Abstract

Microalgae are unicellular photosynthetic organisms that can produce organic carbon from CO₂ using sunlight energy and for their ability to fix atmospheric carbon in organic compounds are considered primary producers in the marine food chain, plankton. The various aquatic species that feed on plankton can represent, due to the incidence of numerous factors, the basis for the proliferation of pathogenic microorganisms and the production of biotoxins, known as phycotoxins, which have attracted the attention of researchers not only for their complex chemical structure and for various pharmacological activities (cytotoxic, anti cancer, antifungicide, antibiotic, etc...), but also for their ability to modify and activate important metabolic pathways. These biomolecules, thanks to the remarkable reproductive speed, formed the substrate to study and understand complex cellular functions, with important effects on human health.

Dinoflagellates are microscopic, unicellular, flagellated algae, which represent one of the most important groups of marine phytoplankton and freshwater and in addition to the Red Tide phenomenon are responsible for the production of highly toxic biotoxins. Important human poisonings occur as a result of the ingestion of bivalve shellfish due to their ability to filter water and, consequently, accumulate pollutants in their body. Some microalgae are able to produce ichthyotoxins that, by acting on gills, can cause prolonged death of fish, and which, if consumed by humans, can lead to serious health risks. Dinoflagellates have attracted the attention of researchers because of the possibility of beneficial use of their metabolites: glycolipids containing polyunsaturated fatty acids are considered molecules responsible for allelopathic effects and therefore these algae are exploited by agronomists in the cultivation of terrestrial plants. Tetradotoxin, although a very toxic molecule, has proven to be important for knowledge of nerve transmission; exerts anesthetic action to block sodium channels; relieves cranial symptoms in cases of heroin withdrawal. Goniatotoxins have been shown to be a safe and effective therapeutic tool as a painkiller as they can be taken for long periods without showing unwanted side effects. Amphidinolids show in vitro a strong cytotoxicity to L1210 murine lymphoma and human epidermal carcinoma cells. The components of these algae also have antibacterial antimicrobial, antioxidant, protect against UV radiation, and for the richness of functional foods, including docosahexaenoic acid can also be used for children's products.

Keywords

Dinoflagellates, Marine toxins, Diarroic shellfish poisoning, Pharmacological activity

Introduction

Fish products are a food of considerable nutritional power, but they can
also be particularly harmful to health, to the presence of pathogenic microorganisms as a result of pollution of the aquatic ecosystem. In fact, they are able to metabolize and accumulate in the tissues various molecules equipped with toxicity for both aquatic fauna and for humans, known as “marine biotoxins”, to which they follow particular disorders that are sometimes even lethal [1].

The substrate for biotoxins synthesis is plankton and the “phytoxins”, i.e. algal biotoxins, are the most important and have attracted the attention of researchers both for the presence of molecules with new chemical structures, and because they have highlighted numerous pharmacological activities [2]. The consumption of bivalve molluscs is considered one of the main culprit of transmission to humans not only of bacterial and viral diseases, but above all of intoxications from algal biotoxins, for the widespread habit of consuming them under-cooked or even raw [3, 4].

Traditional cooking methods (by boiling, steaming, or baking) have little impact on paralyzing toxins because they pass from the tissue in which they are contained to the cooking juices. Industrial processes, if there are very high amounts of toxins, are more effective: both cooking in alkaline pH solution preceded by a detoxification process and thermal treatment followed by canning reduce the presence of most toxins below the safety limits. The boxing process is the most suitable, with no alteration of the sensory characteristics of shellfish.

