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A mini review on challenges and opportunities in dinoflagellates cultivation

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Abstract

Dinoflagellates are photosynthetic protists commonly distributed in marine and freshwater environments and can be found in symbiotic associations. They are a significant primary producer and play a fundamental role in the functioning of aquatic ecosystems – especially for coral reefs. Dinoflagellates can produce a wide variety of secondary metabolites, and their toxins can affect fish, birds and mammals. In recent years these toxins have been found to have potential cytotoxic, anticancer, antibiotics, antifungals activities. This mini review covers the main genera of dinoflagellates, and challenges and advances in their cultivation in addition to prospects for development of dinoflagellates-based products.

Keywords: Microalgae, Biomass, Secondary metabolites, Toxins

Background

Interest in microalgae has increased considerably in recent decades, mainly due to demand for sustainable biomass and bioprocesses, such as aquaculture, where microalgae play essential roles as live food for molluscs, and larvae of crustaceans and fish (Muller-Fuega, 2000; Garrido-Cardenas et al., 2018). Besides other applications, these photosynthetic microorganisms have also aroused interest in wastewater treatment and production of high commercial value molecules (eg., fatty acids, carotenoids and amino acids) and biofuels (Daroch et al., 2013; Salama et al., 2017; Oliveira et al., 2020a). According to Garrido-Cardenas et al. (2018), even with various species of microalgae isolated, global production of and research on microalgae are limited to a small number of taxa, such as *Arthrospira* of Spirulina group, that are intended mainly for human food or as a dietary supplement (Pan-Utai et al., 2018); *Chlorella* spp. for being a potential producer of β-1,3-glucan, an active immunostimulator with antioxidant capacity (Carballo et al., 2019); *Dunaliella salina*, as a source of β-carotene (Ben-Amotz, 2004) and; *Haematococcus pluvialis*, for astaxanthin extraction (Panis & Carreon, 2016).

Dinoflagellates are a eukaryotic group of microalgae common in marine, estuarine and freshwater environments. Besides the species diversity (around 6,000 species), dinoflagellates have various structural shapes (amoeboid, coccoid, palmelloids, etc.), habitats (planktonic, benthic and epicontinental) and nutritional modes (photoautotrophic, heterotrophic, mixotrophic and phagotrophy). They play a significant role as primary producers and contribute to the functioning of aquatic ecosystems, especially coral reefs. The ecological activities of coral reefs heavily depend on the symbiosis between reef-building corals and zooxanthellae (reviewed in Jephcott et al., 2016 and Suggett et al., 2017). In addition, dinoflagellates also receive interest in research because some of their species produce toxins and they also cause harmful algal blooms (HABs) (Gravinese et al., 2018). Despite their great diversity, about 90 species have been reported as potential toxin producers (Burkholder et al., 2008; González-Rodríguez et al., 2010; Speight & Henderson, 2010; Saldarriaga & Taylor, 2017).

Toxins from dinoflagellates can affect human and ecosystem health and, for a long time, this was the main reason for interest in their studies. However, in recent years dinoflagellate toxins have been found to have potential pharmaceutical applications (i.e. cytotoxic, anticancer, antibiotics, antifungals activities). In this context, this mini review reveals key information about dinoflagellates cultivation. The discussion also takes into account the major challenges, new insights and potential of the biomass production of dinoflagellates.

Dinoflagellate Genera

Specific dinoflagellate genera have been studied as a source of bioactive molecules (secondary metabolites): *Alexandrium*, *Amphidinium*, *Gymnodinium*, *Karlodinium* and *Symbiodinium* (Wang & Hsieh, 2002; Parker et al., 2002; Band-Schmidt et al., 2014; Benstein et al., 2014; Lage et al., 2014; Molina-Miras et al., 2018; Langenbach & Melkonian, 2019).

