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Indigenous algae for local bioresource production: Phycoprospecting

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ABSTRACT

Photosynthetic algae represent a large and diverse group of organisms that have only a limited history of characterization and exploitation. The application of resource production from algae is relatively untapped, with the potential to produce fuels, food, fibers and nutraceuticals on a large scale. Methods to screen for indigenous species of algae have improved and can allow communities to prospect for algae suited to regional needs. When cultured locally, indigenous algae are adapted to the prevailing regional abiotic and biotic factors. Native algae commonly inhabit local waste resources and pose no risk of becoming noxious invasives. Methods for culturing algae can utilize anthropogenic waste resources including wastewater nutrients and CO_2 from fossil fuel combustion. While genetic engineering may have a role in helping future algae production succeed, the majority of algae species have yet to be identified or characterized and the genetic diversity of these unknown species may offer significant but currently unknown benefits for bioresource production. Recalcitrant problems of culture stability, biomass density, harvesting, and product refining may be overcome by exploring native biological material. Selecting indigenous algae with intrinsic characteristics amenable to bioresource production and waste mitigation – *phycoprospecting* – is the most sustainable path forward for widespread algae-based bioresource development. Our recent efforts in phycoprospecting of local habitats revealed a diversity of algae with significant lipid content.

Nature's culture collection

Algae are ubiquitous and have been evolving on Earth for billions of years. They are the primary producers for the majority of life on the planet. Exploring this existing, self-maintaining, and vast collection offers a rich base for global biotechnological innovation and application. Native organisms have long been naturally selected to their local regions. Local species are *a priori* adapted to the prevailing regional abiotic and biotic factors, and thus are evolutionarily primed for local bioresource production. They commonly inhabit local waste resources and pose no risk of becoming noxious invasives. Furthermore, local algae provide an ideal platform for additional strain development and optimization. Exploring this biota offers a diverse base of organisms naturally engineered to regions that have needs for waste treatment and bioresource production, facilitating development of regionally-based algal agriculture.

As the conceptual framework of this agricultural industry matures, it is increasingly apparent that the critical factor in its profusion lies in understanding how to adapt the technology to suit the biology. Harnessing algae for societal energy and bioresource production is still in its nascent stages and suffers most from a general lack of application, as opposed to centuries of cultivation and selection experience for the traditional agricultural crops upon which world populations are reliant. Algae are a diverse polyphyletic group of organisms lumped together largely for the sake of convenience. The richness of algal species is spread across evolutionary lineages, with organisms more distantly related than the fungi are to man (Fig. 1). Many of the algae remain unknown to science, giving logical heed to explore this realm for potential application. To further illustrate this point, only 15 of the currently known microalgal species are cultivated in some applied form for use in nutraceuticals, cosmetics, aquaculture feeds, or for wastewater treatment (Raja et al., 2008). Of these 'domesticated' algae, only a few species are cultivated at substantial levels, which are themselves trivial when compared to the annual global production of maize and soya (World Agricultural Supply and Demand Estimates 2010) (Table 1). Furthermore, the estimated number of unknown species for all clades of algae is projected to be two orders of magnitude greater than those currently known (Andersen, 1992; Norton et al., 1996). In order to propel algal biotechnological applications to an agronomically significant, sustainable and robust level of bioresource generation, regional phycological flora should be investigated for potential adaptation to industrial-scale cultivation. *Phycoprospecting* is advocated as a means to explore the regional biota for their inherent phycological resource potential.

Phycological potential

Photosynthetic algae have exceptional potential for remediating waste resources and transforming solar energy into vital carbon-based