The problem of the presence of algal toxins in molluscs is becoming very important from a sanitation point of view, as a result of both the increased development of marine algae species recognized as harmful, and for algal flowering phenomena, presumably linked to the eutrophization of coastal areas. Recent research has revealed a relationship between the presence of pollutants in the water, in particular nitrogen and phosphorus, and the concentration of toxic substances in algae: at low concentrations of these pollutants algal metabolism is aimed at the production of primary components such as proteins and polysaccharides, while a high degree of pollution corresponds to an increase in toxins. The phenomenon of algal blooms has been known since ancient times and is manifested in many areas of the world, linked to the very high proliferation of algal cells, with a change in the colour of the water from red to yellow to brown, depending on the species, as a result of an increase in temperature, brightness and pollution [5, 6].

Algal biotoxins

Biotoxins are substances produced by a living organism capable of inducing, even at low concentrations, harmful biological manifestations on certain living species. Toxic substances are to be considered secondary metabolic products typical of the different species and, according to some authors, they would be produced as a mechanism of defense against any predators as well as being involved in the phenomenon of prolonged algal bloom of the species. The active ingredients have generally been identified based on the macroscopic symptoms of poisoning they provide to humans, or are listed together with the name of the toxin or the species that produces them.

The algae responsible for the intoxication phenomena are red microalgae, the Dinoflagellates (Figure 1), belonging to the group of the Dinoficeae. Often they are phosphorescent algae, provided with a skeleton or shell of a substance similar to cellulose, elegantly sculpted, which extends into long extensions, the flagella, generally two, located in furrows and directed to each other. Their chemical composition is characterized by substances of different nature: amino acids, purinic derivatives, polyethers, polyhydroxylated compounds that have both hydrophilic and lipophilic characteristics [7]. The difference in solubility corresponds to a different expression of toxicity; in fact, water-soluble toxins are responsible for respiratory paralysis (Paralytic Shellfish Poisoning) (PSP) and forms of temporary amnesia (Amnesic Shellfish Poisoning) (ASP), while fat-soluble toxins are primarily responsible for severe and prolonged gastrointestinal disorders (Diarrhetic Shellfish Poisoning) (DSP).

DSP is the most common intoxication, symptoms occur after about thirty minutes after ingestion of contaminated shellfish and the action is commensurate with the amount of mollusk ingested. The syndrome is mainly diarrhetic in nature, caused by polyetheric toxins containing Okadaic acid, \((\text{C}_{11}\text{H}_{22}\text{O}_{11})\) (Figure 1) produced by Dinoflagellates belonging to the Dinoficeae group, genus Dinophysis and Protodinophysis, which are normally present in the seas around the world [8, 9].

With regards the mechanism of action of Okadaic acid, it has been shown that it causes long-lasting contractions in the vascular smooth musculature; this happens without the intervention of neurotransmitters, in fact adrenaline and acetylcholine do not affect the action of that acid. Okadaic acid is held responsible for promoting the tumor on rat skin following local applications and its presence in drinking water induces the formation of tumours in the stomach of rats. Accurate pharmacologica studies have proposed an action mechanism for Okadaic acid and its derivatives, based on inactivation of the function of the tumor suppressor gene function, through hyperphosphorylation, especially at the level of serine and threonine residues, due to inhibition of certain phosphatases. It is likely to result in a build-up of phosphorylated proteins, which are then involved in the promotion of the tumour [10, 11].

DSP toxins are stable to heat, although prolonged boiling can decrease the concentration of Okadaic acid within shellfish. Most of the DSP syndromes have occurred in Japan where shellfish cultivation is widespread and along the eastern and western coasts of North America linked to the “Red Tides” produced by Dinoflagellates.
New poisoning syndromes resulting from Dinoflagellate toxins have recently been characterized due to the presence in addition to the Okadaic acid of other substances, Azaspiracid (C_{47}H_{71}NO_{12}), Yessotoxin (C_{55}H_{82}O_{21}S_{2}) and Palitoxin (C_{129}H_{223}N_{3}O_{54}) (Figure 3-5), compounds that have different toxicological effects and mechanisms of action. Such toxins can be functionally classified as neurotoxins and hepatotoxins, based on their clinical symptoms. Their neurotoxicity is mediated by different and highly specific interactions with the ion channels involved in neurotransmission.