2.1 *Alexandrium*

The genus *Alexandrium* is one of the major harmful algal bloom genera. Three different families of toxins were reported in this genus: saxitoxins (STX), goniodomins and spirolides; but they have not been fully characterized (Balech, 1989; Touzet et al., 2008; reviewed in Anderson et al., 2012). *Alexandrium* spp. are considered opportunistic in relation to nutrition - different species have been found in both nutrientrich (Spatharis et al., 2007) and nutrient-poor waters (Collos et al., 2014). In addition, bacteria and microalgae (dinoflagellate, *Amphidinium carterae*) have been observed to contain food vacuoles (reviewed in Jeong et al., 2010). Moreover, growth under the mixotrophic nutritional mode has also been reported for *Alexandrium catenella* (Legrand & Carlsson, 1998).

2.2 *Amphidinium*

Amphidinium spp. are toxic dinoflagellates found in coastal waters and tempered tropical estuaries (Steidinger & Jangen, 1996). It is known for HABs that may produce mainly ichthyotoxins (Huang et al., 2009) and hemolytic substances (Echigoya et al., 2005). Abundance of peridinin (an apocarotenoid), located in the photosynthetic complex of most dinoflagellates, has been extensively studied in *Aphidinium carterae* (Hofmann et al., 1996); this apocarotenoid possesses strong antioxidant properties and can act against the tumors (Nishino, 1998; Barros et al., 2001). Recently, amphidinols (APDs), secondary metabolites produced by this genus, have aroused a growing interest by presenting potential antifungal, antibacterial, antioxidant and antitumor agents (Satake et al., 2017; Iwamoto et al., 2017; Martínez et al., 2019). Although the structure of APDs is well-documented (Satake et al., 2017), several factors related to the biosynthesis of these molecules are still not well understood.

2.3 *Gymnodinium*

Gymnodinium catenatum is the only dinoflagellate species of this genus that produces paralytic shellfish poisoning (PSP) and its greatest relevance is due to the fact that it can affect human health with neurological and gastrointestinal disorders, usually as a result of the consumption of contaminated shellfish (Band-Schmidt et al., 2008; Martínez et al., 2016). This species is widespread in temperate and tropical waters in many regions of the world (Hallegraeff et al., 2012) and the toxin profile may vary according to environmental factors (Negri et al., 2001; Oliveira-Proença et al., 2001; Holmes et al., 2002; Oh et al., 2010). As for the other dinoflagellates, studies on *G. catenatum* have mainly involved the ecophysiological approach for understanding the influence of environmental factors on the production of toxins.

2.4 *Karlodinium*

In the genus *Karlodinium*, a cosmopolitan species of temperate regions that has been more thoroughly studied is *Karlodinium veneficum* (García-Camacho et al., 2007; Gallardo-Rodríguez et al., 2012; López-Rosales et al. 2015). *K. veneficum* is a producer of karlotoxins (KmTxs) and it can feed by ingesting diatoms and copepods (Bachvaroff et al., 2009; Waters et al., 2010; Place et al., 2012). The KmTxs can be easily isolated, and like APDs, it has hemolytic and ichthyotoxic activity. KmTxs are also more likely to function as anti-grazing and allelopathic. Investigations have shown that *K. veneficum* is able to reconfigure its cellular metabolic machinery and regulate dynamic protein expressions to cope with the stress caused by excess light. This is an interesting strategy for intensive cultivation to produce biomass (Cui et al., 2017). For this reason, *K. veneficum* proves to be a promising species for production of biomolecules.

2.5 *Symbiodinium* (family Symbiodiniaceae)

Symbiodinium spp. were recognized by arbitrary letters (e.g., A, B, C) that became referred to as "clades". Recently, in short, the genus *Symbiodinium,* based on genetics and ecology data, was split into seven new genera belonging to family Symbiodiniaceae, (LaJeunesse et al. 2018). Regardless of taxonomic classification, they are commonly approached for their endosymbiotic association with coral reefs (but they can also be associated with some species of anemones, jellyfish, sponges and others) (reviewed in Stat et al., 2006; Krueger & Gates, 2010). For these associations, most studies have sought to investigate the effect of environmental parameters on endosymbiosis with coral reefs and to clarify the main causes involved in coral bleaching events (McIlroy et al., 2016; Grégoire et al., 2017; Bernasconi et al., 2019). However, peridinin and toxins contents have also aroused, albeit simple, interest in cultivation aimed at the biotechnological applications of *Symbiodinium* spp. biomass (Benstein et al., 2014; Langenbach & Melkonian, 2019; Tsirigoti et al., 2020).