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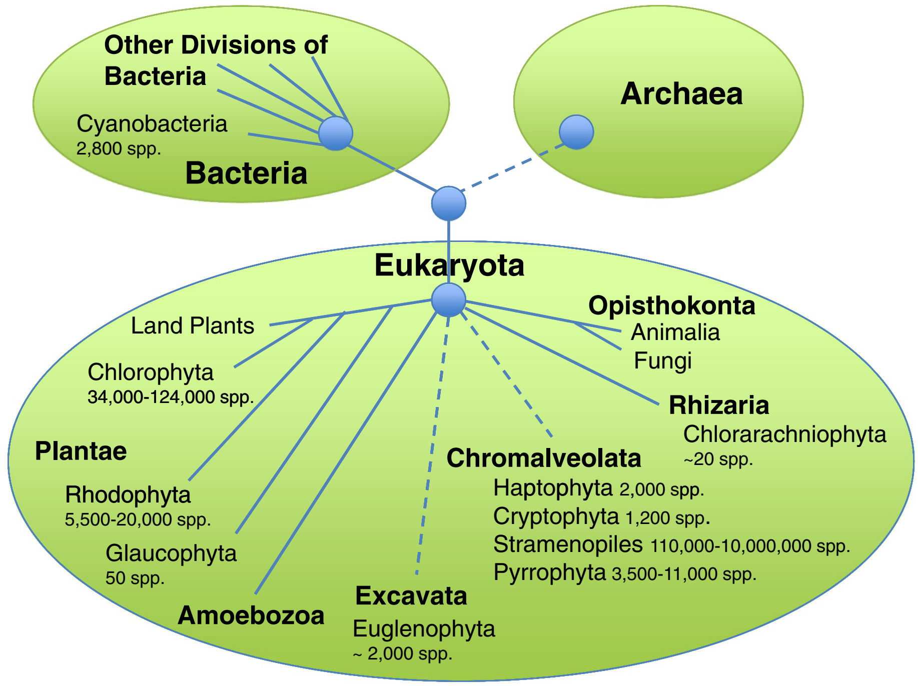

Fig. 1. The diversity of algae. Algae are a polyphyletic group of organisms only grouped together for convenience. Within each algal division, an estimate of the total number of species is given. Other organisms are placed on the diagram to give a sense of the broad diversity of the algae within the tree of life. Adapted from Delsuc et al. (2005), with estimates from Norton et al. (1996).

resources. The possibilities for producing bioresources such as foods, fibers, feeds, fertilizers, pharmaceuticals, and fuels via algae are vast, and depend primarily on: 1) the species of algae cultivated, 2) the method of cultivation, and 3) product demand. Algae with high photosynthetic productivities would make ideal biofuel producers and offer compelling advantages over fossil resources, food-based energy crops, and even physical renewable energy sources. Algal cultivation, when compared with food or dedicated bioenergy crops, is not restricted to arable land or potable water, allowing the creative use of marginal lands, saline aquifers, wastewaters, and oceans for bioresource production. Frequent harvesting of exponentially dividing cells sets algae apart from other crops by allowing a smaller physical and ecological footprint to provide for large-scale bioresource needs. Algae have a potent, underutilized application in remediating anthropogenic wastes. Utilizing waste resources simultaneously abates environmental burdens and substitutes for synthetic nutrients in the algal culturing medium (Lincoln et al., 1996; Wilkie and Mulbry, 2002). Identifying optimal organisms that will serve as future algal crops is a 'work in progress' in the

Table 1
Annual microalgal production in comparison to major terrestrial crops.

Alga[a]/crop[b]	Division	Annual production	Producer country	Application/product
Spirulina	Cyanophyta (cyanobacteria)	3000 tonnes dry weight	China, India, USA, Myanmar, Japan	Human nutrition Animal nutrition Cosmetics Phycobiliproteins
Chlorella	Chlorophyta (green algae)	2000 tonnes dry weight	Taiwan, Germany, Japan	Human nutrition Aquaculture Cosmetics
Dunaliella salina	Chlorophyta (green algae)	1200 tonnes dry weight	Australia, Israel, USA, China	Human nutrition Cosmetics β-carotene
Aphanizomenon flos-aquae	Cyanophyta (cyanobacteria)	500 tonnes dry weight	USA	Human nutrition
Haematococcus pluvialis	Chlorophyta (green algae)	300 tonnes dry weight	USA, India, Israel	Aquaculture Astaxanthin
Crypthecodinium cohnii	Pyrrophyta (dinoflagellates)	240 tonnes DHA oil	USA	Docosahexaenoic acid (DHA) oil
Schizochytrium spp.	Labyrinthista	10 tonnes DHA oil	USA	Docosahexaenoic acid (DHA) oil
Zea mays (Maize)	Magnoliophyta (flowering plants)	798×10^6 tonnes dry weight	Global Production	Human nutrition Animal nutrition
Glycine max (Soya)	Magnoliophyta (flowering plants)	212×10^6 tonnes dry weight	Global Production	Human nutrition Animal nutrition

[a] Adapted from Spolaore et al. (2006).
[b] World Agricultural Supply and Demand Estimates (2010).

application of this promising biotechnology. Selecting indigenous algae with intrinsic characteristics suitable for both bioresource production and waste mitigation is the most sustainable path forward for widespread algae-based bioresource development.