In fact, in addition to Okadaic acid, responsible for DSP syndrome is Prorocentrum minimum (Figure 6) an algae that contains Venerupin, a substance of which the structure is not yet known, but harmful for the liver, which has caused shellfish poisoning resulting in gastrointestinal diseases in the humans. This species, in addition to being responsible for many deaths among humans, is responsible for the remarkable killings of shellfish in Japan, in the Gulf of Mexico, in Florida [12-14]. This algae, in addition to promoting very large red tides due to its high productivity, is also very resistant to changes in temperature and salinity. It has recently been found in the Mediterranean Sea and in particular along the coasts of Adriatic Sea.

Venerupin is a molecule that exhibits hepatotoxic activity and is responsible for VSP (Venerupin Shellfish Poisoning) syndrome. Disorders of this toxin are both gastrointestinal and neurological, but not paralyzing, caused by the ingestion of oysters and clams. Poisoning is characterized by a long incubation 24-48 hours followed by a sudden onset of symptoms: nausea, vomiting, diarrhea, headache, loss of appetite and agitation. In severe cases, liver dysfunction, delirium, liver coma, death can occur [15, 16].

Azaspiracid poisoning (AZA), a group of marine algal toxins first reported by the Netherlands, was caused by the presence of Dinoflagellate Azadinium spinosum, which can accumulate in crustaceans and thus cause disease in humans. Azaspiracid is a polyether poliotoxin that inhibits ion channels of potassium with tension. Shellfish contaminated with AZA as a result of ingestion can cause severe acute symptoms including nausea, vomiting, diarrhea and stomach cramps [17].

AZAs can contaminate various organisms including: scallops oyster mussels, clams, sponges and crabs and through these vector organisms, enter the human food chain, thus posing a potential risk to public health. Oysters are currently the only ones able to accumulate toxin at levels comparable to mussels, the species that accumulates more and the only one that has so far generated intoxication. Toxins can remain
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in shellfish for more than eight months because, although hepatopancreas is the first site where they accumulate, they migrate to other tissues in the body where detoxification occurs more slowly [18].

The structure of AZA was first unveiled in 1998 by Satake’s group and collaborators, following their isolation from Irish mussels (Mytilus edulis). The structure included: a cyclic aminic group (aza), a three-ring spiranic group (spiro) and a terminal carboxylic residue (acid), hence the name “azaspiracid” [19, 20].

Studies of acute oral toxicity were performed using partially purified toxin extracts administered in mice through gastric probe. Mice treated with a dose six times higher than the lethal dose showed no symptoms of poisoning or lethality within 24 hours of treatment, but autopsies of mice sacrificed after 4 hours after treatment showed changes at the gastrointestinal level, with fluid buildup in the ileo and microvilli necrosis: this clinical picture is similar to chronic inflammatory bowel disease, like Crohn’s disease. It was noted that the toxin was absorbed dose-dependent and the highest concentrations were detected after 24 hours in the kidneys, spleen and lungs, followed by those in the liver and heart. After one week AZA levels had dropped significantly in all organs except the kidneys [21].

Several studies have shown the action of AZAs also on voltage-dependent ion channels: they in fact alter the flow of intracellular calcium [22-25]; protons homeostasis [26], causing cell membrane hyperpolymerization [27].

The study of toxins produced by Dinoflagellates has shown that the most representative class of substances is polyethers. Protoceratium reticulatum and Lingulodinium polyedrum (Figure 7 and 8) are two species of Dinoflagellates responsible for the production of Yessotoxins, that are substances that show toxicity to both the heart and the liver and pancreas, digestive organs.

