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Review

Potential strategies and opportunities for the development of *Arthrospira maxima* (Spirulina) processes: A review

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Abstract

The cyanobacterium *Arthospira maxima* (Spirulina) was consumed in Mexico since pre-hispanic times; nonetheless its consumption disappeared after the Spanish conquest. Nowadays, there is the need to look more in depth at the potential that this valuable cyanobacterium offers to several countries with proper climatic conditions for its cultivation. The present review provides an extensive overview of the nutritional and pharmaceutical characteristic of *A. maxima* and discusses some strategies to develop processes oriented to its production in dual purpose systems treating waste and producing biomass for aquaculture / animal feed and more complex strategies oriented to the production of valuable products such as the blue pigment phycocyanin at the industrial level.

Keywords: *microalgae, aquaculture, phycocyanin, biorefineries*.

Estrategias potenciales y oportunidades para el desarrollo de los procesos de *Arthrospira maxima* (*Spirulina*): Una revisión

Resumen

La cianobacteria *Arthrospira maxima* (Spirulina) se consumía en México desde la época prehispánica; sin embargo, su consumo desapareció después de la conquista española. Hoy en día, es necesario profundizar en el potencial que esta valiosa cianobacteria ofrece a varios países con condiciones climáticas adecuadas para su cultivo. En la presente revisión se ofrece un amplio panorama de las características nutricionales y farmacéuticas de *A. maxima* y se examinan algunas estrategias para desarrollar procesos orientados a su producción en sistemas de doble finalidad, tratando los desechos y produciendo biomasa para la acuacultura / alimentación animal, y estrategias más complejas orientadas a la producción de productos valiosos como el pigmento azul ficocianina a nivel industrial.

Palabras clave: *microalgas, acuacultura, ficocianina, biorrefinerías*.

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1. *Spirulina maxima*: History and relevance within the Mexican context

The use of the Mexican microalgal biodiversity, estimated in more than 70 different local species characterized and isolated, could lead to the development of a green circular economy by producing bio products and solving environmental and social development problems (Sosa-Hernández *et al*., 2019). Among microalgal members, cyanobacteria (blue-green algae) are considered as the oldest known organisms with more than 3.4 billion years old of existence (Kiang, 2008). It is considered that after such a long evolution time, cyanobacteria have developed a myriad of mechanisms and chemical compounds to adapt to all habitats on earth and are considered one of the largest and most distinctive groups of photosynthetic bacteria (Nethravathy *et al*., 2019). Cyanobacteria are considered a rich source of valuable biomolecules, which includes high protein content, carbohydrates, essential fatty acids, vitamins, minerals and pigments like carotenoids, chlorophyll a and phyco-biliproteins (Sarma *et al*., 2008). One of the most known and representative cases is Spirulina, a filamentous cyanobacterium recognizable by the spiral-shaped (Fig. 1A), which belongs to the Oscillariacea family (Kulshreshtha *et al*., 2008; Ali and Saleh, 2012). It is important to point out that the name Spirulina has gained popularity due its continue use by marketing companies to sell the product; however, in the last two decades the genus is known now as *Arthrospira* (Vonshak, 1997). In the current review, both names will be used interchangeably with the understanding that Spirulina corresponds now to the genus Arthrospira.

Species of the genus Arthrospira have been isolated from alkaline and saline waters in tropical and subtropical locations. The most known species of the genus are *A. platensis*, mainly found in Africa and *A. maxima*, which was first reported in Mexico (Belay, 2008). The first reference in the history of *A. maxima* is from the pre-Hispanic cultures in the Valley of Mexico by Bernal Díaz del Castillo in 1521, member of the army of the conquer Hernán Cortez (Díaz del Castillo, 1632). In this early reference, it was described that at that period, Spirulina was harvested as a green-blue mat from Lake Texcoco, then it was dried and sold with the name of *"Tecuitlatl"* (stone's excrement), in Tenochtitlan (today Mexico City) market (Fig. 1B). Such edible product "*Tecuitlatl"*, was mentioned until the end of 16th century and then it disappeared from written documents, probably because after the Spanish conquest, the consumption of Spirulina in the Mexican diet was replaced for new protein sources and also because the drainage and loss of lakes in Mexico Valley decreased significantly the natural production (Habib and Hasan, 2008). Nonetheless, during the mid-last century *Arthrospira maxima* was rediscovered accidentally in the tanks (called *"El Caracol"*) of a caustic soda maker factory, named "Sosa Texcoco", located in the same land where Lake Texcoco used to be; and the product used to be extracted in circular ponds where the pumped water from the underground was evaporated. In the early 1970's, Sosa Texcoco established the first large production facility of *A. maxima* for human consumption, being the largest producer worldwide during that time (Ramírez-Moreno and Olvera-Ramírez, 2006) (Fig. 1C). Unfortunately, the production stopped after a labor strike which resulted in the closure of the Sosa Texcoco factory at the end of the last century and with this, the massive production of *Arthrospira maxima* in Mexico ended.

