic plastics.

Antioxidants: Due to the phototrophic life and high oxidative stress, in microalgal cell there are antioxidative scavenger complexes producing antioxidative components. These natural antioxidants have applications especially in sun protecting cosmetics, neutraceuticals and in therapy of oxidation associated diseases (O. Pulz and W. Cross, 2004)

Antibiotics: Bioactive compounds from microalgae like fatty acids (GLA and EPA), pigments (carotenoids) and many others are proved to be antobiotically active as antibacterial, antifungal, antialgal, antiprotozoan substances (Michael A Borowitzka, 1999)

Antivirals: Cyanobacteria are the most significant sources of antivirals. Some of them are identified and reported for the activity against HIV and HSV-2. Calcium spirulan, a sulphated polysaccharide from *Spirulina platensis,* inhibits replication in HSV, HIV etc. Similar activity is also noted from the polysaccharides of red algae (Michael A Borowitzka, 1999).

Anticarcinogens: Cytotoxic and antitumor and antineoplastic substances are mainly isolated from Cyanobacteria, Dinoflagellates and Cryptophytes with inhibitory activity for proliferation of a variety of mammalian carcinomas and tumors.

Microalgae in Bioremediation - Phycoremediation

In Microalgal bioremediation, microalgae are used for the removal or biotransformation of pollutants (nutrients and xenobiotics) from waste water and CO_2 from polluted air. Microalgae can also be used as bioindicators of various pollutants including pesticides and herbicides.

Advantages of microalgae over other methods

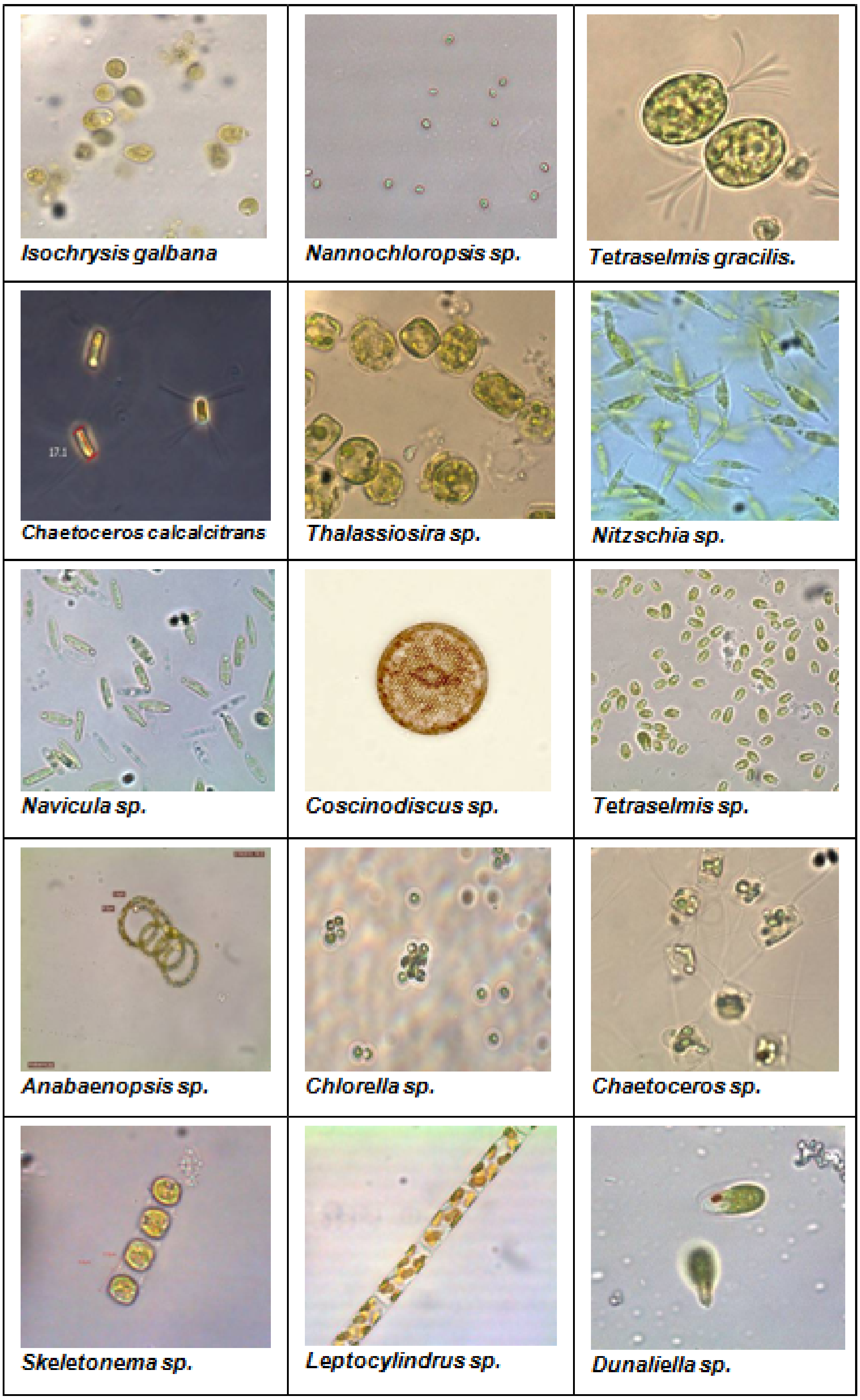
- tackle more than one problem at a time
- natural hence environmentally safe
- more selective and specific in treatments
- no sludge generation
- easy to handle; no need of specialists for operating
- no foul smell
- Oxygenation of environment
- CO_2 removal
- Co-production of biofuels, biofertilizer, etc.

Microalgae in Transgenics

Microalgae represent a much simpler system for genetic manipulations compared with higher plants and other complex eukaryotic hosts. Through metabolic engineering microalgae could be transformed into green cell factories for the production of many heterologous proteins, high quality mammalian proteins (hormones or anibodies) and many other industrially valuable compounds. *Chlamydomonas reinhardtii,* a chlorophyte, is the first and best studied transformation system (for producing antigenic protein by chloroplast transformation) (Rosa Leo Banares, 2004). *Phaeodactylum, Arthrospira, Chlorella, Navicula, Thalassiosira* etc. are some of the other successfully transformed microalgae. Modified organisms are used not only for the production of special compounds but also for bioremediation, Moreover controlled and contained growth of microalgae in photobioreactors minimizes the ethical issues related to GMOs.

Conclusion and future perspectives

Microalgae have been explored for millennia for various purposes like, Arthrospira (as food) in Africa and Nostoc (as fertilizer) in China. Their countless applications range from nutrition and bioactive compounds to cosmetics. Present world is facing challenges like pollution, global warming, water scarcity, health disorders and demand for novel rich nutritional sources. Microalgae can be a unique solution for all such harms and problems. Carbon dioxide - neutral fuel production and discovery of new drugs seems to be the most promising ones. In addition these rich microalgae can be cultured in saline, hyper saline or any other unused water bodies which can save a large volume of fresh water. Among the known 30,000 species (world wide) very few have been explored and cultivated in an extensive way. A country like India can explore marine microalgal diversity and exploit its coastal waters for their production shows great prospective. The genetic modification of microalgae is also a challenging approach. Rather new technical approaches should be developed in mass culture, in designing photobioreactors and in processing methods which will support the extensive production systems. (Spolaore 2006).



Some important microalgal isolates of CMFRI from Indian coast

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