Brevitoxin B, the first toxic compound of which structure was defined exactly by crystallographic analysis, has been isolated from the Dinoflagellate Gymnodinium breve (Figure 9). Its toxicity is due to the stimulation of the nerve fibers that act on sodium channels by increasing the entry of the Na ion by depolarizing the neuronal membrane and inhibition of skeletal muscle activity; if taken for aerosol, due to the presence in the air of droplets of sea water polluted by algae, it produces irritation of airways with rhinorrhea, conjunctivitis and cough [28]. Oysters and mussels are the most infected for the filtering action of seawater and the accumulation of the toxin in the hepatopancreas. The toxin is also resistant to the temperature of 120 °C and to pH values between 2 and 10; the lethal dose for fish is between 0.2-0.5 mg/kg.

Pfiesteria piscicida (Steidinger & Burkholder) (Figure 10) is a species of unicellular algae belonging to the Dinoflagellates, and is responsible for the phenomenon of Red Tides and the death of many fishes and shellfish. It has only been described since 1990 as it can occur in very complex life forms that include cysts, amoeboid forms and toxic zoospores [29, 30].

Figure 7: Protoceratium reticulatum and cysts.

Figure 8: Lingulodinium polyedrum.

Figure 9: Gymnodinium breve.

Figure 10: Pfiesteria piscicida.
Ingestion of fish infected with *Pfiesteria piscicida* can cause nausea, vomiting, abdominal pain, diarrhea, neurological symptoms, psychiatric alteration, eye irritation, skin lesions [31, 32]. Intoxication occurs both through direct contact with fish and polluted water and by aerosol containing toxic particles.

Toxicity depends on various factors related to both the polluted environment, salinity, temperature, light, nutrients, and the stage of life (free, amoeboid, spores) and the presence of prey. Currently there are no specific tests to determine the presence of toxins except biological tests on fish using, in particular, tilapia [33].

**Pharmacological activity of biomolecules**

The seas and oceans have always been considered very important for the research of new drugs from marine organisms: toxins, in fact, are structurally made up of complex molecules with multiple functional groups, each of which is equipped with biological activity and can be applied in the pharmaceutical sector. Many studies have been undertaken to identify the presence of new molecules with anticancer, antibiotic, analgesic, antispasmodic, hypotensive, antiviral activity.

*Dinoflagellates* are unicellular microalgae found in plankton that contain molecules with pharmacological activity, although their notoriety is linked to the presence of biotoxins that make seafood unsafe for health [34]. In recent years, interest has increased in new pharmacologically active biocompounds for use in the biotechnology and microalgae are primary producers that, due to their high productivity, provide at low cost various substances very useful for human health, being able to be used both as medicines and for applications in the biomedical and toxicology sector [35].

*Tetradotoxin* (C$_{11}$H$_{17}$N$_3$O$_8$) (Figure 11), for example, because of its high toxicity, has never been utilized as a drug, but as pharmacological reagent. It is extracted from the fish of the *Tetradontidae* family known as “puff fish”, since it is not possible to extract from the only Dinoflagellate that produces it, *Alexandrium tamarense*. It causes paralysis of peripheral nerve endings due to inhibition of sodium permeability to nerve membranes. This paralysis is reversible, and Tetradotoxin has proved to be an important indicator for the study of the transmission of nerve arousal [36]. *Tetradotoxin* is really a powerful and selective drug, with an analgesic/anaesthetic effect associated with its sodium channel blocking properties. An increase in the activity of live sodium channels is reported in many forms of carcinoma and is an indicator of metastasis [37]. The use of *Tetradotoxin* to block sodium canal activity not only reduces the presence of metastases, but highlights the pathway to finding new drugs that act like toxin, but are less dangerous [38, 39]. *Tetradotoxin* has also been used with minor side effects to relieve cranial symptoms in cases of heroin withdrawal [40].

