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REVIEW
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High Value Phycotoxins From the Dinoflagellate *Prorocentrum*

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Marine dinoflagellates produce chemically diverse compounds, with a wide range of biological activity (antimicrobial, anticancer, treatment of neurodegenerative disease along with use as biomedical research tools). Chemical diversity is highlighted by their production of molecules such as the saxitoxin family of alkaloids ($C_{10}H_{17}N_7O_4$ – 299 g/mol) to the amphipathic maitotoxin ($C_{164}H_{256}O_{68}S_2Na_2$ – 3,422 g/mol), representing one of the largest and most complex secondary metabolites characterized. Dinoflagellates, are most well-known for the production of red tides which are frequently toxic, including okadaic acid and related dinophysistoxins, which are tumor promoters. The mode of action for these phycotoxins, is by specific inhibition of protein phosphatases, enzymes essential in regulation of many cellular processes. Hence, these compounds are being used for vital cell regulation studies. However, the availability of useful amounts of these compounds has restricted research. Chemical synthesis of some compounds such as okadaic acid has been investigated, but the complexity of the molecule resulted in many lengthy steps and achieved only a poor yield. The use of naturally occurring phytoplankton has been investigated as a potential source of these compounds, but it has been shown to be unreliable and impractical. The most practical option is large scale culture with down-stream processing/purification which requires specialist facilities and expertise. This review, describes the biotechnological potential of these organisms and the challenges to achieve useful yields of high quality phycotoxins using *Prorocentrum* spp. as an example to produce okadaic acid.

Keywords: benthic microalgal cultivation, *Prorocentrum lima*, seawater, nutrient limitation, temperature, irradiance

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INTRODUCTION

Over the past decades, the occurrence of harmful algae blooms (HABs) have increased worldwide (Luckas et al., 2005; Hallegraeff, 2010; Griffith and Gobler, 2020). Climate change and anthropogenic pressures are considered responsible for increased frequency, intensity and broader geographic range. Moreover, increased social and scientific awareness due to their associated negative socioeconomic effects intensified monitoring HABs (phytoplankton and toxins) (Anderson et al., 2012; Farabegoli et al., 2018; Brown et al., 2020).

Abbreviations: ASP, amnesic shellfish poisoning; AZA, azaspiracid; AZP, azaspiracid shellfish poisoning; BTX, brevetoxin; CFP, ciguatera fish poisoning; CTX, ciguatoxin; DA, domoic acid; DSP, diarrhetic shellfish poisoning; DTX, dinophysistoxin; GYM, gymnodimines; GTX, gonyautoxin; HAB, harmful algae bloom; LED, Light-emitting diode; Mbp, megabase pair; MTX, maitotoxin; NEO, neosaxitoxin; NSP, neurotoxic shellfish poisoning; OA, okadaic acid; PBR, photobioreactor; PLTX, palytoxin; PnTX, pinnatoxin; PP1, phosphatase 1; PP2A, phosphatase 2A; PP2B, phosphatase 2B; PSP, paralytic shellfish poisoning; PTX, pectenotoxin; Qt, toxin quota; SPX, spirolide; STX, saxitoxin; YTX, yessotoxins.

HABs are typically characterized by rapid proliferation and/or high biomass often known as "red tides" due to their common red color but this color can vary depending on the dominant phytoplankton species involved as well as its concentration and depth. Many of these bloom-forming microalgae produce secondary metabolites, which are toxic (phycotoxins). The purpose of the synthesis of phycotoxins is not clear but it is believed that they may have a role in increasing survival in unfavorable environmental conditions and as a defensive mechanism by inhibiting the growth of co-occurring microalgae or as anti- grazing (Selander et al., 2006; Ajuzie, 2007; Ianora et al., 2011; Anderson et al., 2012; Kohli et al., 2016). Recently, it has also been hypothesized that they could be linked to another trait or process such as photosynthesis II and nitrogen and carbon metabolism (Cassell et al., 2015; Glibert et al., 2016). Nevertheless, the issue arises when phycotoxins bio-accumulate in aquatic edible organisms such as fish and shellfish, leading to eventual poisoning of animal and human consumers (James et al., 2010; Hinder et al., 2011). It is estimated that marine phycotoxins are responsible for up to 60,000 human intoxications per year, with an overall mortality of around 1.5% (Kantiani et al., 2010).

Based on their chemical structure marine phycotoxins are classified into the following groups: (i) linear and macrocyclic polyethers (e.g., okadaic acid (OA) and dinophysistoxins (DTXs)); (ii) ladder-frame polyethers (e.g., ciguatoxins (CTXs) and brevetoxins (BTXs)); (iii) macrocyclic imines (e.g., spirolides (SPXs) and gymnodimines (GYMs)); (iv) tetrahydropurines (e.g., saxitoxins (STXs) and gonyautoxins (GTXs)); and (v) toxic secondary amines (e.g., domoic acid (DA)). Historically, marine phycotoxins were categorized according to the characteristic symptoms they generate in humans after the consumption of contaminated seafood: diarrhetic shellfish poisoning (DSP) (e.g., OA, DTXs, pectenotoxins (PTXs) and yessotoxins (YTXs)), amnesic shellfish poisoning (ASP) (e.g., DA), paralytic shellfish poisoning (PSP) (e.g., STXs and analogs), azaspiracid shellfish poisoning (AZP) (e.g., azaspiracids (AZAs)), neurotoxic shellfish poisoning (NSP) (e.g., BTXs) and ciguatera fish poisoning (CFP) (e.g., CTX and maitotoxin (MTX)).

This review will focus on the dinoflagellate *Prorocentrum* spp. as a potential source of phycotoxins and different biotechnological approaches to enhance their production. We aim to summarize the studies conducted on *Prorocentrum* spp. to enhance the growth rate and DSP toxin production with special emphasis on the influence of nutritional and environmental factors published between 1993 and 2020. It also presents considerations relating to mass cultivation to attain high phycotoxin yields scaling up photobioreactors from bench to large scale.

TOXIC BIOACTIVE METABOLITES FROM DINOFLAGELLATES

Dinoflagellates are a lineage of unicellular eukaryotes of huge ecological and evolutionary significance (Hoppenrath, 2017; Cousseau et al., 2020). Approximately half of the dinoflagellate species are autotrophic possessing chloroplasts for photosynthesis, but they are also able to grow heterotrophically, parasitically, mixotrophically or symbiotically (Hoppenrath, 2017; Cousseau et al., 2020). Most dinoflagellates are pelagic but many are adapted to benthic habitats (Gómez, 2012; Durán-Riveroll et al., 2019). Dinoflagellates represent around 75% of the toxic species responsible for HABs (Smayda, 1997; Cembella, 2003).

Dinoflagellates are known to produce an array of bioactive compounds that are structurally and functionally diverse with valuable toxicological and biological properties (Garcia-Camacho et al., 2007). Most of the toxic secondary metabolites are of polyketide origin, including polycyclic polyketides (e.g., YTXs, CTXs, BTXs, MTXs, and palytoxins (PLTXs)), macrolides (e.g., PTXs and amphidinolides) and linear polyketides (e.g., DTXs and OA) (Verma et al., 2019); but also cyclic imines (e.g., SPXs, GYMs and pinnatoxins (PnTXs)) and alkaloids (e.g., STX, GTX, neosaxitoxin (NEO)). Numerous pharmacological functions, such as analgesic, anesthetic, anticancer, antifungal, anticholesterol, cytotoxic, immune-suppressive and/or neurological disease therapeutics were also attributed to these compounds (Garcia-Camacho et al., 2007; Qian et al., 2015; Assunção et al., 2017). Besides their therapeutic use, they are invaluable tools to study cellular processes but their toxicity is a barrier to reaching clinical studies (Cruz et al., 2013; Assunção et al., 2017).

Toxic Bioactive Metabolites From *Prorocentrum* spp.

To date, there are approximately 60 species of the dinoflagellate genera *Prorocentrum* distributed worldwide, half of them in benthic marine habitats (Hoppenrath et al., 2013; Durán-Riveroll et al., 2019). *Prorocentrum* is known to produce DSP toxins and OA, a major DSP polyketide toxin, hence they are a common chemotaxonomic marker of the toxic *Prorocentrum* spp. (Fernández et al., 2003; Hoppenrath et al., 2013; Luo et al., 2017; Pan et al., 2017; Nishimura et al., 2020). For example, all reported *Prorocentrum lima* strains produce OA and some strains also produce DTX1 as a minor toxin (Jackson et al., 1993; McLachlan et al., 1994; Vanucci et al., 2010; Varkitzi et al., 2010; Hoppenrath et al., 2013; Wang et al., 2015; Hou et al., 2016; Lee et al., 2016; Hoppenrath, 2017; Pan et al., 2017; Aquino-Cruz et al., 2018; Gu et al., 2019). *Prorocentrum belizeanum, caipirignum, concavum, maculosum* and *hoffmannianum* are also known producers of DSP toxins (Morton et al., 1994; Zhou and Fritz, 1994; Hoppenrath et al., 2013; López-Rosales et al., 2014; Luo et al., 2017; Accoroni et al., 2018; Rodríguez et al., 2018; Lim et al., 2019; Lee et al., 2020).