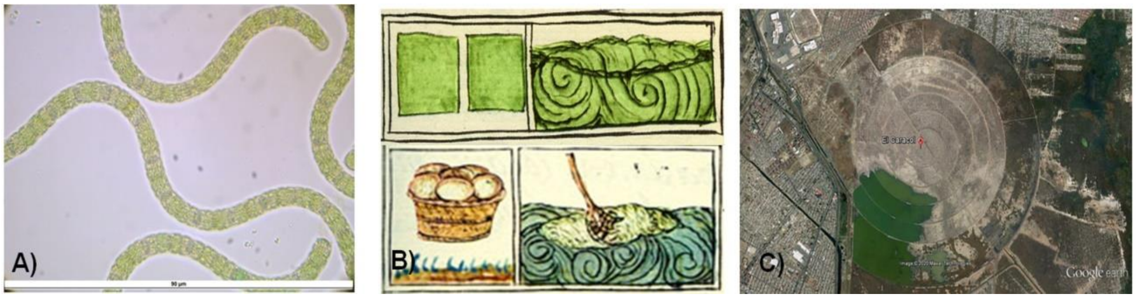

Figure 1. A) Light micrograph of *Arthrospira maxima*, it shows its characteristic spiral-shaped morphology (Environmental Biotechnology Group, INECOL); **B)** The first references of Spirulina came from the Aztec culture which consumed it as kind of cake or bread (Florentine Codex, Book 11), **C)** Current view of *El Caracol*. The last massive production *A. maxima* in Mexico was developed by the Sosa Texcoco factory, during the 70's and 80's of the last century (image source: Google Earth®).

2. Nutritional and therapeutic characteristics of the genus *Arthrospira* (Spirulina)

The supplementation of Spirulina as part of the diet could represent a cost-effective option to provide malnourish population with complementary nutrients and to improve health; it is no surprising that the World Health Organization declared Spirulina as a super food, and the NASA considered it as an excellent food for space travel due its rich nutrient content (Kulshreshtha *et al*., 2008). *A. maxima* represents a rich source of nutritional compounds with the advantage that it is easily digestible due to the absence of cellulose in its cell wall (Raja *et al*., 2008), its chemical composition includes high protein content (50% - 70%), carbohydrates (15% - 25%), essential fatty acids (18%) as gamma-linolenic acid, vitamins like B-12, minerals such as iron, calcium, phosphorous, and pigments like carotenes, chlorophyll-a and phycocyanin as the major complementary pigment (Ali and Saleh, 2012; Gutiérrez-Salmeán *et al*., 2015). Thus, it has been proposed as a functional food (Abd El-Baky *et al*., 2015). It is well documented that compounds extracted from Spirulina have several properties related as anti-oxidant, anti-diabetic, anti-cancer, anti-microbial, anti-viral, anti-inflammatory, modulation of intestinal cholesterol, prevention of cardiovascular disease and other properties like colorants in both food and cosmetic industry (Gantar and Svircev, 2008; Kulshreshtha *et al*., 2008; Ku *et al*., 2013). Several studies in non-human models (such as rats, mice or cellular tissues) have revealed data about the beneficial properties of *A. maxima* consumption. The administration of *A. maxima* in mice has demonstrated its potential benefits in cancer treatment or prevention of relapse with induced genetic damage by Benzo(α)pyrene (Chamorro-Cevallos *et al*., 2014); in citotoxity induced by hydroxyurea conferred moderate genotoxic protection (Álvarez-González *et al*., 2013) and protected against its teratogenic effects (Vázquez-Sánchez *et al*., 2009). In Winstar line rats, the consumption of *A. maxima* biomass has proven its potential benefit properties delaying chronic kidney disease (Memije-Lazaro *et al*., 2018), commonly associated to cardiovascular complications. The *A. maxima* extract, mainly containing phytopigments such as phycocyanin and chlorophyll a, has demonstrated to reduce obesity through suppression of adipogenesis (Seo *et al*., 2018), prevented cell death decreasing oxidative stress (Koh *et al*., 2018), showed powerful neuroprotective effect preventing the radical-mediated cell death (Lee *et al*., 2018), and it has ameliorated cognitive impairments induced by an intracerebroventricular injection of Aβ1-42 in mice (Koh *et al*., 2017).

