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Chlorella, ¿un potencial biofertilizante?

Chlorella, a potential biofertilizer?

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Martha L Ortiz-Moreno[1]; Karen X Sandoval-Parra[2]; Laura V Solarte-Murillo[3]

[1] Biol, MSc, PhD, Departamento de Biología y Química, Facultad de Ciencias Básicas e Ingeniería, Universidad de los Llanos, Villavicencio, Colombia.
[2] Biol, Departamento de Biología y Química, Facultad de Ciencias Básicas e Ingeniería, Universidad de los Llanos, Villavicencio, Colombia
[3] Biol, Departamento de Biología y Química, Facultad de Ciencias Básicas e Ingeniería, Universidad de los Llanos, Villavicencio, Colombia
Email: mlortiz@unillanos.edu.co

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Resumen

Las microalgas son organismos fotoautótrofos con un rápido crecimiento y la habilidad de adaptarse a diversos ambientes. Convierten el dióxido de carbono en biomasa y debido a esto, se considera que tienen gran potencial biotecnológico. La biomasa algal puede usarse en la industria alimenticia y de compuestos bioactivos, en la producción de biocombustibles, en la bioremediación y biofertilización. Como biofertilizantes, las microalgas clorofitas y cianofitas, producen polisacáridos (mucílago) que pueden evitar la erosión, mejorar la estructura y el contenido de material orgánica de los suelos, y aumentar la concentración de iones en los cultivos. Reduciendo de esta forma la necesidad de fertilizantes químicos convencionales. El uso de estas microalgas como biofertilizantes se denomina algalización. Durante este proceso se usan principalmente clorofitas por su alta tasa de crecimiento, la facilidad de su cultivo a gran escala, y su adaptación a las condiciones del suelo. El género *Chlorella* es de gran interés porque diversos estudios han mostrado que puede ayudar en la fijación del nitrógeno, mejorar las propiedades físicas y químicas del suelo, y producir sustancias que promueven el desarrollo de la planta y el control de infecciones. Por esta razón, las microalgas del género *Chlorella* representan una alternativa viable para la biofertilización, generando beneficios no solo para la producción agrícola sino también para el medio ambiente.

Palabras clave: algalización; cianofitas; clorofitas; mejoramiento de suelos

Abstract

Microalgae are photoautotrophic organisms with fast growth and the ability to adapt to different environments. They convert carbon dioxide into biomass and are considered to have great biotechnological potential because of it. Algal biomass can be used in food and bioactive compounds industry, in biofuels production, in bioremediation and biofertilization. As biofertilizers, chlorophytes and cyanophytes microalgae produce polysaccharides (mucilage) that can avoid erosion, improve the

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structure and organic matter content in the soil, and increase the ions concentration for crop plants. Thus, reducing the need for conventional crop chemical fertilizers. The use of this microalgae as biofertilizers is called algalization. Algalization uses mainly chlorophytes due to their high growth rate, their simple large scale cultivation, and their adaptation to soil conditions. *Chlorella* genus is of special interest because research has shown that it can help with nitrogen fixation, improve physical and chemical properties of the soil, and produce substances that can promote plant development and infections control. Therefore, microalgae from *Chlorella* genus are a viable alternative for biofertilization, generating benefits for agricultural production and the environment.

Keywords: algalization; chlorophytes; cyanophytes; soil improvement

Resumo

As microalgas são organismos fotoautotróficos com crescimento rápido e capacidade de adaptação a diferentes ambientes. Eles convertem dióxido de carbono em biomassa e, por isso, são considerados com um grande potencial biotecnológico. A biomassa de algas pode ser usada na indústria alimentar e de compostos bioactivos, na produção de biocombustíveis, na biorremediação e biofertilização. Como biofertilizantes, as microalgas clorófitas e cianófitas produzem polissacarídeos (mucilagem) que podem evitar a erosão, melhorar a estrutura e o conteúdo de matéria orgânica do solo, e aumentar a concentração de iões nas culturas, reduzindo assim a necessidade de fertilizantes químicos convencionais. O uso dessas microalgas como biofertilizantes é chamado de algalização. Durante este processo, usam-se eles principalmente clorofíceas por sua alta taxa de crescimento, facilidade de cultura em larga escala, e sua adaptação às condições do solo. A *Chlorella* é de grande interesse porque vários estudos têm mostrado que pode auxiliar na fixação do nitrogênio, melhorar as propriedades físicas e químicas do solo, e produzir substâncias que promovem o crescimento das plantas e o controle de infecções. Por esta razão, as microalgas do gênero *Chlorella* representam uma alternativa viável para a biofertilização, gerando benefícios não só para a produção agrícola, mas também para o meio ambiente.