OA and DTXs cause gastrointestinal distress with diarrhea, nausea, vomiting and abdominal pain described as the main symptoms (James et al., 2010). They are potent selective inhibitors of protein phosphatases 2A (PP2A), 1 (PP1) and 2B (PP2B) (Bialojan and Takai, 1988; Twiner et al., 2016). It was previously reported that the diarrheic effect of DSP toxins was associated to the inhibitory activity on protein phosphatases

(Cohen et al., 1990) but no links have been found and their mechanisms of toxicity must be re-evaluated (Munday, 2013). Recent reports point to modulation of neurotransmitters that regulate intestinal mobility, along with water and electrolyte secretion as the cause of DSP syndrome (Valdiglesias et al., 2013; Louzao et al., 2015). Protein phosphatases modulate cell signaling pathways so their inhibition by OA and DTXs renders them as an important tool in medical and physiological studies (Meštrović and Pavela-Vrančič, 2003; Cruz et al., 2013; Valdiglesias et al., 2013). In addition, chronic exposure to OA can induce cytotoxicity, neurotoxicity, immunotoxicity, embriotoxicity, genotoxicity and cancer promotion as reviewed by Vilariño et al. (2018) and Fu et al. (2019). The structurally related compounds, DTXs, share the similar negative effects of OA (Vilariño et al., 2018).

Besides OA and DTXs, other phycotoxins have been identified from cultures of *Prorocentrum*. For example in *P. lima* cultures, OA and DTX analogs, as well as formosalides, prorocentrolides and related macrolides, limaol and other non-characterized toxins have been reported (**Table 1**), however, their significance and biosynthesis have not yet been explored. Prorocentrolides and related macrolides are minor but unique constituents of *P. lima* and they should be considered chemotaxonomic markers (Torigoe et al., 1988; Lu and Chou, 2002; Lee et al., 2019; Li et al., 2020). Prorocentrolides are known as "fast-acting toxins" and have been reported to act on nicotinic acetylcholine receptors (Molgó et al., 2017; Amar et al., 2018). Prorocentrolide C exhibited cytotoxicity against cancer cells *in vitro* (HCT-116 and Neuro-2a cells) (Lee et al., 2019) as well as 4-hydroxyprorocentrolide (inhibitory activity against human colon adenocarcinoma DLD-1 and human malignant melanoma RP-MI7951) (Lu et al., 2005). Formosalides and limaol has also shown cytotoxicity activity against tumor cell lines (hepatocellular carcinoma, colon adenocarcinoma, neuroblastoma and T-cell acute lymphoblastic leukemia cells) (Lu et al., 2009; Yang et al., 2017).

BIOTECHNOLOGICAL APPROACHES FOR PHYCOTOXIN PRODUCTION

Despite the potential biological activity of these high-value secondary metabolites, only a few have been produced for research and commercial applications. Currently, commercial availability of phycotoxin standards is low and the cost prices are high depending on purity and supplier (e.g.: ≈1000 – 23000 €/mg of OA; ≈36000 – 296000 €/mg of DTX1; ≈75000 – 667000 €/mg of DTX2). Often, the phycotoxin is present in trace amounts in the producer organism, thus a sustainable supply from dinoflagellate harvesting is not feasible.

Among the different approaches for sustainable phycotoxin production are chemical synthesis, harvesting of natural blooms and large-scale, *in situ*, *in vitro* and transgenic cultivation of the producer organism. The choice of one or another will depend, among other variables, on the structural complexity of the phycotoxin, the nature of the source and its abundance and the biosynthetic pathway.

Chemical Synthesis

Chemical synthesis of some phycotoxins and/or characteristic fragments from dinoflagellates has been attempted (Forsyth et al., 1997; Ley et al., 1998; Dounay et al., 1999; Nicolaou et al., 2006; O'Connor and Brimble, 2007; Crimmins et al., 2009; Fürstner et al., 2009; Pang et al., 2011; Stivala et al., 2012; Wilde et al., 2012; Valot et al., 2015). This approach can be unsuitable as it often involves many steps with low yield, and their extremely complex structures make synthesis unfeasible (Nicholas and Phillips, 2006; Wilde et al., 2012). For example, PLTX contains 64 stereogenic centers and its synthesis involved assembly of seven building blocks in 39 steps, requiring a total of more than 140 steps (Armstrong et al., 1989). The synthesis of some phycotoxins, such as OA, has been pursued within the synthetic community due to the potential broad range of biological activities that OA possesses (Isobe et al., 1986; Forsyth et al., 1997; Ley et al., 1998). Successful total synthesis was also reported for 7-deoxy-okadaic acid (Dounay et al., 1999), DTX2 and 2-epi-DTX2 (Pang et al., 2011). Chemical synthesis has also supported the structural elucidation of several complex phycotoxins as well as help to deduce their mode of action (Usami, 2009; Wilde et al., 2012).

Genetic Engineering

Genetic manipulation and overexpression of specific genes associated with the biosynthesis of phycotoxins to enhance their production within the organism is an attractive approach. In order to genetically manipulate dinoflagellates to increase the production of the metabolites of interest it is essential to understand the biochemical processes occurring within the cells and a genome-scale metabolic model could help (Verma et al., 2019). However, the current level of their functional information is poorly understood which may in part be due to the long and complex genome sequences of microalgae. For example dinoflagellates genome (3–250 pg DNA/cell and 3000–215000 megabase pair (Mbp) in 20–325 chromosomes) is approximately 100 times larger than the human genome (3.2 pg DNA/cell and 3180 Mbp in 23 pairs of chromosomes) (Bhaud et al., 2000; McEwan et al., 2008; Verma et al., 2019). Dinoflagellates genome has a high number of unusual bases with a high degree of methylation, introns, redundant repetitive non-coding sequences, chromosomes are organized into a permanent liquid crystalline form (Rill et al., 1989) and it lacks recognizable promoter features (Guillebault et al., 2002) and common eukaryotic transcription factor binding sites (Bhaud et al., 2000; Moreno Díaz de la Espina et al., 2005; McEwan et al., 2008; Wisecaver and Hackett, 2011). Therefore, genetic manipulation methods used for similar or higher trophic systems cannot be extrapolated to dinoflagellates and it must be developed almost from the start. In addition, the identification of toxin-related genes and proteins is complex as a result of their evolutionary origin (Lin, 2011; Verma et al., 2019). To date, most of the efforts on genetic engineering have been driven toward the increase of lipid production in non-dinoflagellate algae (Lin et al., 2019; Park et al., 2019; Ng et al., 2020).

TABLE 1 | High value secondary metabolites produced by *Prorocentrum lima.*

Bioactive compound	Microalgae	References
OA	*P. lima* (712); (CCAP 1136/11); (Hainan Island); (Cuba isolates); (Paranagua isolates); (Recife isolates); (Spain isolates); (Tahiti isolates); (UK isolates); (Mexico isolates); (New Zealand); (PL11); (PLV2); (Japan isolates)	Murakami et al., 1982; Hu et al., 1992; Rhodes and Syhre, 1995; Bravo et al., 2001; Holmes et al., 2001; Lu and Chou, 2002; Fernández et al., 2003; Nascimento et al., 2005; Paz et al., 2007; Li et al., 2012, 2020; Pan et al., 2017; Moreira-González et al., 2019; Camacho-Muñoz et al., 2020; Nishimura et al., 2020; Tarazona-Janampa et al., 2020
2-deoxy OA	*P. lima*	Schmitz and Yasumoto, 1991
7-deoxy OA	*P. lima*	Schmitz and Yasumoto, 1991; Holmes et al., 2001
C4-diol OA/OA ester (2-hydroxymethyl-allyl okadaate)	*P. lima* (PLV2)	Fernández et al., 2003; Paz et al., 2007
C6-diol OA/OA ester (5-hydroxy-2-methylene-pent-3-enyl okadaate)	*P. lima*	Suárez-Gómez et al., 2005; Paz et al., 2007
C7-diol OA/OA ester (5-methylene-6-hydroxy-2-hexen-1-okadaate)	*P. lima*; (GY-H57)	Yasumoto et al., 1989; Pan et al., 2017
C8-diol OA/OA ester (7-hydroxy-2-methyl-hepta-2,4-dienyl okadaate)	*P. lima* (712); (Mexico isolates)	Hu et al., 1992; Lu and Chou, 2002; Suárez-Gómez et al., 2005; Paz et al., 2007; Tarazona-Janampa et al., 2020
C9-diol OA/OA ester (5,7-dihydroxy-2,4-dimethylene-heptyl okadaate)	*P. lima* (GY-H57)	Suárez-Gómez et al., 2005; Paz et al., 2007; Pan et al., 2017
C9-diol OA/OA ester (5-hydroperoxy-7-hydroxy-2,4-dimethylene-heptyl okadaate)	*P. lima* (PLV2)	Suárez-Gómez et al., 2005; Paz et al., 2007
C9-diol OA/OA ester (7-hydroxy-4-methyl-2-methylene-hept-4-enyl okadaate)	*P. lima* (712)	Hu et al., 1992; Paz et al., 2007
C9-diol OA/OA ester (7-hydroxy-2,4-dimethyl-hepta-2,4-dienyl okadaate)	*P. lima*	Norte et al., 1994; Pan et al., 2017
C9-diol OA/OA ester (7-hydroxy-4-methyl-2-methylene-hept-4-enyl okadaate)	*P. lima*	Norte et al., 1994; Paz et al., 2007
C9-diol OA	*P. lima*	Yasumoto et al., 1987; Lu and Chou, 2002
C10-diol OA/OA ester (5,7-dihydroxy-2,4-bis(methylene)heptyl okadaate)	*P. lima* (GY-H57); (Hainan Island)	Pan et al., 2017; Li et al., 2020
Methyl okadaate/OA Methyl ester/Methyl OA	*P. lima* (PL11); (PL2V); (712)	Hu et al., 1992; Lu and Chou, 2002; Fernández et al., 2003; Paz et al., 2007; Li et al., 2020
Ethyl okadaate/OA Ethyl ester/Ethyl OA	*P. lima* (712)	Hu et al., 1992
OA ester	*P. lima* (Spain isolates)	Bravo et al., 2001
Norokadanone	*P. lima* (PL2V); (CCAP 1136/11)	Fernández et al., 2003; Paz et al., 2007; Camacho-Muñoz et al., 2020
DTX1	*P. lima* (CCAP 1136/11); (Cuba isolates); (Paranagua isolates); (Spain isolates); (UK isolates); (Mexico isolates); (PL2V); (Japan isolates)	Hu et al., 1992; Bravo et al., 2001; Lu and Chou, 2002; Nascimento et al., 2005; Paz et al., 2007; Pan et al., 2017; Moreira-González et al., 2019; Camacho-Muñoz et al., 2020; Nishimura et al., 2020; Tarazona-Janampa et al., 2020
35S-DTX1	*P. lima* (GY-H57)	Pan et al., 2017; Camacho-Muñoz et al., 2020
DTX1-a	*P. lima* (CCAP 1136/11)	Camacho-Muñoz et al., 2020; Tarazona-Janampa et al., 2020
DTX2	*P. lima* (Spain isolates)	Hu et al., 1992; Bravo et al., 2001; Pan et al., 2017
DTX4	*P. lima* (UK isolates)	Hu et al., 1995; Nascimento et al., 2005
DTX6	*P. lima* (PL2V)	Suárez-Gómez et al., 2001; Paz et al., 2007
C8-diol DTX1-a	*P. lima* (Mexico isolates)	Tarazona-Janampa et al., 2020
C8-diol DTX1	*P. lima* (Mexico isolates)	Tarazona-Janampa et al., 2020
C9-diol DTX1	*P. lima* (PL-KNUAL-23)	Lee et al., 2015