In humans, several benefits have been confirmed too, especially in obese, high blood pressure and diabetic patients (Ku *et al*., 2013). The effect of *A. maxima* consumption (4.5 gd^{-1} during 6 weeks) was evaluated in subjects between 18 - 65 years old by Torres-Durán *et al*., (2007); the study demonstrated a hypolipemic effect with significant reduction in the levels of triacylglycerol, total cholesterol and cholesterol associated to high density lipoprotein; additionally systolic and diastolic blood pressure were reduced. Torres-Durán *et al*., (2012) studied the effect of *A. maxima* as alternative treatment (5 gd^{-1} during 15 days) to improve lipid clearance in young subjects (10 - 26 years old); their results concluded that *A. maxima* decreased post prandial lipemia and the youngest subjects were the most responsive to the beneficial effects. Szulinska *et al*., (2017) reported the administration (2 gd^{-1} during 3 months) of *A. maxima* biomass to obese patients with hypertension; those patients reported a significant decrease in body mass, lower-density lipoprotein cholesterol and interleukin-6 concentration; in addition, patients considerably improved total antioxidant status and insulin sensitivity ratio. Considering these results, Spirulina was suggested as a promising therapeutic compound to increase health in obese-related hypertension patients. Additionally, this particular algal species has both immunity improving capacity and also it is capable of suppressing the viral activities in humans including type II Herpes Simplex, HIV, and it could probably be utilized to fight against novel coronavirus COVID-19 (Elaya-Perumal and Sundararaj, 2020).

Considering all the above-mentioned nutritional characteristics of *Arthrospira*, some attempts have been conducted to incorporate its biomass into acceptable meals. Fradique *et al*., (2010) aimed at incorporating microalgae biomass (*Chlorella vulgaris* and *Spirulina maxima*) into fresh pasta. This strategy resulted in the enhancement of nutritional and sensorial quality in pasta, while cooking and textural properties were not affected. Finally, it is important to point out that the consumption of Spirulina has been approved as safe by many food administrations all over the world. The Dietary Supplements Information Expert Committee has awarded Spirulina with a grade “A” safety rating as safe food to be consumed (Marles *et al*., 2011)

The beneficial properties of Spirulina consumption in human health are especially relevant in developing countries. For example, in Mexico, major death causes in the population are related to heart problems (high blood pressure), diabetes, cancer and liver diseases, which account with more than 30% of total deaths (INEGI, 2018). Thus, a Program for increasing the consumption of Spirulina as a diet supplement, could help to decrease such health problems.

3. Opportunities for economic and social developments based on *Arthrospira* (Spirulina) production

Data available of the whole world Spirulina annual production is hard to summarize, however, according to Selvendran (2015), it was inferred that it was over 30 thousand tons per year in 2015, with an annual growth rate of 13% - 14%.Thus, these authors put forward a projection indicating that it could reach between 60 - 70 thousand tons per year by 2025, being the United States the leader in the world production, followed by Asian countries like China, India or Thailand.

Despite all the benefits reported from Spirulina biomass, its use and consumption has not received serious consideration as a potential key crop in many regions, especially in tropical / subtropical coastal areas with proper conditions for its growth and where traditional agriculture crops struggle the effect of land salinization, water shortages and the presence of vulnerable social malnourished groups or lack of job opportunities (Habib and Hasan, 2008). These conditions are potentially exploitable in a country like Mexico with a costal line extension of 11,122 km and a geographical position with several regions with tropical and arid conditions with annual average temperatures between 20 - 30 °C and abundant solar irradiance which can be exploited for Spirulina culture (Fig. 2). It is estimated that the production cost of Spirulina for human consumption can be reduced in at least 50% in tropical regions with abundant and yearly sun, proper temperature and presence of alkaline water (Henrikson, 2010).