In many marine *Dinoflagellates*, such as *Alexandrium catenella* and *Alexandrium tamarense* (Figure 12 and 13) there are phytotoxins that accumulate in molluscs and the intake of these causes paralyzing symptoms and even death for humans. The mechanism of action of these phytotoxins was studied, highlighting that their toxicity is due to a blockage of neuronal transmission influencing the permeability of sodium in nerve cells. Researchers have highlighted the group of *Goniautotoxins* (C$_{10}$H$_{17}$N$_7$O$_8$S) (Figure 14) among the paralyzing toxins and have demonstrated their action as scarring and painkillers. They have shown that local applications of small amounts of paralyzing toxins produce a reversible paralysis of the striatum muscle, which turns out to be dependent dose. Patients with chronic tension-type headache were infiltrated locally with 50 mg of *Goniautotoxins* at the site where the pain was present and after a few minutes they showed a clear attenuation of pain [41]. Patients did not need to use other drugs and no adverse side effects were reported, and no second infiltration was carried out over a long period of about eight weeks, so the use of *Goniautotoxins* proved to be a safe and effective therapeutic tool as a painkiller.

Patients treated with an infiltration of *Goniautotoxins* during a surgery of arthroplasty in the knee showed a greater decrease in pain than those treated with conventional pain protocol, purchasing early the complete extension of the knee. No adverse effects were reported during the three-day hospital stay, during which patients did not experience any pain from the prolonged action of the *Goniautotoxins* [42].
Gastrointestinal pathology is a very complex form, the onset of which is little known. Visceral pain is a characteristic symptom of functional disorders such as irritable bowel syndrome and inflammatory bowel disease that affect many individuals around the world. The use of drug therapies often does not get good results for the lack of effectiveness and to cause many unwanted adverse effects. Scientists have recognized some receptor sites of the perception of painful sensations on which new biologically active natural compounds could more effectively act. Marine toxins represent, in fact, high affinity and selectivity to different molecular mediators of visceral pain, acting in particular on ion channels and receptors involved in pain generation [43]. Their use is very useful for studying the properties of ionic channels and receptors involved in pain perception, improving knowledge of their pathophysiological properties. A major disadvantage is that the toxins have low oral bioavailability, so injecting is required, which is generally unwelcome to the patient, high production costs and low conservation stability.

Dinoflagellates also produce many cytotoxic and/or long-chain polychetid macrolide: Amphidinolids (Figure 15) and Cholopsinols that are products of the genus Amphidinolids. The Amphidinolids show strong cytotoxicity towards cells L1210 murine lymphoma and in vitro epidermal human carcinoma; in particular, a N-type macrolide ampidinolide has been isolated from Amphidinolids operculatum var. November Gibbosum (Figure 16). The metabolic extract of a variety of Dinoflagellate cells such as Amphidinolids carterae (Figure 17), highlighted hemolytic, antifungal and cytotoxic properties, particularly towards Candida albicans (MIC = 64 µg/mL) [44-46].

The genus Karenia consists of unicellular, photosynthetic, planktonic organisms found in marine environments known mainly for their dense blooms of toxic algae and red tides that cause considerable ecological and economic damage causing serious animal mortality. Karenia brevis (Figure 18) is known to cause respiratory distress and poisoning in humans by neurotoxic crustaceans to build up toxins in tissues [47]. Karenia brevis (Figure 18) is found all over the world in oceanic and coastal waters and when algal blooms are formed and the availability of nutrients decreases, the genus Karenia begins to die releasing their neurotoxins that are destructive to the nervous system. Toxins characterized as Brevetoxins (C50 H70 O14) (Figure 19) are liposoluble and act by activating the tension-sensitive sodium channels and causing them to stay open for long periods of time with uncontrolled depolarization of the neural membrane and persistent neuronal arousal [48-50].
No deaths have been recorded in association with Brevetoxins, but serious effects have been noted, such as nausea, vomiting and a variety of neurological symptoms, confused language, skin irritation directly exposed to water, irritation ocular. Exposure to Brevetoxins occurs by ingestion or inhalation: Karenia brevis cells are weak, so the action of the waves can break the cells, releasing the Brevetoxins as aerosols.