(Continued)

TABLE 1 | Continued

Bioactive compound	Microalgae	References
DTX2 ester	*P. lima* (Spain isolates)	Bravo et al., 2001
DTX4 diol ester	*P. lima* (UK isolates)	Nascimento et al., 2005
Formosalide A	*P. lima* (PL040104002)	Lu et al., 2009
Formosalide B	*P. lima* (PL040104002)	Lu et al., 2009
Limaol	*P. lima* (Korea isolates)	Yang et al., 2017
Prorocentrin	*P. lima* (PL021117001 clone)	Lu et al., 2005
Prorocentrolide	*P. lima* (PL01); (PL11)	Lu and Chou, 2002; Li et al., 2020
Procentrolide 30-sulfate	*P. lima*	Torigoe, 1990
Prorocentrolide C	*P. lima* (YD-5)	Lee et al., 2019
14-*O*-acetyl-4-hydroxyprorocentrolide	*P. lima* (PL01)	Lu and Chou, 2002
3,5-dihydroxy-6,7-megastigmadien-9-one	*P. lima* (PL11)	Li et al., 2020
4-hydroxyprorocentrolide	*P. lima* (PL01); (YD-5)	Lu and Chou, 2002; Lee et al., 2019
9,51-dihydroprorocentrolide	*P. lima*	Torigoe, 1990
Apo-9′-fucoxanthinone	*P. lima* (PL11)	Li et al., 2020
Polyketide unidentified	*P. lima* (PL11)	Li et al., 2020
Terpenoid unidentified	*P. lima* (PL11)	Li et al., 2020

Culture of Dinoflagellates

Dinoflagellates in general grow slower than other protists such as diatoms, are quite shear-sensitive and relatively inefficient at nutrient uptake (Smayda and Reynolds, 2003). It has been speculated that the low chlorophyll a to carbon ratio is responsible for the slow growth of dinoflagellates (Tang, 1996) but the chlorophyll a content per unit mass of dinoflagellates is similar to diatoms (Gallardo-Rodríguez et al., 2012). Several studies have focused on small-scale cultures of benthic dinoflagellates to optimize growth rates and toxic production (Jackson et al., 1993; McLachlan et al., 1994; Morton et al., 1994; Yang et al., 2008; Zhong et al., 2008; Li et al., 2009; Vanucci et al., 2010; Varkitzi et al., 2010; López-Rosales et al., 2014; Wang et al., 2015; Hou et al., 2016; Accoroni et al., 2018; Aquino-Cruz et al., 2018; Gu et al., 2019; Lee et al., 2020). In a typical photosynthetic culture the maximum biomass concentration reached was well below 1 g/L and regarding phycotoxin production it is of the order of picograms (Gallardo-Rodríguez et al., 2012). Therefore, to obtain sufficient secondary metabolites mass culture in large-scale bioreactors is needed although it has proven challenging (Gallardo Rodríguez et al., 2010; Wang et al., 2015).

Similar to other phototrophic organisms, dinoflagellates require energy from light for their biosynthesis processes. Vertical migration and photo-acclimation allow them to travel and grow at the bottom and the upper layer of the aquatic system to gain access to nutrient-rich deeper layers and well-lit surface waters (Smayda and Reynolds, 2003; Smayda, 2010; Orchard et al., 2016; Shikata et al., 2020).

Typical dinoflagellate cell cycle involves an initial growth phase with high cell metabolic activity first (G1 phase), followed by DNA synthesis (S phase). Then, a second growth phase (G2 phase) in which cells accumulate nutrients needed for the mitosis (M phase) and cytokinesis (Pan et al., 1999; Bhaud et al., 2000). The biosynthesis of phycotoxins typically varies during different growth phases, with the highest production rates during the end of the exponential phase prior entering the stationary phase (Pan et al., 1999; Jia et al., 2019; Lee et al., 2020). Circadian rhythms are also known to influence growth rates and biosynthesis of secondary metabolites (Jacob-Lopes et al., 2009; Jia et al., 2019).

Dinoflagellate tolerance and acclimatization to different temperatures are strain specific and may depend on their original habitat as it was found that dinoflagellates from tropical habitats have lower tolerance to low temperatures (below 20°C) than dinoflagellates from temperate habitats (Jackson et al., 1993; McLachlan et al., 1994; Accoroni et al., 2018).

OA and DTXs are essentially produced via the polyketide pathway (Perez et al., 2008; Gallardo-Rodríguez et al., 2012). Therefore, the interaction between dinoflagellates (i.e., *Prorocentrum*) and their co-existing microbiome encoding the polyketide synthase gene may influence intracellular phycotoxin production (Perez et al., 2008; Lee et al., 2016). However, to date, there is little evidence that supports that extracellular bacteria influence growth and phycotoxin production (Tarazona-Janampa et al., 2020). A sound knowledge of cell cycle regulation and metabolism, synchronization of the cell cycle, use of optimum nutritional requirements and culture conditions, awareness of stress factors and optimized configurations of bioreactors are fundamental to mass culture of dinoflagellates for phycotoxin production.

CULTURE OF *Prorocentrum* spp. FOR PHYCOTOXIN PRODUCTION

The effects of key environmental factors (e.g., nutrients, temperature, salinity, and light availability) on the phycotoxin production are species dependent (Vanucci et al., 2010; Varkitzi et al., 2010; Wang et al., 2015; Aquino-Cruz et al., 2018; Gu et al., 2019; Lee et al., 2020). Nutrient limitation is the most common strategy used to boost phycotoxin production, however as a result of nutrient limitation cell growth usually declines.

Phycotoxin production was deemed to be a dependent of growth rate rather than due to the environmental or nutritional stress applied (Pistocchi et al., 2012). Therefore, a compromise must be reached between high phycotoxin production and maximum growth rate, leading generally to two-step culture strategies, with an initial phase based on biomass accumulation (nutrient replete conditions) followed by a phase of phycotoxin production (under deprivation conditions). Alternative strategies include changes in the light cycle, pH, temperature or salinity to boost phycotoxin production.

Effect of Culture Media Composition

Media

Natural seawater enriched with nutrients and trace metals is frequently used as a base for culture medium of dinoflagellates (Harrison and Berges, 2005). However, its continuous supply may be inconvenient so synthetic seawater is commonly used instead when a natural seawater supply is unfeasible (e.g., geographic limitations). Although composition of synthetic seawater can be easily controlled and modified, trace organics present in natural seawater are difficult to reproduce in its artificial counterpart. L1, L1-Si, f/2, f/4, K medium have been used to enrich the natural or synthetic seawater for the cultivation of *Prorocentrum* spp. (**Tables 2–6**). All these media contains similar nutritional elements (nitrogen, phosphorus, sulfates and trace metals and vitamins) but in different ratios. According to Guillard and Morton (2003) L1 and f/2 medium are more suitable for coastal planktonic species, while L2 and K media are favorable for growing benthic, epiphytic and oceanic planktonic species. Although the use of artificial seawater is acceptable for cultivating marine dinoflagellates, its long term replacement for natural seawater was deemed to lower production of bioactive metabolites (Hsieh et al., 2001). When *P. lima* was cultured in f/2 media prepared in natural seawater, artificial seawater (Guillard and Morton, 2003), and salt water (NaCl 35 g/l) it was able to grow in all types of prepared medium. However, the use of salt water affected the cell integrity in the culture that was indicated by lack of pigmentation and high levels of OA in the medium (Praptiwi, 2014). Long-term artificial seawater cultivation resulted in a substantial reduction of OA production of almost 70% after the second cycle of sub-culturing (Praptiwi, 2014) suggesting that artificial seawater may lack trace elements or nutrients present in natural seawater necessary to support phytotoxin production.

Nitrogen

Microalgae are able to obtain nitrogen from different sources. Benthic *Prorocentrum* spp. has been cultured using nitrate, ammonium and urea (**Table 2**) (McLachlan et al., 1994; Pan et al., 1999; Zhong et al., 2008; Li et al., 2009; Varkitzi et al., 2010; Hou et al., 2016; Accoroni et al., 2018; Gu et al., 2019; Lee et al., 2020). Whereas some studies suggest that nitrogen concentration is directly proportional to the maximum cell density but not growth rate (Zhong et al., 2008; Vanucci et al., 2010; Hou et al., 2016), others found that it is directly proportional to both (McLachlan et al., 1994; Li et al., 2009).