Cultivation for resource production

With appropriate biological and technological innovations, renewable resources can be produced from algae without compromising agricultural land and freshwater resources, or promoting land degradation. A technology of this robust nature is equally applicable in developing and developed nations. Modern algal culture can be tailored to meet regional needs or deficiencies, providing the raw feedstocks for bioresource processing. Many species produce high-quality proteins, essential fatty acids, or vitamins that can supplement local dietary needs. Indeed, the original phycoprospectors were the Aztecs of Lake Texcoco and the Chinese, who harvested naturally prolific cultures of *Spirulina* and *Nostoc*, respectively. These local algae were presumably cultivated and eaten to provide a supplemental protein source.

Algae make up the majority of the trophic base for aquatic ecosystems. As such, techniques developed within the aquaculture industry have become the foundation of many algal isolation and cultivation techniques. Cultivated phytoplankton supply primary nutrition to nursery-reared larval fish, shellfish, crustaceans, and zooplankton. Nutritional qualities of many algae have been investigated for application to aquaculture. Early work in algal isolation and cultivation methods established the cultivation of unialgal and axenic cultures (Allen and Nelson, 1910). Unialgal cultures are often combined to provide the optimal levels of proteins, essential fatty acids, vitamins, and mineral content in aquaculture feeds. The difficulty and cost associated with cultivating large volumes of unialgal and axenic microalgal cultures presages the high value of microalgal-derived aquaculture feeds (Muller-Fuega, 2000). The aquaculture industry now cultivates several major genera for the production of primary and supplemental aquacultural feeds (*e.g. Isochrysis*, *Tetraselmis*, *Thalassiosira*, *Pavlova*, and *Skeletonema*). The combined global production of algal biomass cultivated for aquaculture feed has been estimated at 1000 t dry weight annually (Borowitzka, 1997; Muller-Fuega, 2004). However, this annual production amount falls short of the total need of aquaculture itself, as evidenced by the continued and increasing reliance on terrestrial crops for the production of aquaculture feeds (Naylor et al., 2009).

Modern mass cultivation techniques are currently in a period of rapid development with a plethora of novel ideas almost as diverse as the algae themselves. Large-scale commercial cultivation is, however, limited to only a few species (Table 1). Each of these commercially produced algae is typically cultivated using techniques adapted to the specific organism of interest. Thus, only a few cultivation systems are commonly used on a commercial scale: the classic raceway and paddlewheel (*Spirulina*), center-pivot ponds (*Chlorella*), shallow brine ponds (*Dunaliella salina*), enclosed tubular reactors (*Haematococcus pluvialis*), and enclosed heterotrophic fermentation (*Crypthecodinium cohnii* and *Schizochytrium* spp.). Regional algae farming may need to adapt existing techniques or employ new approaches for the cultivation of local phycoprospects.

The cultivation of marine macroalgae is also a significant industry (Lüning and Pang, 2003). Marine macroalgae are cultivated for the production of high-value foods and functional polysaccharides (*e.g.* carrageenan and alginate). Macro and microalgae are botanically distinguished by: 1) the size of the organism, and 2) the presence of differentiated tissues. To illustrate, kelps are macroalgae that have fronds and rhizoids, analogous to the leaves and roots of terrestrial vascular plants. In terms of bioresource production, macro and microalgae are distinguished by the radically different methods