Exposure to Brevetoxins is all the more harmful the greater the contact time and the death of marine mammals is due to the ingestion of organisms that have accumulated high concentrations of Brevetoxins in their tissues. Humans are at risk, mainly through respiratory exposure which can result in a severe inflammatory response of bronchial mucous [51, 52]. Respiratory symptoms highlighted for exposure to marine aerosol containing Brevetoxins are coughing, involuntary sneezing, tearing, rhinorrhea, burning sensation in the throat and nose, and breathing difficulties [53-56]. Various forms of Brevetoxins are known to have cytotoxic activities with DNA damage; they affect cell proliferation in a dose-dependent way, are genotoxic and cause cell death through an apoptotic mechanism.

Some experimental work has also shown that aerosol causes inflammation of the smooth bronchial musculature and broncho constriction even to animals that have been exposed [57, 58].

Dinoflagellates of the genus Amphidinolids carterae contain a carotenoid, Peridinin, which forms a complex with chlorophyll that is responsible for the brown coloration of algal blooms. In fact Peridinin absorbs light at wavelengths between 470 -550 nm, of blue-green color and is able to transfer energy to chlorophyll molecule by giving it fluorescence [59]. Peridinin, like other carotenoid structure pigments act as sunscreen for both corals and algae with which they live in symbiosis.

The obtained fluorophore is very stable and finds different applications in immunological tests and flow cytometry for cell counting, determining cellular characteristics and their function, detection of microorganisms, diagnosis of pathologies such as blood tumors, etc.

Peridinin being a carotenoid is equipped with antioxidant and anticancer activity as has been demonstrated in a study on the proliferation and survival of lines of T cells infected with the HTLV-1 leukemia virus. Results showed an inhibition of cell proliferation dependent on the dosage of Peridinin used. Sugawara et al., highlighted colon cancer cell apoptosis for treatment with Peridinin isolated from Dinoflagellates, Heterocapsa triquetra (Figure 20) [60, 61]. Photoactivated
Porphyrones, pigments with a molecular structure similar to chlorophyll and hemoglobin, found in Dinoflagellates also showed antimicrobial activity [62, 63]. Heterocapsa circularisquama (Figure 21) is a Dinoflagellate toxic to bivalves but not to fish: it contains hemolytic porphyrin, which shows light-dependent cytotoxicity towards cancer cells. His antibacterial activity was also highlighted preferentially towards light-dependent gram+ [64].

Cryptothecodinium chonii (Figure 22) is the only non-toxic Dinoflagellate that is used industrially for the production of docosahexaenoic acid (DHA), high value omega-3 polyunsaturated fatty acid that possesses various physiological and nutritional functions, used for the enrichment of baby products. The acid is contained entirely within cells and is distributed in both phospholipids and conservation lipids and therefore the extraction method is very long and complex. Particular attention should be used, then, to prevent oxidation phenomena that would not guarantee the purity of the final product [65]. During the production of docosahexaenoic acid this algae also produces polysaccharides, of which are known antioxidant, anti-inflammatory, antiadhesive, anti-coagulant, anti-cancer, anti-viral and immunomodulating properties [66-68]. Numerous studies on various cancer cell lines show that marine polysaccharides have high cytotoxicity and apoptogenic activities that can be considered a future alternative for the production of natural antitumor drugs compared to synthetic drugs.

Conclusions

Toxic algal blooms are a serious problem for all aquatic environments. They are not a new phenomenon, but they currently occur very frequently, and have taken on a strong expansion in Asian countries and America. The main cause is environmental degradation, but climatic change, the misuse of fertilisers and pesticides, industrial discharges, overcrowding, and engineering work also contribute [69, 70].