Under, routine culture conditions, maximum phycotoxin levels have been recorded at the end of the exponential growth phase and start of the stationary phase (McLachlan et al., 1994; Li et al., 2009; Varkitzi et al., 2010). However, when microalgae is grown under stress conditions, such as nutrient depletion, they synthesize multiple secondary metabolites, phycotoxins among them, to increase the possibility of survival under these unfavorable conditions. In general, limited nitrogen availability leads to an increase in phycotoxin production (McLachlan et al., 1994; Hou et al., 2016; Accoroni et al., 2018; Gu et al., 2019; Lee et al., 2020). For instance, in a nitrogen-depleted medium, intracellular concentration of OA in *P. hoffmannianum* was 3.7 times basal nitrogen level (883 μM) during the stationary phase (45.38 pg/cell) and 3.4 times this during its maximum growth (21.38 pg/cell) (Lee et al., 2020) (**Table 2**). Whereas in a medium with ten times more nitrogen (8830 μM) intracellular concentration of OA was similar during the stationary and the maximum growth phase (both ≈16.38 pg/cell) but the phycotoxin levels were higher in the maximum growth phase (2.6 times) than in the stationary phase (1.3 times) (Lee et al., 2020). Vanucci et al. (2010) demonstrated a similar trend of increased phycotoxin production as the concentration of nitrogen was decreased. In this case the effect was more noticeable in the production of DTX1 than in OA, with DTX1 production increased up to 3.3 times (0.39 pg/cell) and OA production up to 1.9 times (12.5 pg/cell) in a culture of *P. lima* when the concentration of nitrogen was reduced to 17.7 μM (**Table 2**) from basal levels. The difference on OA yield observed between *P. hoffmannianum* and *P. lima* when changing N concentrations could be due to be strain specific or due to the small differences between the culturing conditions.

In addition, the chemical nature of nitrogen may affect the phycotoxin production (**Table 2**). It has been suggested that ammonia may be a preferred source of nitrogen for benthic *Prorocentrum* spp. as it does not need enzymatic fixation although it does not seem to increase phycotoxin production (Pan et al., 1999; Zhong et al., 2008; Varkitzi et al., 2010). Gu et al. (2019) compared OA production of *P. lima* cultured with nitrate, urea or ammonium. Maximum cell density (47000 cells/mL) and OA production (25 pg/cell) were obtained using nitrate as a source and the lowest with ammonium. Similar effects were obtained by Varkitzi et al. (2010) when the same concentration of nitrate or ammonium were used for growing *P. lima*. Zhong et al. (2008) also reported the highest OA production using 100 μM nitrates (OA 338 pg/cell) but no differences were observed among 100 μM urea (OA 113 pg/cell) and 100 μM ammonium (OA 100 pg/cell). In the study of Gu et al. (2019) the use of different nitrogen sources led to different phycotoxin production, maximum cell densities and growth rates. In general, nitrates gave the maximum OA production and cell density followed by urea and finally ammonium.

Phosphorus

Phosphorus is an essential nutrient involved in several metabolic processes in dinoflagellates including photosynthesis, cell membrane synthesis, signal transduction and catabolism of sugars and fatty acids, among others (Lin et al., 2016).

TABLE 2 | Effect of nitrogen and phosphorus on the growth and toxin content of *Prorocentrum* spp.

Strain	Culture	Nitrogen source	Phosphorus source	N:P	Growth rate (μ/day)	Maximum cell density (cells/mL)	Toxin content (pg/cell)	References
P. hoffmannianum (CCMP2804)	L1-Si medium in seawater; salinity 30 ppt; 22°C; 3000 lux; 12/12h light/dark cycle; initial cell density 1000 cells/mL	NO_3^- (883 μM)	PO_4^{3-} (36.2 μM)	24.4	0.07	40200 (Day 42)	OA: 6.31 (Day 28); 12.40 (Day 50)	Lee et al., 2020
		NO_3^- depleted L1-S1 medium	PO_4^{3-} (36.2 μM)	0	n.a.	1910 (Day 42)	OA: 21.38 (Day 28); 45.38 (Day 50)	
		NO_3^- (8830 μM)	PO_4^{3-} (36.2 μM)	244	n.a.	53100 (Day 42)	OA≈16.38 (Day 28); 16.38 (Day 50)	
		NO_3^- (883 μM)	PO_4^{3-} depleted L1-Si medium	-	n.a.	3230 (Day 42)	OA: 18.36 (Day 28); 67.03 (Day 50)	
		NO_3^- (883 μM)	PO_4^{3-} (362 μM)	521	n.a.	49000 (Day 42)	OA: 12.57 (Day 28); 14.74 (Day 50)	
P. lima (CCMP2579)	F/2 medium; 20°C; 58 μmol photons/m^2/s; 12/12h light/dark cycle; initial cell density 12000 cells/mL (no seawater)	Control F/2 medium	Control F/2 medium	24.4	≈0.043[a]	45000 (Day 50)	OA≈12 (Day 20); ≈25 (Day 38); ≈60 (Day 50)	Gu et al., 2019
		N-limited (17.7 μM)	PO_4^{3-} (36.2 μM)	0.49	≈0.036[a]	38000 (Day 50)	OA≈12[c] (Day 20); ≈40[c] (Day 38); ≈38[c] (Day 50)	
		NO_3^- (883 μM)	P-limited (1.81 μM)	488	≈0.023[a]	30000 (Day 50)	OA≈8[c] (Day 20); ≈35[c] (Day 38); ≈175[c] (Day 50)	
		NO_3^- (883 μM)	PO_4^{3-} (36.2 μM)	24.4	≈0.039[a]	47000 (Day 50)	OA≈25[c] (Day 38)	
		Urea (883 μM)	PO_4^{3-} (36.2 μM)	24.4	≈0.036[a]	35000 (Day 50)	OA≈18[c] (Day 38)	
		NH_4^+ (883 μM)	PO_4^{3-} (36.2 μM)	24.4	no growth	13000 (Day 12)	OA≈13[c] (Day 38)	
		NO_3^- (883 μM)	Glycerophosphate (36.3 μM)	24.3	≈0.021[a]	45000 (Day 50)	OA≈18[c] (Day 38)	
		NO_3^- (883 μM)	NaH_2PO_4 (36.3 μM)	24.3	≈0.052[a]	45000 (Day 50)	OA≈35[c] (Day 38)	
		NO_3^- (883 μM)	ATP (36.3 μM)	24.3	≈0.032[a]	49000 (Day 50)	OA≈30[c] (Day 38)	
P. hoffmannianum (PHKL0414; isolates from Florida Keys, United States)	Modified f/4 medium: Si free, f/4 macronutrients, Se in seawater and trace metals, iron, vitamins (H, B1 and B12) and HEPES pH 7.1 at levels of f/2 medium; 27°C; 90–100 μmol photons/m^2/s; 12/12h light/dark cycle; initial cell density 500 cells/mL	NO_3^- (441 μM)	PO_4^{3-} (18.1 μM)	24.4	0.28 at 21°C	2611 (Day 12)	OA: 49.57	Accoroni et al., 2018
					0.19 at 27°C	5363 (Day 28)	OA: 106.91	
		NO_3^- (441 μM)	PO_4^{3-} (0.6 μM)	735	0.13 at 21°C	736 (Day 12)	OA: 262.49	
					0.12 at 27°C	752 (Day 16)	OA: 642.57	
		NO_3^- (14.7 μM)	PO_4^{3-} (18.1 μM)	0.81	0.21 at 21°C	809 (Day 12)	OA: 51.91	
					0.14 at 27°C	713 (Day 8)	OA: 56.88	

(Continued)