Palavras chave: algalização; clorofíceas; cianofíceas; melhoramento do solo

Introduction

Microalgae are a polyphyletic microscopic size photosynthetic group, consisting of eukaryotic organisms and prokaryotic cyanobacteria. Microalgae have unique advantages, including high growth rates, easy cultivation, low growth costs and the ability to adapt to different environments, which allow their cultivation to be established in small areas and in regions normally unsuitable for agricultural crops (Wang *et al.*, 2014; Odjadjare *et al.*, 2017). They obtain nutrients from the soil or aquatic habitats, absorb sunlight, capture CO^2 from the air, and produce about 50% of the atmospheric oxygen (Rizwan *et al.*, 2018).

Microalgae convert carbon dioxide into biomass, and are, therefore, considered to have great biotechnological potential with several industrial applications, and also, to contribute with greenhouse effect mitigation. From algal biomass a wide variety of practical and potential products can be obtained: food supplements, lipids, enzymes, proteins, starch, phycocolloids, polymers, toxins, pigments, vitamins, antioxidants, stable isotope biochemicals, and green energy products such as biofuels and bio-ethanol (Chen *et al.*, 2014a; Bleakley and Hayes, 2017; Moreno-Garcia *et al.*, 2017; Pemmaraju *et al.*, 2018). Therefore, are commercially used in human food, nutraceuticals, animal and aquatic feed, personal skin and cosmetics products, and in biomedicine and pharmaceutical industries for the synthesis of anti-inflammatory, antithrombogenic, antiatherogenic, anticoagulant, antiviral, antibacterial and anticancer treatments (Suganya *et al.*, 2016; Wells *et al.*, 2017; Sassi *et al.*, 2019).

Use of algae has been extended to the treatment of wastewaters (removing heavy metal ions), environmental toxicants monitoring, bioassays, effective bioremediation of organic or recalcitrant pollutants, and even as the photosynthetic gas exchangers for space travel (Iyovo *et al.*, 2010; Subashchandrabose *et al.*, 2011; Rizwan *et al.*, 2018). Additionally, microalgae are thought to have great potential as novel low-cost and environmentally friendly expression systems; for instance, production of PHB bioplastic in diatoms and development of composite materials using *Chlorella vulgaris* as filler in various polymers (Wijffels, 2013). Some of the major microalgal species used for commercial production include *Arthrospira* (Spirulina), *Chaetoceros*, *Chlorella*, *Dunaliella* and *Isochrysis*. Nevertheless, microalgae are versatile, unexplored and diverse microorganisms, and hence, there may be seve-ral features which are yet to be discovered and exploited (Odjadjare *et al.*, 2017; Rizwan *et al.*, 2018).

Microalgae can also be employed in agriculture as biofertilizers and soil conditioners. In this role, they have capabilities such as fixing nitrogen, which is used in tropical lowlands, and controlling erosion in temperate climate zones (Iyovo *et al.*, 2010; Odjadjare *et al.*, 2017). Moreover, some allelopathic compounds from microalgae can act as environment-friendly herbicides or biocontrol agents (Liu *et al.*, 2016b).

Microalgae as biofertilizers

The use of chlorophytes and cyanophytes as biofertilizers is called "algalization", a term developed by G.S Venkataraman in the 1970s. In recent years, more and more attention has been paid worldwide to the use of these microorganisms to intensify organic plant production, particularly in the context of a changing climate (*Grzzesik and Romanowska-Duda, 2015*). Algalization has a unique potential to enhance productivity in a variety of agricultural and ecological situations and plays an important role in building up soil fertility, consequently increasing the yield. Use of microalgae as a soil additive has been shown to significantly improve germination, nitrate reduction potential, root volumes, chlorophyll formation, carotenoid accumulation, grain yields, shoots dry weight and plant height (Tripathi *et al*., 2008; Lin *et al*., 2013; Odjadjare *et al*., 2017). This alternative has provided better results than traditional chemical fertilizers and uses mainly chlorophytes due to their high growth rate, their simple large-scale cultivation, and their adaptation to soil conditions. Moreover, it has become an essential component of organic farming, maintaining long-term soil fertility and sustainability ensuring *economic viability (*Kumar *et al*., 2015; *Grzzesik and Romanowska-Duda, 2015).*