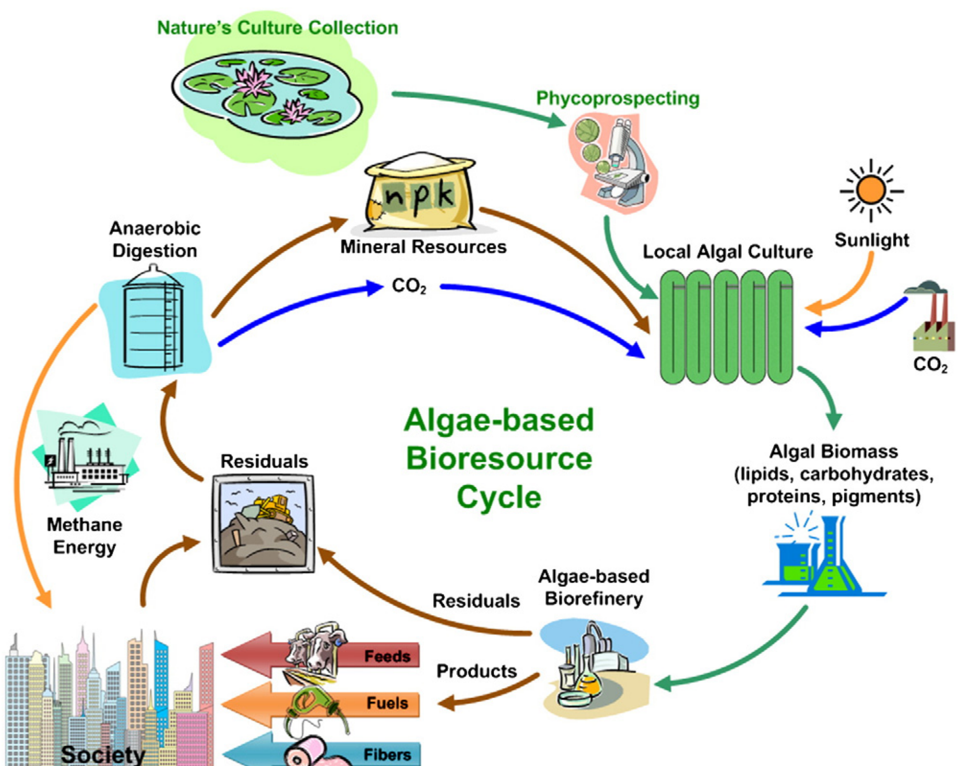

Fig. 2. The algae-based bioresource cycle. Algae with favorable characteristics are sourced and cultivated from the regional flora, resulting in algal biomass which can then be processed in an algae-based biorefinery into consumable products. Sunlight drives the synthesis of algal cells using carbon dioxide and nutrients from local sources. Carbonaceous residuals from processing and consumption can be recycled through anaerobic digestion, producing energy and remineralizing elements required for algal culture operations.

applied in their cultivation and harvesting. Macroalgae typically need a substratum on which to attach and grow, whereas microalgae are typically cultivated in suspension. The large thalli (fronds) of the macroalgae make harvesting relatively simple and less energy intensive when compared to the centrifugation methods commonly used in harvesting microalgae. However, marine macroalgae are commonly devoid of large lipid deposits, instead storing energy in various starches. The application of these organisms for biofuel production relies on the fermentation of starches to alcohol, hydrogen, or methane, which are less valuable than the starches themselves. Discovering macroalgae with the potential to store large amounts of photosynthetic energy as lipids would significantly reduce the cost of harvesting and drying algal biomass. Phycoprospecting promotes the discovery of such novel organisms, which may define the preferred regional methods of algal cultivation.

Algae-based bioresource cycle

Algal bioresource generation can be integrated with human communities to form a sustainable permaculture ecosystem, or an *algae-based bioresource cycle*. As shown in Fig. 2, local species of algae are sourced and investigated from 'Nature's culture collection' for bioresource production. Algal farmers can utilize locally available waste resources (wastewaters, CO_2, and heat) to cultivate desired native algae for biomass feedstock production, which can then be harvested and regionally processed at an algae-based biorefinery into consumable products. Algal cultivation systems integrated with algae-based biorefineries can yield a diversity of bioresources (biodiesel, green gasoline, biojet fuel, isolated proteins, food starches, textiles, organic fertilizers, *etc.*), which mitigate the cost of biofuel production. For example, the alga could be an indigenous variety of *Chlorella* that is grown on local nutrients from municipal wastewater treatment plant effluent and captures CO_2 from nearby sources such as the combustion of coal/oil/natural gas, fermentation and industrial facilities, cement plants, landfill gas, or biogas from anaerobic digestion. This alga might produce lipids, proteins or starches that could be processed into biodiesel, nutritional supplements, and food products. The organic residuals produced during processing and after consumption can be anaerobically digested to produce biogas (methane and CO_2) and solubilized mineral nutrients. The CO_2 and the nutrients can be reused directly by the algal culture, avoiding the costs associated with supplying these external inputs. In addition to community use as a renewable fuel, the methane can provide energy for on-site processing, including harvesting, drying, heating, or mixing the algal culture.