TABLE 2 | Continued

Strain	Culture	Nitrogen source	Phosphorus source	N:P	Growth rate (μ/day)	Maximum cell density (cells/mL)	Toxin content (pg/cell)	References
P. lima (CCMP2579)	F/2 medium; 20°C; 58 μmol photons/m^2/s; 12/12h light/dark cycle; initial cell density 12000 cells/mL	NO_3^- (882 μM)	PO_4^{3-} (36.3 μM)	24.3	≈0.097[a]	≈40000[c]	≈17.5[b,c]	Hou et al., 2016
		NO_3^- (17.7 μM)	PO_4^{3-} (36.3 μM)	0.49	≈0.103[a]	≈35454[c]	≈30.1[b,c]	
		NO_3^- (882 μM)	PO_4^{3-} (1.81 μM)	487	≈0.021[a]	≈25909[c]	≈35.2[b,c]	
P. lima (isolates from coastal lagoon of Goro, Italy)	F/2 medium in seawater; salinity 25%; 20°C; 90 μmol photons/m^2/s; 16/8h light/dark cycle; initial cell density 300–400 cells/mL	NO_3^- (17.7 μM)	PO_4^{3-} (36.3 μM)	0.49	0.22	7040	OA: 12.5[b] DTX1: 0.39[b]	Vanucci et al., 2010
		NO_3^- (44.2 μM)	PO_4^{3-} (36.3 μM)	1.22	0.22	≈10000[c]	OA≈11[b,c] DTX1≈0.25[b,c]	
		NO_3^- (88.3 μM)	PO_4^{3-} (36.3 μM)	2.43	0.22	≈14000[c]	OA≈11.74[b,c] DTX1≈0.18[b,c]	
		NO_3^- (294 μM)	PO_4^{3-} (36.3 μM)	8.10	0.22	≈24000[c]	OA≈10.34[b,c] DTX1≈ 0.15[b,c]	
		NO_3^- (882 μM)	PO_4^{3-} (36.3 μM)	24.3	0.22	33100–35000	OA: 6.69–6.87[b] DTX1: 0.12[b]	
		NO_3^- (882 μM)	PO_4^{3-} (0.73 μM)	1208	0.22	6250	OA: 10.75[b] DTX1≈ 0.26[b,c]	
		NO_3^- (882 μM)	PO_4^{3-} (1.81 μM)	487	0.22	≈11000[c]	OA: 15.80[b] DTX1≈ 0.32[b,c]	
		NO_3^- (882 μM)	PO_4^{3-} (3.63 μM)	243	0.22	≈17000[c]	OA≈11.27[b,c] DTX1≈ 0.26[b,c]	
		NO_3^- (882 μM)	PO_4^{3-} (12.1 μM)	72.9	0.22	≈31000[c]	OA≈9.7[b,c] DTX1≈ 0.16[b,c]	
P. lima (CCAP1136/11)	Modified f/2 medium by adding H_2SeO_3 and reducing the $CuSO_4$ conc. in seawater; salinity 38 psu; 20°C; 35 μmol photons/m^2/s; 16/8h light/dark cycle; initial cell density ≈1000[c]	NO_3^- (882 μM)	PO_4^{3-} (36.3 μM)	24.3	0.22	20950	≈6[b,c] (Day 34)	Varkitzi et al., 2010
		NH_4^+ (882 μM)	PO_4^{3-} (36.3 μM)	24.3	0.21	10790	≈4.7[b,c] (Day 20)	
		NO_3^- (300 μM)	PO_4^{3-} (72.5 μM)	4.14	0.11	≈18000[c]	≈5.0[b,c] (Day 34)	
		NO_3^- (1450 μM)	PO_4^{3-} (18.1 μM)	80.1	0.2	≈16000[c]	11.27[b,c] (Day 34)	
		NO_3^- (1450 μM)	PO_4^{3-} (18.1 μM)	80.1	0.17	20690	OA: 4.63 DTX1: 0.55	Varkitzi et al., 2017
P. lima (clone from Mahone Bay, Nova Scotia)	F/2 medium buffered with Tris in seawater; 23°C; 80–90 μmol photons/m^2/s; 16/8h light/dark cycle; initial cell density 5000–6000 cells/mL	NO_3^- (0 μM)	PO_4^{3-} (36.3 μM)	0	≈0.013[a]	≈8077[c]	OA + DTX1: 34[b] (Day 20)	McLachlan et al., 1994
		NO_3^- (300 μM)	PO_4^{3-} (36.3 μM)	8.26	≈0.032[a]	≈20000[c]	OA + DTX1: 25[b] (Day 30)	
		NO_3^- (1000 μM)	PO_4^{3-} (36.3 μM)	27.6	≈0.033[a]	≈20833[c]	OA + DTX1: 7[b] (Day 30)	
P. lima (CCMP2579)	n.a.	NO_3^- (88.2 μM)	PO_4^{3-} (36.3 μM)	2.43	≈0.029[a]	≈5220[c]	OA: 192.69[b]	Li et al., 2009
		NO_3^- (882 μM)	PO_4^{3-} (36.3 μM)	24.3	≈0.039[a]	≈10000[c]	OA: 100.66[b]	
		NO_3^- (882 μM)	PO_4^{3-} (3.63 μM)	243	≈0.029[a]	≈3333[c]	OA: 268.68[b]	
		NO_3^- (882 μM)	PO_4^{3-} (36.3 μM)	24.3	≈0.039[a]	≈10000[c]	OA: 100.66[b]	

(Continued)

TABLE 2 | Continued

Strain	Culture	Nitrogen source	Phosphorus source	N:P	Growth rate (μ/day)	Maximum cell density (cells/mL)	Toxin content (pg/cell)	References
P. lima (CCMP2579)	n.a.	NO_3^- (12 μM)	PO_4^{3-} (36.3 μM)	0.33	0.058	≈13180[c]	OA≈210[b,c]	Zhong et al., 2008
		NO_3^- (25 μM)	PO_4^{3-} (36.3 μM)	0.69	0.058	≈13180[c]	OA≈275[b,c]	
		NO_3^- (50 μM)	PO_4^{3-} (36.3 μM)	1.38	0.058	≈14540[c]	OA≈240[b,c]	
		NO_3^- (100 μM)	PO_4^{3-} (36.3 μM)	2.75	0.058	≈15000[c]	OA≈338[b,c]	
		NH_4 (12 μM)	PO_4^{3-} (36.3 μM)	0.33	0.059	≈13210[c]	OA≈85[b,c]	
		NH_4 (25 μM)	PO_4^{3-} (36.3 μM)	0.69	0.059	≈11740[c]	OA≈68[b,c]	
		NH_4 (50 μM)	PO_4^{3-} (36.3 μM)	1.38	0.059	≈12500[c]	OA≈85[b,c]	
		NH_4 (100 μM)	PO_4^{3-} (36.3 μM)	2.75	0.059	≈13620[c]	OA≈100[b,c]	
		Urea (12 μM)	PO_4^{3-} (36.3 μM)	0.33	0.06	≈13880[c]	OA≈100[b,c]	
		Urea (25 μM)	PO_4^{3-} (36.3 μM)	0.69	0.06	≈13330[c]	OA≈58[b,c]	
		Urea (50 μM)	PO_4^{3-} (36.3 μM)	1.38	0.06	≈15550[c]	OA≈55[b,c]	
		Urea (100 μM)	PO_4^{3-} (36.3 μM)	2.75	0.06	18330	OA≈113[b,c]	
P. lima (CCMP2579)	n.a.	NO_3^- (882 μM)	PO_4^{3-} (0.5 μM)	1764	0.059	≈12230[c]	OA≈88[b,c]	Yang et al., 2008
		NO_3^- (882 μM)	PO_4^{3-} (1 μM)	882	0.059	≈13290[c]	OA≈76[b,c]	
		NO_3^- (882 μM)	PO_4^{3-} (2 μM)	441	0.059	≈14200[c]	OA≈86[b,c]	
		NO_3^- (882 μM)	PO_4^{3-} (5 μM)	176	0.059	≈15000[c]	OA≈60[b,c]	
		NO_3^- (882 μM)	PO_4^{3-} (10 μM)	88.2	0.059	≈16000[c]	OA≈68[b,c]	
		NO_3^- (882 μM)	Gly PO_4^{3-} (0.5 μM)	1764	0.048	≈11840[c]	OA≈116[b,c]	
		NO_3^- (882 μM)	Gly PO_4^{3-} (1 μM)	882	0.048	≈11840[c]	OA≈97[b,c]	
		NO_3^- (882 μM)	Gly PO_4^{3-} (2 μM)	441	0.048	≈11440[c]	OA≈100[b,c]	
		NO_3^- (882 μM)	Gly PO_4^{3-} (5 μM)	176	0.048	≈13600[c]	OA≈94[b,c]	
		NO_3^- (882 μM)	Gly PO_4^{3-} (10 μM)	88.2	0.048	≈16310[c]	OA≈88[b,c]	
		NO_3^- (882 μM)	ATP (0.5 μM)	1764	0.053	≈13160[c]	OA≈51[b,c]	
		NO_3^- (882 μM)	ATP (1 μM)	882	0.053	≈13830[c]	OA≈57[b,c]	
		NO_3^- (882 μM)	ATP (2 μM)	441	0.053	≈17000[c]	OA≈57[b,c]	
		NO_3^- (882 μM)	ATP (5 μM)	176	0.053	≈18160[c]	OA≈60[b,c]	
		NO_3^- (882 μM)	ATP (10 μM)	88.2	0.053	≈19160[c]	OA≈57[b,c]	

n.a.: not available; [a] *The specific growth rate is calculated according to the following formula:* $\mu = ((\ln N_1)-\ln(N_0))/(t_1-t_0)$ *where* N_0 *and* N_1 *are the cell density at time* t_0 *and* t_1*;* [b] *The toxin level was determined at stationary phase;* [c] *The data were estimated from data in the corresponding publication; OA: okadaic acid; DTX1: dinophysistoxin-1; N: nitrogen; P: phosphorus.*

Limited availability of phosphorus in culture medium has also proved to hinder the growth of *Prorocentrum* spp. (**Table 2**) (Hou et al., 2016; Accoroni et al., 2018; Gu et al., 2019). Whereas production of OA and DTX was promoted when dinoflagellates were grown under P-limitation (Vanucci et al., 2010; Varkitzi et al., 2010; Hou et al., 2016; Accoroni et al., 2018; Lee et al., 2020). In general, studies evaluate the effect of P-depletion on the growth rate, maximum cell density and phycotoxin production but not the effect of P-augmentation. Lee et al. (2020) observed that 362 μM of P had no great impact on the maximum cell density (20% increase) and phycotoxin production (1.2 times greater) of *P. hoffmannianum* in comparison to the effect of P-depletion had (maximum cell density was decreased by 92% but phycotoxin content increased 5.4 times).

The chemical nature of phosphorus also influence growth rates and cellular phycotoxin levels in *P. lima* (Gu et al., 2019; Yang et al., 2008) (**Table 2**). Maximum cell growth and OA production (35 pg/cell) was observed using sodium dihydrogen phosphate (NaH_2PO_4), followed by ATP (30 pg/cell) and finally glycerophosphate (18 pg/cell) (Gu et al., 2019). However, maximum cell density was similar regardless of the phosphorus source used (Gu et al., 2019). Yang et al. (2008) observed a similar effect on the growth rate and the maximum cell density of *P. lima* after different phosphorus sources were used. However in this case the phosphorus concentrations evaluated (0.5 to 10 μM) were below typical culture media (36.3 μM) and the maximum OA cell content increased as the concentration decreased (from 10 to 0.5 μM) after using glycerophosphate (from 88 pg/cell to 116 pg/cell of OA), and NaH_2PO_4 (from 68 pg/cell to 88 pg/cell) but not after using ATP (from 57 pg/cell to 51 pg/cell).