Soil-forming characteristics

Chlorophytes and cyanophytes are soil-forming agents because they secrete polysaccharides and mucilaginous substances with high aggregating capacity, which provide the cohesiveness for binding soil mineral particles and thereby help in soil structure formation (Ghosh, 2018). These polysaccharides are the first biological cementing agents providing an initial stabilization of the soil surface, are the first source of organic carbon during soil development, and are also able to decrease rivers bed load (Mager 2010; Arce *et al*., 2019). This mechanism is more potent in microalgae than in other types of organic matter such as compost; since it is capable of avoiding water and wind erosion in the soil, even in desert zones, because the substances exuded contribute significantly to the immobilization of unstable sandy soils and consequently, sand dunes are formed, increasing soil strength, resistance, and fertility (Cólica *et al.,* 2014; Felde *et al*., 2018; Lan *et al*., 2014). Overall, the contribution of these microorganism to soil stabilisation make them essential components of precarious environments such as arid, semi-arid, polar and alpine areas (Chamizo *et al*., 2016; Williams *et al*., 2016) and suitable elements for restoring degraded dryland ecosystems, trigger soil rehabilitation and counteract desertification (Antoninka *et al*., 2016; Adessia *et al*., 2018).

Some of those adhesives polysaccharides exhibit strong intrinsic mechanical properties, and in addition to avoiding erosion, they are able to improve soil structure by inducing microaggregates formation, which can entangle clay particles and form clusters. These clusters or microaggregates, in turn, grow and take the shape of macroaggregates and subsequently of larger soil aggregates. These macroaggregates allow gas exchange and water percolation, and therefore, can release nutrients from mineral particles (Mager and Thomas, 2011). Generally, high soil aggregate stability and greater amounts of stable aggregates are desirable for sustaining agricultural productivity and protection of environmental quality, because they favor high water infiltration rates, provide adequate aeration and enhance root growth (Awale *et al*., 2017; Baumann *et al*., 2017).

Simultaneously, microalgae were reported to reduce the water penetration into the soil by inducing surface sealing and pore clogging, thus impinging on its hydraulic conductivity, allowing the creation of moistened microenvironments that retain water for longer periods than the surroundings and form dew (Fischer *et al*., 2012). Therefore, they can affect water retaining properties of soil, mainly due to their hygroscopicity, becoming a key factor for reducing evapotranspiration, increasing water availability, slowing down water loss and possibly contributing to expedite the recovery of microbial activities after dry periods (Maqubela *et al*., 2009; Lan *et al*., 2010).

Mineral and nutrients source

Besides improving soil properties, polysaccharides and mucilage from microalgae represent a huge source of utilizable carbon for the heterotrophic microbial soil community and adjacent plants, being also involved in physically and chemically trapping nutrients, and so, the level of soil microbial activity is increased (Mager and Thomas, 2011; Chen *et al*., 2014b). These organic matter accumulation leads to rising soil buffer capacity, enhance thermal conductivity and buffer temperature oscillations (Nain *et al*., 2010). In general, soil fertility is improved by the organic matter produced by microalgae (Lin *et al*., 2013).

Furthermore, microalgae play an important role as one of the major sources of nitrogen atmospheric fixation, converting it into bioavailable forms like ammonium, which is required for plant growth. Moreover, mucilage from chlorophytes and cyanophytes, present in the cell wall, allows the concentration and mobilization of macro and micronutrients and ions, which can then be made available to plants and soil by exudation, autolysis and microbial decomposition (Zhuang *et al*., 2014). This permits to reduce the needed amount of crop chemical fertilizers, increase the soil cation exchange capacity (CTC), and improve pH and electrical conductivity of the soil (Osman *et al*., 2010; Rizwan *et al*., 2018). Likewise, microalgae cause a higher phosphorus content due to their ability to produce excretions of enzymes or acidic metabolites that make this element available to plants, thereby increasing their efficiency and availability (Sahu *et al*., 2012; Liu *et al*., 2016a). Additionally, soil microalgae are ameliorators in the reclamation of saline and metals, thereby improving soil quality as well (Lin *et al*., 2013).