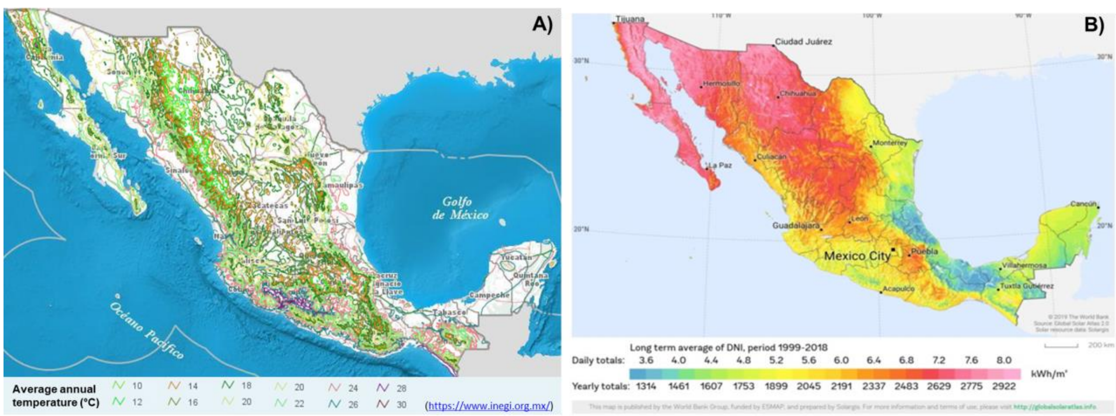

Figure 2. The geographical location of Mexico provides good conditions of **A)** temperature (source: https://www.inegi.org.mx/temas/climatologia/default.html#Mapa), and **B)** solar irradiance (source: (https://solargis.com/maps-and-gis-data/download/mexico) with an abundant costal area for Spirulina massive cultivation.

It is also important to consider the cost of the infrastructure and production facility. It is known that the investment cost depends on several conditions including: final product (biomass of low or high quality, high value products), local facilities, land cost, the goal of production (commercial, social development), and scale of production, just to mention some of them. Production can be designed at different scales, from small ponds to intensive commercial development over large areas. The scale of production can be classified according to the annual productivity as: micro farms (< 2 tones / year), small farms (2 - 20 tones / year), large farms (20 - 200 tones/year) and very large farms (> 200 tones/year) (da Silva and Reis, 2015). It is not easy to estimate the costs involved in a facility building, since such information is not shared by companies. Nonetheless, Selvendran (2015) estimated an investment cost of approximately US$10,000 for stablishing a rural Spirulina farm with 400 m^2 of production area, capable of an annual production of 1 ton of dried biomass per year, with the potential to satisfy nutritional demands in rural areas of 12,000 children (1 g / 100 days) or about 6000 children and 2000 adults (3 g / 100 days).

As different conditions are involved in the cost for running an *Arthrospira* (Spirulina) production plant, two different scenarios could be envisaged:

1) Integrated production within a biorefinery following the strategy of dual-purpose systems proposed by Olguín (2012) in which *Arthrospira* biomass is produced at low cost using sea water and digestates from the anaerobic treatment of animal wastes as source of nutrients. Such biorefinery scheme was described extensively and included the production of *Arthrospira* as feed for aquaculture, biogas from animal waste, and other biofuels from other microalgae species. The production of high yields of *Arthrospira* in such dual-purpose systems was previously demonstrated to be feasible at small scale (Olguín *et al*., 1994, 1997) and at pilot scale during three consecutive years (Olguín *et al*., 2003).

2) On the other hand, an industrial strategy with higher investment in infrastructure and automatization can be oriented towards the cultivation of *Arthrospira* for high quality biomass or value-added products such as phycocyanin. It would be expected that such projects could be financed by government, private enterprises or small spin-off companies oriented to larger markets national and / or international. It is important to mention that the production oriented towards high value-added products extracted from the biomass, increases its value in the market (Fig. 3).