Trace Metals

In microalgae, trace metals play important roles in numerous metabolic processes including electron and oxygen transport, nutrient acquisition, anti-oxidative mechanisms, cell division and integrity (Bruland et al., 1991; Raven et al., 1999; Sunda, 2012). Growth and cell morphology may be altered by a deficiency of trace metals but an excess could induce cell death and cyst formation (Tian et al., 2018).

TABLE 3 | Effect of light on the growth and toxin content of *Prorocentrum* spp.

Strain	Culture	Light(h)/ dark(h) cycle	Illuminance	Growth rate (μ/day)	Maximum cell density (cells/mL)	Toxin content (pg/cell)	References
P. lima (CCMP 2579)	F/2 medium; salinity 30%; 25°C; 100 μmol photons/m^2/s; initial cell density 5000 cells/mL	8/16	100 μmol photons/m^2/s	n.a.	≈11000 (Day 35)	OA≈1.5[b,c]	Wang et al., 2015
		12/12	100 μmol photons/m^2/s	n.a.	≈25500 (Day 35)	OA≈1.5[b,c]	
		16/8	100 μmol photons/m^2/s	n.a.	≈26000 (Day 35)	OA≈1.2[b,c]	
P. belizeanum (VGO1029), Isolates from La Puntilla, Las Palmas de Gran Canaria)	L1 medium in seawater; 40 μmol photons/m^2/s; initial cell density 10000 cells/mL	12/12	20 μmol photons/m^2/s at 18°C	≈0.075[a,c]	≈60000[c] (Day 25)	n.a.	López-Rosales et al., 2014
		12/12	40 μmol photons/m^2/s at 18°C	≈0.120[a,c]	≈120000[c] (Day 25)	OA≈4.75[b,c]	
		12/12	80 μmol photons/m^2/s at 18°C	≈0.120[a,c]	≈120000[c] (Day 25)	n.a.	
		12/12	120 μmol photons/m^2/s at 18°C	≈0.105[a,c]	≈80000[c] (Day 25)	n.a.	
		12/12	20 μmol photons/m^2/s at 25°C	≈0.070[a,c]	≈35000[c] (Day 25)	n.a.	
		12/12	40 μmol photons/m^2/s at 25°C	≈0.200[a,c]	≈135000[c] (Day 20)	OA≈1.1[b,c]	
		12/12	80 μmol photons/m^2/s at 25°C	≈0.170[a,c]	≈100000[c] (Day 20)	n.a.	
		12/12	120 μmol photons/m^2/s at 25°C	≈0.120[a,c]	≈60000[c] (Day 20)	n.a.	
		12/12	20 μmol photons/m^2/s at 28°C	≈0.070[a,c]	≈15000[c] (Day 15)	n.a.	
		12/12	40 μmol photons/m^2/s at 28°C	≈0.120[a,c]	≈35000[c] (Day 15)	OA≈2.625[b,c]	
		12/12	80 μmol photons/m^2/s at 28°C	≈0.100[a,c]	≈35000[c] (Day 15)	n.a.	
		12/12	120 μmol photons/m^2/s at 28°C	no growth	-	n.a.	
P. hoffmannianum (882a, Isolates from Little Lameshur Bay, St John, US Virgin Island)	Modified K medium in seawater (no Tris, Cu and Si); salinity 36%; 27°C	16/8	2000 lux	≈0.06–0.2[c] (max at 27°C)	n.a.	OA≈10–53.8[c] (max at 23°C)	Morton et al., 1994
		16/8	3000 lux	≈0.1–0.425[c] (max at 29°C)	n.a.	OA≈11.3–38[c] (max at 29°C)	
		16/8	4000 lux	≈0.07–0.425[c] (max at 27°C)	n.a.	OA≈5–45[c] (max at 29°C)	
		16/8	5000 lux	≈0.08–0.53[c] (max at 27°C)	n.a.	OA≈2.5–17.5[c] (max at 25 and 31°C)	

n.a.: not available; [a] *The specific growth rate is calculated according to the following formula:* $\mu = ((\ln N_1)-\ln(N_0))/(t_1-t_0)$ *where* N_0 *and* N_1 *are the cell density at time* t_0 *and* t_1*;* [b] *The toxin level was determined at stationary phase;* [c] *The data were estimated from data in the corresponding publication.*

TABLE 4 | Effect of salinity on the growth and toxin content of *Prorocentrum* spp.

Strain	Culture	Salinity (%)	Growth rate (μ/day)	Maximum cell density (cells/mL)	Toxin content (pg/cell)	References
P. lima (CCMP 2579)	F/2 medium; salinity 30%; 25°C; 100 μmol photons/m^2/s; initial cell density 5000 cells/mL	15	n.a.	≈11500 (Day 35)	OA≈1.0[b,c] DTX1≈4.7[b,c]	Wang et al., 2015
		30	n.a.	≈25000 (Day 35)	OA≈1.8[b,c] DTX1≈11.0[b,c]	
		45	n.a.	≈7000 (Day 35)	OA≈3.0[b,c] DTX1≈22.5[b,c]	
P. hoffmannianum (882a, Isolates from Little Lameshur Bay, St John, US Virgin Island)	Modified K medium in seawater (no Tris, Cu and Si); salinity 36%; 27°C; 3000 lux; 16/8h light/dark cycle	28	≈0.125[c]	n.a.	OA≈7.5[c]	Morton et al., 1994
		31	≈0.135[c]	n.a.	OA≈4.5[c]	
		34	≈0.220[c]	n.a.	OA≈3.2[c]	
		37	≈0.160[c]	n.a.	OA≈3.5[c]	
		40	≈0.135[c]	n.a.	OA≈4[c]	

n.a.: not available; [b] *The toxin level was determined at stationary phase;* [c] *The data were estimated from data in the corresponding publication; OA: okadaic acid; DTX1: dinophysistoxin-1.*

In routine medium recipes, the composition of trace metals has been formulized to meet the biochemical requirements of most dinoflagellates culture. Yet, in some cases, modifications are needed as each *Prorocentrum* spp. has its own distinctive growth requirements. Furthermore, trace metal composition may discourage or promote phycotoxin production by dinoflagellates (Rhodes et al., 2006; He et al., 2010). For example, Se at pM level induced an increase of OA ester quota per cell in *P. lima* by approximately 50% (Rhodes et al., 2006). Gu et al. (2019) observed that an increase of Cu concentration (from 40 to 5039 nM) limited the growth of *P. lima* by 36% but increased the cellular OA concentration level 1.8 times (27 pg/cell). The effects of trace metals on dinoflagellate culture and their implications for mass cultivation and bioactive metabolite production strategies require further exploration.

Effect of Physical Factors

Other than nutritional factors, physico-chemical factors such as light, salinity and temperature have been shown to impact on the growth and the phycotoxin production of dinoflagellates (Morton et al., 1994; López-Rosales et al., 2014; Tan and Ransangan, 2015; Wang et al., 2015).

Light

As with other photoautotrophic phytoplankton, growth of dinoflagellates is directly affected by the availability and irradiance of light. In general, artificial light is chosen to culture dinoflagellates as it provides better control of the light spectrum, irradiance and photoperiod (**Table 3**) (Morton et al., 1994; López-Rosales et al., 2014; Wang et al., 2015). Light-emitting diodes (LEDs) are an excellent alternative as they outperform fluorescent light. LEDs are adjustable to fit the design of the photobioreactors (PBRs), provide effective light without generating too much heat, are low-cost and non-toxic. A few studies have shown the potential of LEDs to culture dinoflagellates (Schulze et al., 2014; López-Rosales et al., 2016; Molina-Miras et al., 2018).

Light intensity and photoperiod influence not only the growth and cell cycle of *Prorocentrum* spp. but also their cellular phycotoxin composition (**Table 3**). In the study of López-Rosales et al. (2014) excessive light intensity led to the photoinhibition of *P. belizeanum*. Maximum growth of *P. belizeanum* was found at 40 μmol photons/m^2/s regardless of the temperature (18–28°C). *P. hoffmannianum* growth rate increased as the irradiance increased from 2000 lux (0.20 μ/day) to 5000 lux (0.53 μ/day). High phycotoxin production has been observed at low light intensity conditions. Intracellular content of OA by *P. hoffmannianum* was higher (53.8 pg/cell) at low light intensity (2000 lux) in comparison to higher light intensities (5000 lux; OA: 17.5 pg/cell). The most commonly used light/dark cycles to study phycotoxin production are 8h/16h, 12h/12h and 16h/8h. Wang et al. (2015) reported that the light duration is directly proportional to maximum cell density. Maximum cell density of *P. lima* increased from 8h (≈11000 cells/mL) to 12h (≈25500 cells/mL) and remained fairly constant after 16h (≈26000 cells/mL) of light. However, cellular OA content was lower when the light exposure was increased up to 16h. Little is known about the relationship between phycotoxin production and photosynthesis that could be potentially associated to cell cycles (Pan et al., 1999). OA was suggested to be biosynthesized in the chloroplast (Zhou and Fritz, 1994) and stored in them or in peripheral vacuoles of *P. lima* (Barbier et al., 1999). Wang et al. (2015) indicated that both photosynthesis and dark respiration are needed for DSP toxin biosynthesis. Pan et al. (1999) indicated that DSP production was instigated by light. DSP toxin amount per cell (OA, OA C8-diol-ester, DTX1 and DTX4) increased soon after *P. lima* culture was exposed to light after a dark synchronization period (no changes during dark). Different DSP toxins were produced at different phase of the photocycle. A rise in DTX4 levels (initiated in the G1 to S phases "morning") preceded an increase in the cellular content of OA and DTX1 (produced during S and G2 phases "afternoon") 3 to 6h later. Based on the sequential pattern in the biosynthesis of DTX4, OA, OA-C8 diol-ester and DTX1, DTX4 was considered the precursor toxin in the biosynthetic cascade through a light-mediated stepwise enzymatic reactions. In Praptiwi (2014), light and dark cycle at low to medium

TABLE 5 | Effect of temperature on the growth and toxin content of *Prorocentrum* spp.