Growth-promoting agents

Soil microalgae excrete growth-promoting bioregulators such as hormones, vitamins, sugars, amino acids, and organic acids that benefit plants and may be responsible for their high productivity (Osman *et al*., 2010; Maqubela *et al*., 2012; Awale *et al*., 2017). Specifically, microalgae from *Chlorella* genus produce phytohormones similar to cytokinins identified as iso-pentenyladenine, zeatin, and its conjugated ribosides, which influence cell division and differentiation, and are also important in chloroplast development, apical dominance and delay of senescence. These molecules were identified in tests with hypocotyls from cucumber and bean, showed to increase growth and development of gillyflower and grapevine, and has also exhibited effectiveness in controlling cell division in plants by *in vitro* tests with lilies and rice (Shanan and Higazy 2009; Tarkowsky *et al*., 2009; Hussain *et al*., 2010; Romanowska-Duda *et al*., 2010). In this order of ideas, Hussain and Hasnain (2012), observed that phytohormones of microalgal origin proved to be better at inducing adventitious roots and shoots on internodal and petiolar segments of cabbage than regular plant-derived phytohormones.

Antipathogen activities

In addition to promoting plant growth, chlorophytes and cyanophytes can also help control infections in plants and animals. For example, *Chlorella* and *Nostoc* genus have compounds with antimicrobial and anti-herbivorous activity, such as *C. vulgaris* clorelin, that control the development of bacteria such as *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa;* and phycocyanins that inhibit pathogenic growth of bacteria and fungi (Grzzesik and Romanowska-Duda, 2015; Liu *et al*., 2016b). Besides this direct mechanism, extracts of chlorophytes and cyanophytes can induce phytoalexins production in plants favoring their response against pathogenic microorganisms (Beltrame and Pascholati, 2011). It has also been reported microalgae elicitors that inhibit growth and adhesion of pathogenic bacteria and fungi and triggered systemic acquired resistance in crop plants such as wheat (Rana *et al*., 2012; Raposo *et al*., 2013), and tomato (El Modafar *et al*., 2012). Further, they may support the development of beneficial microorganisms in the rhizosphere of plants and thus avoid colonization by pathogens. For instance, extracts of *C. pyrenoidosa* had shown to stimulate the colonization and development of arbuscular mycorrhizae in papaya and passion fruit (Grzzesik and Romanowska-Duda, 2015).

Antioxidant activity

Microalgae can develop a defense system by producing polysaccharides to cope with oxidative stress. Cyanophytes that develop naturally in the soil have a high tolerance to oxidative stress because their biomass contains different antioxidant molecules (Mohamed 2008). Aqueous extracts of *C. vulgaris, N. ellipsosporum,* and *N. muscorum* have also reported antioxidant activity (Hajimahmoodi *et al*., 2010). To explain this tolerance to oxidative stress, a variety of mechanisms such as extra-cellular detoxification, reduced uptake, efflux and sequestration by polysaccharides have been proposed (El-Sheekh *et al*., 2012). This characteristic makes application of chlorophytes and cyanophytes in seeds good for germination, since in the process of lysing the endosperm and in infections by pathogenic fungi, where high amounts of free radicals are produced, and they can ultimately be neutralized with the algae extracts (Osman *et al*., 2010).