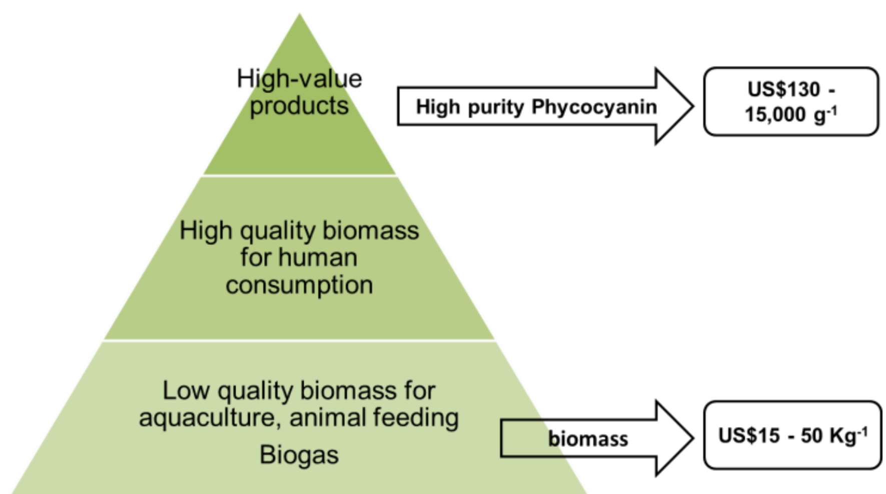

Figure 3. The value of *Arthrospira* increases from low-quality biomass to high-value products such as pure pigments like phycocyanin.

4. Non-conventional low-cost media for the cultivation of *Arthrospira maxima* (*Spirulina*)

The synthetic medium mostly used for Spirulina cultivation is Zarrouk (1966) medium, since it provides nutrients and a high concentration of sodium bicarbonate to mimic the conditions found in alkaline lakes. However, the cost of synthetic salts for large scale projects increases the production cost. It has been reported (Andrade *et al*., 2019), that approximately 15% of the production cost of cultivating Spirulina is related to nutrients and another 2.6% on water, thus almost 20% is related to the culture medium. As a strategy to counteract such costs, and also as a way to treat the wastes in dual purpose systems as mentioned above, the cultivation of *Arthrospira maxima* using wastes of different origin has been reported by some authors.

Early work focusing in taking advantage of using animal waste as source of nutrients, led to the development of integrated agricultural systems for the cultivation of *Arthrospira* using digestates from pig waste treated anaerobically (Olguín, 1982). Later on, following a similar strategy, Olguín *et al*., (1994) reported the use of sea water supplemented with anaerobically treated pig slurry (2% v/v) with a high alkalinity for the cultivation of *A. maxima* in high rate oxidation ponds (20 L) under light intensity (60 - 70 µmol $m^{-2}s^{-1}$) and temperature (30 °C) control. These authors found that under optimum agitation conditions (20 rpm), they obtained 0.42 gL^{-1} biomass concentration with high protein content of 71%. Further on, the same research group reported the use of high rate oxidation ponds exposed outdoors under temperate conditions (Olguín *et al*., 1997) and tropical conditions for the cultivation of *A. maxima*. Under the later conditions (Olguín *et al*., 2003), using a similar culture medium supplemented with pig waste digestates under a fed-batch mode, the average productivity of semi-continuous cultures during summer was 15.1 $gm^{-2}d^{-1}$ with a depth of 0.20 m. Additionally, the average protein content of the biomass was 48.9% ash-free dry weight.

Concerning the use of another waste commonly found in tropical regions around the world, dos Santos *et al*., (2016) studied the feasibility of using sugar cane vinasse as a supplement in the cultivation medium of *A. maxima*. They adopted a cyclic two-stage cultivation strategy, alternating an autotrophic phase (12 h, 70 - 200 µmol $m^{-2}s^{-1}$) and heterotrophic dark phase (12 h, 3% v/v vinasse). At the end of the seventh day of cultivation, the biomass concentration was around 0.495 - 0.609 gL^{-1} and it showed a high protein content (74.3% - 77.3%).