Dinoflagellates, responsible for algal blooms known as “Red Tides”, are unicellular microalgae that produce biotoxins that make seafood toxic, as well as being responsible for a high death of fish. The increase in algal blooms in recent decades requires greater surveillance of seawaters and the need to take appropriate action to study this phenomenon, in an attempt to avoid serious repercussions on the environment, the economy and, above all, on the health of men. There are no specific therapies against algal biotoxins because, being ionophores, they affect the transport of ions (sodium and potassium pumps) at the cellular level. Currently, the only intervention, if the intoxication is reported in a timely manner, is to resort to the elimination of toxic residues from the digestive system by gastric lavender or with activated charcoal dust. In more severe cases, when neurological symptoms are present and respiratory paralysis is feared, it is necessary to resort to intubation of the patient to to subject him to mechanical ventilation [71-73].
In addition, detoxification methods are provided for health, especially in relation to mussels, before they are put on the market. The most commonly used method involves the transfer of toxic shellfish into waters free of toxic plankton, to allow self-purification, but it is a method that involves a long time; the transfer of shellfish is very tiring and expensive. Electric shocks or the use of chlorine reduce the duration of contamination, but one runs the risk of altering the sensory properties of the product, decreasing its appeal. The use of ozone has recently been proposed, which has been shown to be effective in preventing the accumulation of toxins by shellfish, without any alteration of them, but has shown no an efficient action towards invertebrates species that accumulate cysts of microorganisms, or that bind toxins to their tissues for long periods of time. There is still no effective, rapid and universal method of detoxification for all shellfish and as the costs of such treatments are still high, monitoring areas exposed to algal blooms, mussels, is still high [74–76].

Dinoflagellates are often studied because they are related to harmful algal blooms but are also capable of producing bioactive compounds for the treatment of human pathologies. In addition to proteins, fatty acids, vitamins and pigments they contain bioactive compounds such as carotenoids, polysaccharides, vitamins, lipids and powerful neurotoxins that can be applied as drugs by showing activities, analgesic, anticaner, anti-cholesterol, cytotoxic, anti-infective, immunosuppressants or as nutraceuticals. As primary producers, these marine microalgae are also rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), polyunsaturated fatty acids known as omega-3 and are responsible for many human health benefits, particularly in reducing heart disease such as arrhythmia, stroke and hypertension, as well as acting on depression, rheumatoid arthritis and asthma [77–79]. Industrially they are added to infant milk formula or other foods, they are used as food additives for the presence of dyes, such as feed, even in aquaculture, pharmaceutical compounds, cosmetics and potentially as a source of biofuels [80–82].

Recent research has highlighted in Amphidinolids carterae the presence of enzymes of biotechnology interest such as polyketides synthase (PKS) which are both responsible for the synthesis of toxins and other polyketides with interesting ecological and biotechnological functions such as antiprifer, allelopathic, anti-cancer, antifungal activity and/or beneficial effects for the treatment of Alzheimer's disease [83].

L-asparaginase is a polyketide enzyme synthase that catalyzes the hydrolysis of L-asparagine into L-aspartic acid and is used to treat acute lymphoblastic leukemia, acute myeloid leukemia, non-Hodgkin’s lymphoma; malignant cells having a reduced ability to produce asparagine synthase, they use the asparagine present in the blood. By limiting the supply of asparagine, the growth of cancer cells is inhibited [84]. This enzyme also has applications in the food industry to reduce acrylamide, a carcinogenic substance, in many foods: adding L-asparaginase to chips, biscuits, crispy bread is effectively reduced the formation of acrylamide [85].

The study of toxins, in addition to helping to reduce cases of food poisoning, can facilitate the research and development of new drugs by studying the routes of action and receptors to which they claim. To ensure the safety of shellfish it is not sufficient to subject them to analysis to identify the nature and extent of contamination, but it is essential to monitor aquatic environments to identify the toxic algal species present and the risks to human health. Should not be neglected, then the analysis of Red Tide aerosols for the identification of toxins and microorganisms present in it, to assess the risks related to time and exposure dose for human health.