Strain	Culture	Temperature (°C)	Growth rate (μ/day)	Maximum cell density (cells/mL)	Toxin content (pg/cell)	References
P. lima (isolates from Moonfleet on the Fleet Lagoon, United Kingdom)	F/2 medium in seawater ($NO_3^- + NO_2^-$: 928–1000 μM; PO_4^{3-}: 32–40 μM) ; 50 ± 15 μmol photons/m^2/s; 12/12h light/dark cycle;	5	0.05	600	OA free: 0.99–1.78[e] OA total: 2.05–4.52[e] DTX1 free: 0.94–2.60[e] DTX1 total: 0.82–1.99[e]	Aquino-Cruz et al., 2018
		10	0.13	10500	OA free: 1.51–5.12[e] OA total: 3.77–10.99[e] DTX1 free: 1.31–5.61[e] DTX1 total: 2.07–5.96[e]	
		15	0.18	11500	OA free: 1.41–6.04[e] OA total: 2.78–10.69[e] DTX1 free: 1.18–4.92[e] DTX1 total: 1.43–5.32[e]	
		20	0.14	9600	OA free: 1.30–2.20[e] OA total: 2.62–7.50[e] DTX1 free: 0.97–2.97[e] DTX1 total: 0.97–3.16[e]	
		25	0.17	10100	OA free: 1.16–3.11[e] OA total: 2.68–6.71[e] DTX1 free: 0.82–2.60[e] DTX1 total: 0.95–2.66[e]	
		30	0.14	4100	OA free: 0.98–4.09[e] OA total: 2.2–10.54[e] DTX1 free: 0.88–3.58[e] DTX1 total: 0.84–3.31[e]	
P. lima (CCMP 2579)	F/2 medium; salinity 30%; 100 μmol photons/m^2/s; 12/12h light/dark cycle; initial cell density 5000 cells/mL	15	≈0.048[a]	≈16000 (Day 35)	OA≈5.5[b,c] DTX1≈14.1[b,c]	Wang et al., 2015
		20	≈0.050[a]	≈25000 (Day 25)	OA≈1.5[b,c] DTX1≈11[b,c]	
		25	≈0.056[a]	≈25000 (Day 25)	OA≈1[b,c] DTX1≈4.7[b,c]	
		30	≈0.036[a]	≈8200 (Day 7)	OA: 12.371[b,c] DTX1: 16.587[b,c]	
P. belizeanum (VGO1029, isolates from La Puntilla, Las Palmas de Gran Canaria)	L1 medium in seawater; 40 μmol photons/m^2/s; 12/12h light/dark cycle; initial cell density 10000 cells/mL	18	≈0.120[a,c]	≈120000[c] (Day 25)	≈4.75[b,c]	López-Rosales et al., 2014
		25	≈0.200[a,c]	≈135000[c] (Day 20)	≈1.1[b,c]	
		28	≈0.120[a,c]	≈35000[c] (Day 15)	n.a.	
P. lima (isolates from Nova Scotia, Canada)	n/a	5	≈0.000[a]	≈1000 (Day 28)	OA: 8 (Day 28)	Jackson et al., 1993
		10	≈0.073[a]	≈7800 (Day 28)	OA: 2.5 (Day 28)	
		15	≈0.150[a]	≈11000 (Day 16)	OA: 4.4 (Day 16)	
		20	≈0.198[a]	≈23800 (Day 16)	OA: 2.5 (Day 16)	
		25	≈0.229[a]	≈39000 (Day 16)	OA: 1.4 (Day 16)	
P. hoffmannianum (PHKL0414; isolates from Florida Keys, United States)	Modified f/4 medium: Si free, f/4 macronutrients, Se in filtered seawater and trace metals, iron, vitamines (H, B1 and B12) and HEPES pH 7.1 at levels of f/2 medium; 27°C; 90–100 μmol photons/m^2/s; 12/12h light/dark cycle; initial cell density 500 cells/mL	21	0.28	2611 (Day 12)	OA: 49.57	Accoroni et al., 2018
		27	0.19	5363 (Day 28)	OA: 106.91	

n.a.: not available; [a] *The specific growth rate is calculated according to the following formula:* $\mu = ((lnN_1)-ln(N_0))/(t_1-t_0)$ *where* N_0 *and* N_1 *are the cell density at time* t_0 *and* t_1*;* [b] *The toxin level was determined at stationary phase;* [c] *The data were estimated from data in the corresponding publication;* [e] *Ranges consider lag, exponential and stationary growth phases; OA: okadaic acid; DTX1: dinophysistoxin-1.*

TABLE 6 | Bioreactor type, culture mode and operational conditions for growth and toxin production of *Prorocentrum* spp.

Strain	Type of reactor	Reactor operation	Reactor size (L)	Type of aeration/agitation	T^a (°C)	Irradiance and light regime	References
P. lima spp.	Carboy	Batch	19	Gentle aeration/ No stirring	18	- 90 ± 5 μmol photons/m^2/s; Continuous dark for 17 days; 14h:10h light/dark cycle for the following 13 days	Pan et al., 1999
P. lima(CCMP 2579)	Vertical flat PBR	Batch	100	Air bubbling (0.08 vmm)/No stirring	20	4W fluorescent lamps with white light; 12h/12h light/dark cycle	Wang et al., 2015
P. lima(CCAP1136/11)	Carboy	Batch	4	Gentle agitation by hand every 24h	20	Cool white fluorescent tubes; 35 μmol photons/m^2/s; 16/8h light/dark cycle	Varkitzi et al., 2010
P. lima(CCAP1136/11)	Polypropylene bag	Batch	300	Aeration with air pumps at 100 L/h/Manual agitation every 24h	20	Cool white fluorescent tubes; 35 μmol photons/m^2/s; 16/8h light/dark cycle	Varkitzi et al., 2017

PBR, photobioreactor.

light intensities (25 and 50 μmol/m^2/s) were proved to have no substantial effect to the total yield of OA and DTX-1, when compared to cultures with continuous light exposure. It did, however, improved the low yield gained in cultures exposed to high light intensity (95 μmol/m^2/s), with approximately 36% increase for OA and 66% increase for DTX-1 when using 12 hours light and dark cycles compared to continuous cycle. Despite this, the study (Praptiwi, 2014) further found that these improvements in yields are still significantly lower than those obtained from cultures exposed to low and medium light intensities (25 and 50 μmol/m^2/s) at both continuous light and 12 hours light/dark cycles.

Salinity

Salinity influences biochemical processes in the cell such as nutrient uptake and transportation system inside cell (Hsieh et al., 2001). Variations in ion strength and ionic composition induces distinctive responses on the growth of dinoflagellates due to osmoregulation (Stefels, 2000). Growth tolerance under a range of salinities content appears to be different for each species and is attributed to its geographic origin (D'Ors et al., 2016; Monti-Birkenmeier et al., 2019).

In principle, changes in salinity could affect phycotoxin production and content, either by direct effect on the biosynthetic pathways, or indirectly, by modulating cell growth which in turn indirectly affect cellular phycotoxin content (Parkhill and Cembella, 1999; Aguilera-Belmonte et al., 2013). This is supported by evidence that despite the same salinity, phycotoxin production appeared to be variable throughout the growth phase; with a constant level during the exponential growth phase which starts to increase at early stationary phase (Parkhill and Cembella, 1999). However, it was argued by Lim et al. (2011) that phycotoxin production is not a result of salinity-dependent-growth rate, but rather governed by cell osmoregulation and utilization of amino acid. Lim et al. (2011) further explained that evidence of this was shown in their results of toxin quota (Qt) obtained from the two lowest growth cultures at 5 and 35 psu, where much lower Qt observed in 5 psu than 35 psu.

Very few studies (**Table 4**) have dealt with the effect of salinity on growth rate and phycotoxin production by *Prorocentrum* spp. and they show contradictory results. In general growth rate decreased with salinity (0.125 μ/day at 28% and 0.105 μ/day at 37%; at 40% there was 0.120 μ/day) (Morton et al., 1994). The maximum cell density during the stationary phase also declined with increasing salinity (Wang et al., 2015). The cellular phycotoxin content in *P. lima* increased with increasing salinity. The Qt of OA increased from ≈1.0 pg/cell to ≈3.0 pg/cell and DTX1 from ≈4.7 pg/cell to ≈22.5 pg/cell when the salinity was increased from 15 psu to 45 psu (Wang et al., 2015). On the other hand, cellular content of OA in *P. hoffmannianum* was inversely proportional to salinity content and growth rate (Morton et al., 1994). The maximum cellular OA level (≈7.5 pg/cell) was reached at 28 psu and the lowest (≈3.2 pg/cell) was found at 34 psu which coincided with the highest growth rate (≈0.22 μ). As the salinity was increased up to 40 psu the growth rate declined (≈0.135 μ) and the OA cellular content slightly increased up to ≈4.0 pg/cell).