Biomass Production

Difficulties in reaching high biomass concentrations in cultures of dinoflagellates limit the commercial applications. This is mainly due to the sensitivity of many dinoflagellates to shear forces. Recently, the application of twin-layer porous substrate bioreactor (TL-PSBR) has been investigated in the laboratory. However, although projections are commonly made for large TL-PSBR (g m-2), operation of this bioreactor on an industrial scale is still doubtful (Langenbach & Melkonian, 2019). In addition to the TL-PSBR, bubble column photobioreactors (BC-PBR) have been used successfully for the biomass production of dinoflagellates (López-Rosales et al. 2016, 2017). The BC-PBR also controls shear stress, ensuring healthy growth of dinoflagellate cells. Moreover, the BC-PBR is likely to be more productive than the TL-PSBR because they have a larger photosynthetically active area than the biofilm of TL-PSBR. The improvement of photobioreactors for the intensive cultivation of dinoflagellates is still a basic process necessary for the development of this production chain.

Potential Aplications in Aquaculture and Future Directions

Due to the production of allelopathic compounds and the ability to grow under mixotrophic nutritional mode the dinoflagellates have a great potential to treat wastewaters. The microalgae, because of their use in wastewater treatment, have attracted increasing attention; they can convert inorganic compounds into polyunsaturated fatty acids (PUFA), carotenoids, amino acids and others biomolecules, in addition to the secondary metabolites (Zeller et al., 2013; Oliveira et al. 2020b). This potential has not been sufficiently explored (Molina-Miras et al. 2020). In the case of PUFA, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), exhibit biological activities and are considered in the treatment of heart disease, cancer, type 1 diabetes and others (reviewed in Mendes et al., 2009). Hitherto, fish oil is the most widely used product of this category in the market even with some negative characteristics (e.g. distracting odor, allergic reactions, high refinery costs etc.). In addition, this use amounts to unsustainable exploitation of wild prey fish in aquaculture of fish and shrimp feed (Naylor et al., 2000). Based on this potential, a simplified model for production of dinoflagellate biomass using aquaculture wastewater is shown in **Figure 1**.

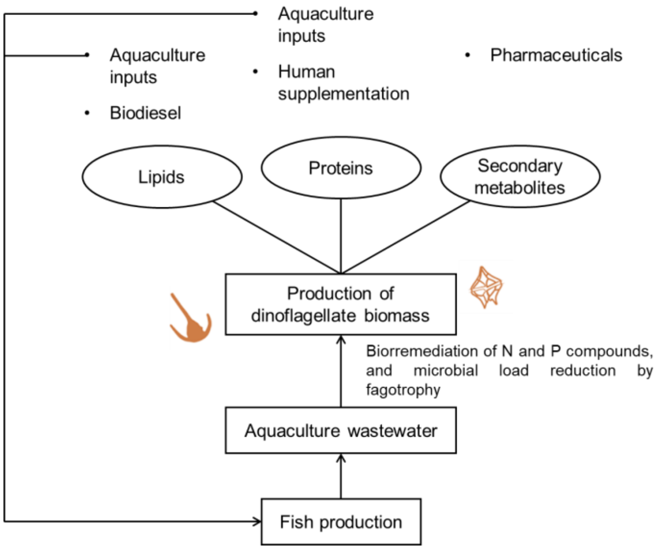

Figure 1. Simplified integrated model for the production of dinoflagellate biomass using aquaculture wastewater.

Recent interest in the cultivation of dinoflagellates has already resulted in substantial improvements and technological advances in the production processes. Limitation on commercial application of pigments and secondary metabolites produced by dinoflagellates is due to the lack of a reliable natural source of these macromolecules, since industrial-scale cultivation of dinoflagellates still faces barriers. Addressing some of these constraints will be a significant step towards the large-scale development of new inputs and drugs.

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