Utilizing the energy, nutrients and CO_2 held within residual waste materials to provide all necessary inputs except for sunlight, the cultivation of algae becomes a closed-loop engineered ecosystem. Developing this biotechnology is a tangible step towards a waste-free sustainable society. For example, the Israeli company Seambiotic (www.seambiotic.com) is currently cultivating marine algae by utilizing waste CO_2 contained in the flue gas from a coal-burning power station located on the coast in Ashkelon, Israel. Seawater used in cooling the power plant is recycled as the culture medium for the algae. Another example of co-location synergy is provided by the New Zealand company AquaFlow (www.aquaflowgroup.com) which is cultivating wild algae on municipal wastewaters. In the latter case, naturally occurring algae are harvested and converted to "Green Crude™", which is then refined to produce various bioresources. These are examples of sustainable algae-based bioresource cycles in development.

Fuels from flora

Biofuels from algae have attracted a revitalized attention worldwide in both the scientific (Chisti, 2007; Hu et al. 2008; Pienkos and Darzins, 2009; Posten and Schaub, 2009) and popular consciousness. The recognized need for fossil fuel replacements is undoubtedly the driving force behind this surge of interest in algae-based fuels, such as biodiesel, biogas, and hydrogen. The U.S. government has recently endorsed (EPA, 2010) and invested (DOE, 2010) in algal fuel technologies. Local algal culturing operations could provide the opportunity to displace fossil fuel consumption, reducing dependence on nonrenewable resources and oil imported from politically volatile sources. Regional algal bioresource generation supports community stability and provides secondary benefits (protein and fiber) not available when petroleum products are used. The U.N. has recently released a document exploring the potential of algae-based biofuels and possible co-products to provide resources for developing nations (van Iersel et al., 2009). Bioresource production via algae is already technically feasible in the laboratory and at a larger scale in the nutraceutical market, for example *Spirulina* production as a health food product. However, recent critical analysis (van Beilen, 2010) shows that the current state of large-scale algal production has a poor energy balance and needs both technical and biological breakthroughs to achieve successful application on a globally significant scale.

Selecting optimal algae

Phycoprospecting of indigenous species has advantages over other methods of sourcing algae from type culture collections and from genetically engineered organisms (Table 2). Screening native algae for species with desirable traits gives a robust biological platform for bioresource production. This biological platform comes equipped with millions of years of adaptation to the local climate and biota, meaning less energy expended on methods of environmental control and sterile techniques. Through optimization efforts of breeding, selected native strains may yield superior organisms for bioresource production. Additionally, in the mass production of bioresources, unialgal cultures may not be advantageous. The cost of maintaining unialgal or axenic status may easily outstrip the value of the product, especially for low-value commodities. In addition to screening individual algae, algal polycultures and their symbiotic interactions may lead to stable and productive "algal agro-ecologies". A sustainable ecological production system would imitate a mature forest ecosystem with steady-state productivity and several dominant species. A polyculture with one or more dominant species may also have an advantage if the species produce different compounds of interest. A hypothetical polyculture might contain a native species of *Chlorella*, high in lipids, and a species of *Euglena*, high in astaxanthin esters. The simultaneous processing of the algal biomass containing the two organisms would give lipids for biofuel production as well as a high-value compound (astaxanthin) to mitigate the cost of the operation. The use of polycultures is being widely encouraged in sustainable agricultural practices, where it is commonly referred to as intercropping. Such a technique encourages natural pest management, pollination, and crop stability. Similar benefits may be realized in the cultivation of algae, yet the current understanding of organism-level interactions is limited and the optimization of polyculture dynamics is certainly in need of further research.

Specific criteria of selection for the production of biofuels from indigenous algae should include biomass productivity, lipid productivity, harvestability of the organism, and oil extractability. Phycoprospecting may improve the efficiency of lipid extraction by yielding organisms with traits amenable to oil recovery. Many algal species lack cell walls, greatly simplifying efficient solvent extraction or cell fractionation. Some algae excrete extracellular lipids (*e.g. Botyrococcus* and some zooxanthellae), which are much easier to recover. Some algae may also be tolerant of certain solvents and allow the extraction of oil from live cells without cell destruction. Huerlimann et al. (2010) investigated several marine microalgae in terms of biomass and lipid productivity on a volumetric basis in different growth media. Such evaluation is needed

Table 2
Advantages and disadvantages of methods for sourcing algae.