Temperature

Effects of temperature on growth and phycotoxin production of dinoflagellates appear to be similar to salinity, where each species has a different temperature range that enables optimum growth and bioactive compound production, which is a dependent on its original habitat (Koike et al., 1998; Aguilera-Belmonte et al., 2013). Benthic *Prorocentrum* spp. grow in tropical regions (≈30°C), whereas epiphytic strains grow in colder waters. Hence, *Prorocentrum* spp. are able to adapt to a broad range of temperatures (**Table 5**). For instance *P. lima* isolated from temperate areas (Canada) can grow between 10 and 25°C (Jackson et al., 1993), whereas if it is a strain isolated from tropical regions (United States) it grows between 21 and 27°C (Accoroni et al., 2018). In particular, 25°C is the optimal growth temperature for *P. lima* and *P. belizeanum* (Jackson et al., 1993; López-Rosales et al., 2014; Wang et al., 2015; Aquino-Cruz et al., 2018). Morton et al. (1994) observed that *P. hoffmannianum* can tolerate temperatures between 21 and 36°C, and gave 27°C as the optimal temperature.

Temperature has been found to influence growth, enzymatic activities and phycotoxin production in *Prorocentrum* spp. (López-Rosales et al., 2014; Lee et al., 2016).

Stress caused by changes in temperature promote phycotoxin production in *Prorocentrum* spp. Several studies have shown that maximum growth and cell density of *Prorocentrum* spp. obtained at specific temperatures were disassociated from maximum cellular phycotoxin levels (Jackson et al., 1993; López-Rosales et al., 2014; Wang et al., 2015; Aquino-Cruz et al., 2018) (**Table 5**). Disagreement on the influence that temperatures have above or below the optimal growth temperature to obtain the highest OA production have been published. For instance, temperatures above the optimal growth temperature of *P. lima* led to an increase of up to 12 times of the cellular OA level and up to 3.5 times of DTX1 (Wang et al., 2015) and up to 2.2 times of OA in *P. hoffmannianum* (Accoroni et al., 2018). In contrast, lower temperatures led to an increase of the cellular phycotoxin content of OA and DTX1 in *P. lima* and *P. belizeanum* (Jackson et al., 1993; López-Rosales et al., 2014; Aquino-Cruz et al., 2018). Extreme temperatures led to an increase of the cellular phycotoxin content although the relationship between temperature and phycotoxin production is not clear (Lee et al., 2016).

MASS CULTIVATION OF DINOFLAGELLATES

Early studies on secondary metabolites from dinoflagellates mostly attempted to understand how environmental and nutritional parameters affect the production of phycotoxins as a control strategy for HABs and their related health impacts. Nevertheless, the potential applications of these phycotoxins had shifted the interest to optimize their production at large scale.

For example, various attempts were carried out to find the most efficient way of producing OA for commercial demands. Total synthesis of OA was successfully demonstrated by a study (Ley et al., 1998) which assembled three chemical fragments in a convergent manner but this method is somewhat time-consuming, requires many steps with low final yield, and may be impractical for a large-scale production. Another approach was to extract OA from natural blooms of microalgae in seawater by a large-scale pumping method, as shown in a study by Rundberget et al. (2007). Nevertheless, considering the small quantity of purified phycotoxin obtained (2.7 mg OA and 1.3 mg DTX2 from ≈10250 L of seawater containing ≈2×10^4 cells/L of Dinophysis acuta), this method can be unsustainable as it consumes a lot of energy. The limitations of these approaches highlight the convenience and practicality of culturing organisms that produce OA and other bioactive compounds.

One of the biggest challenges in microalgae mass cultivation is to design and operate a cost-effective production system that allow the control over the growth rate and product yield. To address low product yields and upscaling the main parameters to control are nutritional and environmental factors, light availability and mass and heat transfer as well and efficient PBR design.

Traditionally, microalgae have been cultivated in shallow open PBRs using a raceway design and basic mixing. Unfortunately, they are not suitable for toxic microalgae as they pose a considerable risk for the ecosystem and are prone to contamination (Gallardo-Rodríguez et al., 2012). Conversely, closed PBRs allow more control over operating conditions and less chance of contamination. Various configurations of closed PBRs have been used for culturing dinoflagellates including carboys, stirred-tanks, airlift, bubble column and flat-panel type, with the last two the most typically used (Wang et al., 2015; López-Rosales et al., 2016; Molina-Miras et al., 2018). Selection of closed PBRs will depend on the characteristics of the culture organism, the desired product and other parameters such as capital and maintenance costs. The culture can be operated as batch, fed-batch, continuous, or semi-continuous modes. Continuous systems are attractive due to the possibility of maintain optimum growth and automatization however, they need constant supply of fresh media, and they are complex to install and run as well as being more expensive. In semi-continuous mode fresh media is introduced as required, at interval periods, using much less volume of media than the continuous system. However, to date only a few studies described the cultivation of *P. lima* to produce OA and all of them operate in batch mode (**Table 6**).

Light is one of the key parameters to enhance the productivity of phototropic microalgae. Light should be provided at the appropriate intensity, wavelength and duration (Carvalho et al., 2011). Light attenuation and/or mutual shading effects should be limited to ensure culture efficiency and lower energy consumption. It is necessary to design a PBR that has a high surface to volume ratio, and relatively low culture thickness (López-Rosales et al., 2014). Wang et al. (2015) designed a vertical flat bioreactor with an illumination system arranged at the bottom of the PBR. This design promoted the light energy absorption and utilization of benthic microalgae (Wang et al., 2015). Light and dark cycles allow the cells to perform photosynthesis during the light regime and respiration during the dark regime. An alternative to continuous light availability is

intermittent and "flashing" light which have proven to efficiently promote photosynthesis rate (Acién Fernández et al., 2013) and increase the productivity of secondary metabolites from microalgae (Carvalho et al., 2011). LEDs have been satisfactory used in pilot-scale bubble column PBRs for dinoflagellate culturing (Schulze et al., 2014; López-Rosales et al., 2016; Molina-Miras et al., 2018).

Phototrophic microalgae produce oxygen and uptake carbon dioxide during photosynthesis altering the culture medium and the pH. Therefore, it is essential to remove the produced oxygen from the medium and to supply enough CO_2 as the carbon source (Posten, 2009). In addition, as benthic microalgae grow, they form lumps that attach to the bottom of the bioreactor forming dense mats and some strains (e.g., *P. belizeanum*) produce extracellular mucus that can cover the bottom of the reactor forming a thin layer (López-Rosales et al., 2014). Gas injection is essential to increase mass transfer but designing a suitable culture system for dinoflagellate production can be particularly difficult in ensuring that the cell viability is maintained. Dinoflagellates are extremely sensitive to fluid turbulence in the culture media (García Camacho et al., 2007) and most of them do not readily grow in closed PBRs due to this (Gallardo-Rodríguez et al., 2009, 2012). Small-scale turbulence may affect growth rate and morphology and excessive turbulence may result in flagella impairment due to physical damage (Berdalet et al., 2007; García Camacho et al., 2007; Wang and Lan, 2018). Not only the intensity of shear force should be taken into account but also its period and interval (Gibson and Thomas, 1995). Detrimental effects of shear stress include growth inhibition; increased time for recovery, reduced photosynthetic activity, disturbed cell cycles, production of reactive oxygen species (ROS), changes in the fluidity of the cell membrane and even cell lysis (García Camacho et al., 2007; Gallardo-Rodríguez et al., 2009; Gallardo Rodríguez et al., 2010). No adverse effects were observed when the specific energy dissipation rate was below 1 cm^2/s^3 (Berdalet et al., 2007). The mechanism of shear-induced damage on cells has been hypothesized in previous studies. It has been suggested that damage is linked to their enormous genomes packed in relatively small volumes, to the alteration of metabolic processes that induce the production of intracellular ROS, to the reduction of time-integrated light exposure of individual cells or to direct impact on the integrity of cellular organelles (Juhl et al., 2001; Garcia-Camacho et al., 2007; Gallardo-Rodríguez et al., 2009). Antioxidant agents and additives to alter the fluidity of the media have been proven useful at low concentrations to protect the cells against shear stress (Gallardo-Rodríguez et al., 2009, 2012; Gallardo Rodríguez et al., 2010). Despite this, fluid turbulence is an important aspect in a bioreactor to maintain mass transfer within the system. It ensures good mixing of nutrients and gas exchange, and prevents deposition of cells at the bottom of the reactor, as well as forcing the cells to move between light and dark zones of the PBR. Overall, selecting the cultivation system should reflect on a design that is able to provide sufficient mass transfer whilst maintaining cell viability of the culture.

CONCLUSION

Despite the negative impact of phycotoxins from dinoflagellates on human health, they have shown valuable pharmacological and biotechnological potential. OA and its derivatives are needed for research studies mainly due to their activity as selective protein phosphatases inhibitors. Besides OA, DTXs and analogs, *Prorocentrum* spp. produce many other secondary metabolites such as prorocentrolides and formosalides with interesting bioactive potential that remain neglected. Limited quantities in the producer organism as well as the variability of these high value secondary metabolites content urge a suitable production method to ensure adequate and consistent supply. As chemical synthesis, harvesting of natural blooms and genetic engineering strategies currently have considerable limiting factors (e.g., complex, low yield, expensive and scalability issues) culturing has proven to be the most suitable process to obtain high quantities of phycotoxins. Considerable efforts have been made into culturing to enhance phycotoxin production by *Prorocentrum* spp. at lab scale but just a few studies have demonstrated significant increase in yields. Discrepancies observed among performance highlight the absence of generic culturing protocols (e.g., age, cell density of inoculum) to maximize the growth rate of *Prorocentrum* spp. and the production of valuable secondary metabolites. Some advances have been made on the design of PBRs, in terms of nutritional and operational factors, however a few technological limitations still need to be addressed to grow this extremely shear sensitive organisms.

AUTHOR CONTRIBUTIONS

DC-M: writing – original draft and writing – review and editing. RP: writing – review and editing. LL: writing – review and editing and conceptualization. CE: writing – review and editing, conceptualization, and funding acquisition. All authors contributed to the article and approved the submitted version.

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