Another non-conventional source of nutrients reported was oyster shell. Jung *et al*., (2014) reported a modified Zarrouk medium with elements from oyster shell and soil extract for the cultivation of *A. maxima*. They reported an increase in biomass, chlorophyll, and phycocyanin by 17%, 16%, and 64 %, respectively. Authors indicated, oyster shell and soil provided sufficient amounts of calcium and trace metals to sustain cultivation of *A. maxima*.

The use of non-conventional medium will depend on the presence and abundance of wastes or residues in the local area, to formulate the proper medium, one attractive alternative could be to adapt Spirulina production plants around animal breeding farms. However, it is important to point out, that according to the Mexican normativity (NMX-F-508-1988), the biomass produced under these conditions is excluded for human consumption. Thus, the potential use of this biomass could be oriented to the production of biofuels, animal feed or aquaculture projects.

4.2 Biomass for aquaculture and animal feed

As mentioned previously, the Spirulina biomass produced under non-conventional medium is not recommended for human consumption, but it can be integrated into the food chain through animal / fish consumption. Spirulina is a cheaper protein and nutrient ingredient than those of animal origin (Habib and Hasan, 2008); thus, it can be used as a partial or complete replacement for animal / fish nutrition. Several documents support the use of *A. maxima* biomass as supplement or replacement in fish and animal diets.

It has been reported that the use of Spirulina in aquaculture improves the appearance and enhance skin color in several species of commercial interest like shrimp, koi carp, prawns, salmon, tilapia and yellow tail tuna (Henrikson, 2010). Olvera-Novoa *et al*., (1998) suggested the replacement of up to 40% of the fish meal protein in Tilapia (*Oreochromis mossambicus*) diets for a proper growth and feeding performance, showing that the consumption of *A. maxima* increased the pigmentation aspect which is a valuable property in the market. Rincón *et al*., (2012) reported that the substitution of 30% of the normal diet of red Tilapia (*Oreochromis* sp.) by *Arthrospira maxima* biomass showed a higher feed conversion. More recently, Edirisinghe *et al*., (2019) described the enhancement of the immune response and a higher disease resistance in larvae and adults of Zebra fish (*Danio rerio*) after consuming a novel pectin isolated from *A. maxima*.

As part of animal diets, some reports discussed the effects on poultry and swine. The consumption by hens enhances the yellow skin coloration and increases the deep yellow color of egg yolks (Henrikson, 2010). It has also been reported that eggs fortified in Fe, Zn and Mn have been obtained after a Spirulina diet (Saeid *et al*., 2016). In swines, it has been reported the use of enriched biomass of *A. maxima* with Cu as the source of valuable nutrients in swine feeding (Saeid *et al*., 2013a), and for reducing total cholesterol in contrast to conventional diets using inorganic salts (Saeid *et al*., 2013b).

Considering the benefits described above, the design of integrated systems including Spirulina cultivation, waste treatment and production of animal / fish feed show a great potential for the improvement of social, environmental, economic and nutritional conditions in various types of communities, especially those close to the cost with abundance of sea water and proper climatic conditions.

5. Characteristics of Phycocyanin from the genus *Arthrospira* (*Spirulina*)

Phycocyanin (PC) is a blue accessory pigment located at the structure called phycobilisome inside the thylakoid membrane of blue-green algae; it is usually attached to other phycobiliproteins such as allophycocyanin and phycoerythrin. These phycobiliproteins are classified according to their spectral features (Wang *et al.*, 2001) as: phycoerythrins (PE; λ_{max} = 565 nm), phycocyanins (PC; λ_{max} = 617 nm) and allophycocyanins (APC; λ_{max} = 650 nm). These phycobiliproteins are distributed in the antennae rods of phycobilisomes in the order of APC at the core, PC in the middle and PE at the tip