Method	Advantages	Disadvantages
Culture collections	Recognized organisms Unialgal and axenic cultures Allows comparison between laboratories Can select for organisms known to produce lipids or high-value compounds	Limited number of species available Unadapted to local climates and outdoor cultivation May not be able to grow on local wastes Easily overtaken by native algae in open ponds May invade local ecosystems
Genetic engineering	Possibility of increased lipid productivity Production of high-value compounds May simplify harvesting by excretion of lipids or high-value compounds Modification of traits to increase productivity	Limited genomic data for algal species Unadapted to local climates and outdoor cultivation High cost of development and containment Negative public perception Risk of genetic transfer May invade local ecosystems
Phycoprospecting	Vast diversity of species available Adapted to local climates and outdoor cultivation Adapted to local wastewaters and aquatic environments Adapted to local biota Native polycultures possible May provide unique traits amenable to bioresource production Applicable in any region regardless of access to culture collections No charge for procurement	Screening practices must be intensive Optimization may take dedicated breeding programs Experiments based on multispecies consortia difficult to translate across laboratories

for utilization of native organisms and local anthropogenic wastes. Additional considerations include the evaluation of waste toxicity on indigenous algae as well as the effectiveness of waste remediation by the algae. Thorough investigations of local species for the dual purpose of waste remediation and bioresource production are limited. Phycoprospecting encourages investigators and future algal farmers to explore, examine, and evaluate their own local culture collections.

Modern methods for screening algae are being adapted from the high-throughput methods developed in the medical and materials sciences. These include fluorescent and infrared analyses using data acquisition instruments such as 96-well plate readers and flow cytometers (Chen et al., 2009; Dean et al., 2010; Mendoza et al., 2008). Fluorescent lipid stains, such as Nile Red (9-diethylamino-5H-benzo [α]phenoxazine-5-one) and BODIPY 505/515 (4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene), as well as the starch stain Safranin O, highlight desirable metabolites and can give a rapid characterization of the subject alga (Cooper et al., 2010; Dean et al., 2010; Huang et al., 2009). With these techniques, portable microscopes and fluorometers can be taken to the field for *in-situ* analyses of standing algal biomass. Alternatively, infrared analysis, which does not depend on stain application but rather detects specific molecular absorption bands to give approximate concentrations, can be used for the detection of many metabolites (*e.g.* lipids, starches, and proteins). This method has recently been applied to detecting changes in algal cell composition during nitrogen starvation (Dean et al., 2010).

A recent review by Mutanda et al. (2011) discusses some of the many different screening methods in detail. These rapid techniques are substantial improvements over traditional analytical methods, which are time consuming and require pure biomass that may not be readily available in the exploration stage of indigenous flora sampling. Applying these modern techniques for rapid algal analysis gives the phycoprospector advanced tools for screening the indigenous algal diversity of any particular region. Local algae are naturally adapted to local conditions and given their diversity may provide the biological breakthroughs needed to advance the successful development of algae-based bioresources. Further, CO_2 and waste nutrient resources are available from electric power and wastewater treatment plants – locations with impacted ecosystems containing indigenous species that may be suitable for mass culture utilizing these resources.

Depending on the criteria of selection, a range of useful materials can be produced from indigenous algal biomass. If the selected organisms are oleaginous, algal lipids can be used to produce non-toxic, biodegradable, and potentially carbon-neutral petrochemical alternatives. Selection for other characteristics such as valuable constituents (*i.e.* carotenoids, polyunsaturated fatty acids, functional proteins or starches, *etc.*), rapid biomass production, and specific wastewater or combustion-gas tolerances will be determined primarily by the local anthropogenic industries and the indigenous algae. Natural selective pressures from human industry on native algae yield organisms primed for waste remediation within the local context.

Microalgae are currently cultivated for such high-value compounds as astaxanthin, beta-carotene, lutein, *etc.* Selection of algae for high-value secondary metabolites has the potential to defray the economic burden of algal mass cultivation, specifically for biofuels. As an example, we have phycoprospected a native species of *Euglena cf. sanguinea* (Fig. 3), which has abundant levels of carotenoids (presumably astaxanthin esters) and large paramylon carbohydrate deposits. This organism was found growing in a pond highly enriched from agricultural run-off.