Use of *Chlorella* as biofertilizer

Out of all microalgae, *Chlorella* genus has been most used for biofertilization so far and was the first microalga to be cultivated (Wijffels, 2013). Mainly because *Chlorella* provides with high amounts of macro and micronutrients, constituents or metabolites, such as carbohydrates and proteins (Elarroussia *et al*., 2016), and growth promoting factors, like cytokinins (Kholssi *et al*., 2018). Due to these multiple benefits, several research has been done. Faheed and Fattah (2008), evaluated its effect on lettuce and found an increase of up to 186% in several characteristics like growth, and biomass and pigment content in seedlings that were fertilized with *Chlorella vulgaris*. Chacón (2010), tested suspensions of *C. vulgaris* lyophilized cells and their effect on functional phytochemical compounds content in broccoli, finding increases of up to 100% in ascorbic acid, sulforaphane, and β-carotene in seedlings. *C. vulgaris* was also found to improve germination and growth of rice plants (Zayadan *et al*., 2014; Rajasekaran *et al*., 2015). In 2016, Özdemir *et al*., used this same species as biofertilizer in organically grown tomato production in greenhouses obtaining an increase in plant growth, yield and some fruit qualities like dry weight, total soluble solids, titratable acidity, and vitamin C. Agwa *et al*., (2017) showed that *C. vulgaris* application is efficient and economical in improving soil nutrients for greater productivity of okra. Okra seeds had higher moisture, organic matter and phosphorus when compared to control, and exhibited lower germination time. Moreover, okra plants displayed higher protein, lipid and chlorophyll content, also a higher plant height and fruit number. Furthermore, Schreiber *et al*., (2018) described too, the potential of *C. vulgaris* to accumulate phosphorus and fertilize nutrient-poor soil substrates. Lastly, leaf application of *C. vulgaris* has shown suppression of diseases in strawberry (Kim *et al*., 2014), cucumber seedlings (Abd Elhafiz *et al*., 2015), and grapevine (Bileva 2013).

Other species such as *Chlorella sorokiniana*, produced a higher percentage of germination, root and stem weight, length and total plant biomass when applied in wheat. A greater number of leaves with the bigger surface area were observed in soybean seedlings irrigated with *Chlorella pyrenoidosa* (Dubey and Dubey, 2010). Organic Chinese chives and spinach treated with *Chlorella fusca* showed an increased number and thickness in leaves, better mineral content, and improvement of commercialization yield because of higher fresh weight. Besides, gray mold disease severity was reduced by treatment with *C. fusca* (Kim *et al*., 2018). Positive effects had also been seen in willow plants when biofertilized with *Chlorella*, rooting of cuttings, number and length of formed roots or shoots, plant growth, physiological activity, and fresh and dry weight were found to be significantly increased. Indeed, plants developed faster and showed a higher health status, indicating that *Chlorella* contains a potential source of bioactive compounds that activate several metabolic processes, regulating the growth and development of plants (Grzzesik and Romanowska-Duda, 2015). Similar results were obtained by Grzzesik *et al*., (2017) in this same plant with foliar application of *Chlorella sp*. Two strains of *Chlorella* (*C. oocystoides* and *C. minutissima*), were recommended as biostimulator and liquid biofertilizer for maize crops as well (Al-Shakankery *et al*., 2014; Taher and Mohammed, 2015). Parallel effects were seen in grapes, sunflower, and corn (Grzzesik and Romanowska-Duda, 2015). This overall positive influence on the seed germination and plant growth in different species is possible because of *Chlorella* positive effect on the nutrient uptake which enhances all physiological reactions (Grzzesik and Romanowska-Duda, 2014; Ghiloufi *et al*., 2016; Borchhardt *et al*., 2017).

Finally, *C. vulgaris* has too been tested mixed with other microalgae such as *Spirulina platensis*, both playing a key role in improvement of rice plants, in terms of plant height, number of leaves, leaf area, fresh and dry weight, availability of nitrogen, phosphorus and potassium in plant soil, performance during germination and yield characters (Dineshkumar *et al*., 2018). *C. vulgaris* has also been tried in consortium with plant growth-promoting bacteria such as *Pseudomonas putida*, *Serratia proteomaculans,* and *Stenotrophomonas maltophilia*, in clove pelleted seeds, finding a better development and increased root biomass when comparing to the control (Raposo and De Morais, 2011). Further, this microalga along with cow dung manure has shown effects on growth performance, soil characteristics, macro and micronutrients, and the microbial population at the flowering stage of maize (Dineshkumar *et al*., 2017).

Conclusion

Although application and quantities of algal inoculum, in addition to the experimental conditions, are dissimilar, an overall positive effect of *Chlorella* on plant growth is established in all of the experiments mentioned above. In this context, biofertilizers from these microalgae can provide a suitable supplement to the chemical fertilizers, and organic farming can become a reality in the future with cleaner and healthy harvests, securing food production and human health, as well as protecting the environment and the natural resources.

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Martha Ortiz:https://orcid.org/0000-0003-0172-9111
Karen Sandoval:https://orcid.org/0000-0002-3431-8549
Laura Solarte:https://orcid.org/0000-0002-9097-0734