(Fig. 4,A). Their collective range of absorption spans the entire visible spectrum of sunlight and increases the potential light harvesting by the photosynthetic apparatus. The tertiary fold and general architecture of phycobiliproteins is remarkably conserved in all of them. This general architecture consists in α and β subunit polypeptides which exhibit high affinity for one another and associate into αβ monomers, which then aggregate into $(\alpha\beta)_3$ trimers and $(\alpha\beta)_6$ hexamers (Eriksen, 2008) (Fig. 4,B). The rods in the phycobilisome normally include two or more phycocyanin hexamers, but in some species, rods also contain phycoerythrin or phycoerythrin hexamers. *Arthrospira platensis* contains only two phycobiliproteins, APC and PC, where PC is the major light-harvesting pigment present in the antenna rods and APC is a minor component present only at the core (Padyana *et al*., 2001). Different phycobiliproteins contain different kinds and different numbers of chromophores. The chromophore is a moiety that causes a structural change of the molecule when is hit by light, and these are open-chain tetrapyrroles linked to cysteine residues via thioester bonds (Fig. 4,C). Chromophores are classified by its structure as phycoerythrobilin (PEB), phycocyanobilin (PCB), phycoviolobilin (PVB) and phycourobilin (PUB). Phycocyanin has three PCB chromophores attached to one monomer through thioester linkages (Wang *et al*., 2001). A graphical overview of previous description is presented in the Fig. 4.

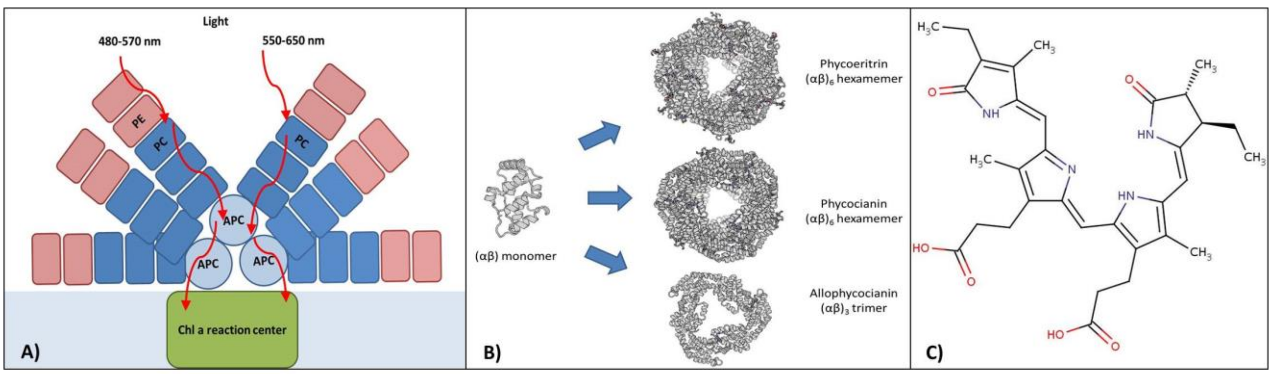

Figure 4 A) The structure of the phycobilisome includes the accessory pigments phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC); **B)** The general architecture of phycobiliproteins consist in α and β subunit polypeptides which associate into αβ monomers, then aggregate into $(\alpha\beta)_3$ trimers and $(\alpha\beta)_6$ hexamers. **C)** The chromophore phycocyanobilin is attached in to the phycocyanin hexamer complex. (source: Protein Data Bank https://www.rcsb.org/).

6. Conclusions and final remarks

The information discussed herewith provides an overview of some of the most relevant topics and issues related to *A. maxima*. Although consumption by ancient Aztecs in Mexico is a good example of the use of this cyanobacterium in the human diet, the introduction of this valuable biomass into the current Mexican diet or any other rural population worldwide offers several challenges and should not be promoted as an overnight solution to malnourishment. Instead, evidence is provided in this review that the cultivation of *A. maxima* in dual purpose systems in which valuable biomass can be produced using digestates of anaerobically treated animal waste offers the advantage of production of feed for aquaculture at a low cost, simultaneously to the treatment of the animal waste. Furthermore, a second strategy is also discussed in which production of *A. maxima* at large scale within an industrial facility can provide not only valuable biomass but also high added value products such as phycocyanin. Mexico and many other countries offering the proper climatic conditions could promote projects either directed towards the production of *A. maxima* as a feed in aquaculture or the production of this valuable cyanobacterium as a supplement for human consumption and for the isolation of high added value products such as phycocyanin.

Finally, to make feasible the expansion of *A. maxima* cultivation worldwide, the interactions of all actors involved such as the academia with the transfer of knowledge and technology, the governmental agencies with financial support for social development projects, proper regulation and market opportunities and the entrepreneurs and producers developing new skills and financing large scale projects, are required.

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