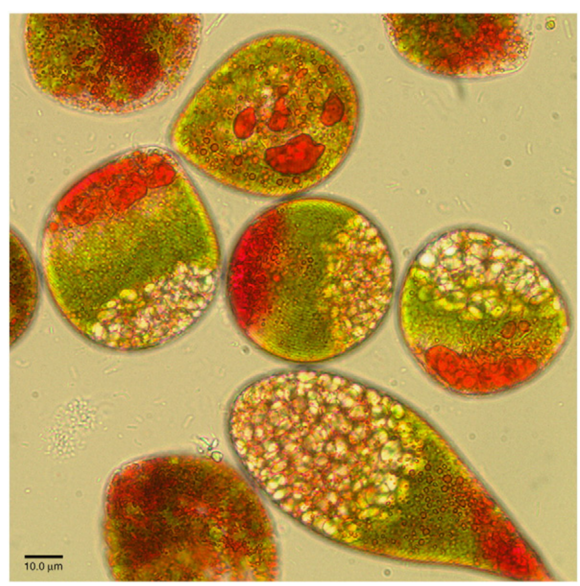

Fig. 3. Indigenous alga with high-value compounds. *Euglena cf. sanguinea* collected from a pond enriched by agricultural run-off. The photo, taken under brightfield transmission illumination, shows distinct regions of red carotenoids (presumably astaxanthin esters), green photosynthetic chlorophyll, and clear paramylon carbohydrate granules (storage material).

In contrast, recent publications on selecting algae for biofuel production propose the use of 'type' strains obtained from culture collections (Rodolfi et al., 2009; Song et al., 2008). While such cultures may have uniform laboratory appeal, they present major challenges to agricultural-scale application. Organisms transferred to new locales often fail to prosper as they are unadapted to climatic or aquatic conditions, or are overwhelmed by competition, predation, and parasitic/pathogenic interactions with the native biota. Often indigenous algae, which have not been selected for advantageous traits, will out-compete the desired algae in open systems (Sheehan et al., 1998). On the other hand, transferred organisms that do thrive in open conditions may pose an unknown exotic-invasive risk to public waterways and aquatic ecosystems. Type strains in closed systems (*i.e.* photobioreactors) need prohibitive inputs of energy *ad infinitum* to maintain their foreign growing conditions and exclude native organisms. Further, type strains would require sterilized wastewater to eliminate indigenous species. Such intensive pretreatment would nullify the economic and environmental advantages afforded by utilizing wastewater in the first place. Type strains may be useful for understanding the dynamic interactions of algae and their environments in repeatable laboratory studies but, due to abiotic and biotic pressures, will likely be impractical for waste treatment or bioresource generation on a globally significant scale.

The use of genetically engineered (GE) organisms is repeatedly proffered as a panacea for the growing pains of algae-based fuels (Beer et al., 2009; Rodolfi et al., 2009; Rosenberg et al., 2008). Genetically tailoring algae as a means to increase desired end-product partitioning, herbicide resistance, light tolerance, oxidation tolerance, or end-product secretion is extolled as the end-all solution for industrial-scale fuel production. While of great intellectual and scientific value, research into GE algae may be severely limited in terms of practical large-scale bioresource production except for the production of high-value pharmaceuticals in closed photobioreactors. Commercial success has been limited despite decades of effort and significant research and development invested. High-lipid algae, modified and grown in laboratory conditions, are notoriously difficult to successfully transfer into mass-culture systems. This is accompanied by the persistent challenge of simultaneously growing a sufficient quantity of biomass and maintaining high lipid productivity, goals which the literature suggests are mutually exclusive (Roessler, 1990; Sheehan et al., 1998; Shifrin and Chisholm, 1981) and which may prove improbable to simply engineer in practice.

Even if successful, several challenges to implementing the practical cultivation of GE algae arise. Algae are naturally adaptable to wide variations in aquatic and soil ecosystems, and can easily spread through abiotic (wind and rain) and biotic means. The escape and spread of GE algae represents an unknown risk and may pose ecological as well as public concerns for natural and commercial waterways, depending on the nature of the genetic modification chosen. Like bacteria and yeast, both eukaryotic algae and cyanobacteria have high generation rates and consequently have the potential to increase the extent of the genetic pool with an engineered trait through both genetic transfer and genetic adaptation. This means that the genetic manipulation of algae to increase herbicide resistance, for example, is unlikely to have long-term sustainable benefits, as indigenous species have a high probability of rapidly acquiring this resistance. The containment of GE algae, like containment requirements for other GE microorganisms, would demand using an enclosed bioreactor system. However, cultivating GE algae for biofuel production would require significant production areas and an escape of GE cells and cellular materials would be inevitable.

While advances in this field will undoubtedly provide key scientific insights, GE algae are not a proven necessity for the production of algae-based bioresources and the threat of escaped GE algae is an unexamined and potentially significant risk. If the GE algae path is chosen and applied on a mass scale for biofuel production, the economic burden of containment and the threat of environmental impacts may severely limit widespread adoption of the technology. Public concern over GE organisms, especially organisms associated with environmental and health calamities (*e.g.* harmful algal blooms), may further suppress the full potential of the technology. Finally, the genetic diversity of algae that have yet to be explored suggests that genetic manipulation of algae may be premature since a wealth of genetic information has yet to be revealed in the many unknown species that await characterization. Genetic engineering tools may, however, be useful in phycoprospecting to test wild strains for known genetic traits that are likely to enhance bioresource production. As an example, molecular probes for ATP:citrate lyase (ACL) genes in a well-characterized species such as *Chlamydomonas reinhardtii* could be used to test prospected cultures for this trait. No oil-accumulating microorganism has yet been reported that does not have ACL activity (Ratledge, 2004).

Phycoprospecting results

To demonstrate that phycoprospecting of indigenous algae has a reasonable chance of success, we phycoprospected various local

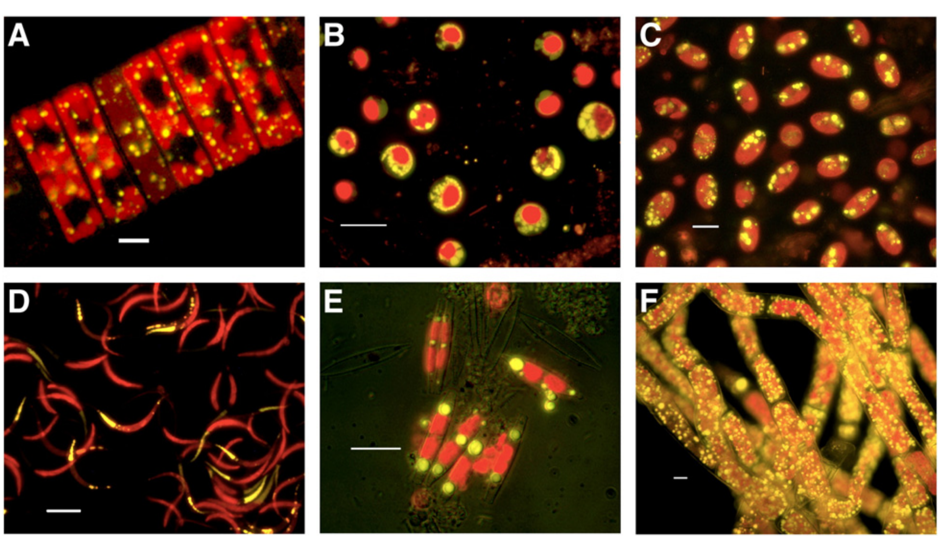

Fig. 4. Diversity of lipid-rich indigenous algae. Algae stained with Nile Red and photographed under epi-fluorescent illumination. Yellow coloration indicates stained lipid bodies and red coloration shows chlorophyll auto-fluorescence. (A) *Fragilaria* sp. from a riverine system adjacent to a major highway. (B) Unidentified chlorophyte collected from agricultural soils. (C) *Chlorella cf. ellipsoidea* from municipal solid waste landfill leachate. (D) *Ankistrodesmus* sp. from municipal solid waste landfill leachate. (E) *Navicula* sp. from a manure lagoon. (F) *Rhizoclonium* sp. from a wastewater treatment facility. (Scale bar = 10 μm).

habitats by sampling the standing algal biomass. Samples were stained for lipids with Nile Red following the methods developed by Cooksey et al. (1987) and observed under epi-fluorescent illumination (Fig. 4A–F). Habitats prospected ranged from human-impacted natural water bodies, agriculturally impacted soils, municipal solid waste landfill leachate, manure lagoons, and wastewater treatment facilities. The diversity of algae found to accumulate significant quantities of lipids, as well as the diversity of habitats sampled, exemplifies the tremendous potential of phycoprospecting.

Conclusion

Given the great diversity of algae, our phycoprospecting hypothesis predicts that many local organisms are potential candidates for bioresource generation, and are simply yet to be investigated or identified as such. Indeed, many algae with favorable characteristics may already inhabit local wastewaters, but lack proper domestication for agricultural production. A concerted scientific effort in regionally-based phycoprospecting for indigenous algae with advantageous characteristics will increase the rate and application of sustainable biotechnological solutions from algae. Recalcitrant problems of culture stability, biomass density, harvesting, and product refining may be overcome by exploring native biological material, facilitating development of bioresource production from algae.

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