Conflict of Interest

The author declares she has no conflicts of interest.

References

1. Vilarino N, Louza MC, Vieyes MR, 2010. Botana LM. Biological methods for marine toxin detection. Anal Bioanal Chem 397(5): 1673–1681. https://doi.org/10.1007/s00216-010-3782-9
2. Farabegoli F, Blanco L, Rodríguez LP, Vieites JM, Cabado AG. 2018. Phytotoxins in marine shellfish: origin, occurrence and effects on humans. Mar Drugs 16(6): 188. https://doi.org/10.3390/md16060188
3. Blanco J. 2018. Accumulation of Dinophysis toxins in bivalve mussels. Toxins (Basel) 10(11): 453. https://doi.org/10.3390/toxins10110453
4. Tangen K. 1983. Shellfish poisoning and the occurrence of potentially toxic dinoflagellates in Norwegian waters. Sarsia 68(1): 1–7. https://doi.org/10.3808/s68-4827.1983.10420550
5. Hallegraeff GM. 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. J Physiol 46: 220–235. https://doi.org/10.1111/j.1529-8817.2010.00815.x
6. Hallegraeff GM. 2014. Harmful algae and their toxins: progress, paradoxes and paradigm shifts, toxins and biologically active compounds from microalgae. In: Rossini GP (ed) Toxins and biologically active compounds from microalgae, CRC Press, Boca Raton, Florida, USA, pp 3–20.
7. Gallardo-Rodriguez JJ, Sánchez-Mirón A, García-Camacho F, López-rosales L, Chisti Y, et al. 2012. Bioactives from microbial dinoflagellates. Biotechnol Adv 30(6): 1673–1684. https://doi.org/10.1016/j.biotechadv.2012.07.005
8. Tachibana K, Scheuer PJ, Tsukitani Y, Kikuchi H, Van Engen D, et al. Okadaic acid, a cytotoxic polymer from two marine sponges of the genus Halichondria. J Am Chem Soc 103(9): 2469–2471. https://doi.org/10.1021/ja00399a082
9. Valdiglesias V, Prego-Faraldo MV, Pásaro E, Mendez J, Lafon B 2013. Okadaic acid: more than a diarrheic toxin. Mar Drugs 11(11): 4328–4349. https://doi.org/10.3390/md11114328
10. Suganuma M, Okabe S, Marino MW, Sakai A, Sueoka E, et al. 1999. Essential role of tumor necrosis factor alpha(TNF-α) in tumor promotion as revealed by TNF-α-deficient mice. Cancer Res 59(18): 4516–4518.
11. Fuji, H, Sueoka E, Watanabe T, Suganuma M. 2018. The concept of the okadaic acid class of tumor promoters is revived in endogenous protein inhibitors of protein phosphatase 2A, SET and CIP2A, in human cancers. J Cancer Res Clin Oncol 144(12): 1–8. https://doi.org/10.1007/s00432-018-2765-7
12. Tangen K. 1980. Brown water in the OSfloord, Norway; in September 1979 caused by the toxic Prorocentrum minimum and other dinoflagellates Blyttria. 38: 145–158.
13. Marasovic I, Pucher-Petkovic T. 1985. Effects of eutrophication on the coastal phytoplankton community. Rapp Comm Int M Int Mer Medit 29: 137–139.
14. Heiet CA, Gilbert PM, Fan C. 2005. Prorocentrum minimum (Pavillard) Schiller—a review of a harmful algal bloom species of growing worldwide
Ferrara.

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28. Cheng YS, Zhou Y, Irvin CM, Pierce RH, Naar J, et al. 2005. Azaspiracid in female NMRI mice. Toxicon 56(2): 109-116. https://doi.org/10.1016/j.toxicon.2012.04.351

27. Vale C, Nicolau KC, Frederick MO, Vieytes MR, Rotana LM. 2010. Involvement of caspase activation in Azaspiracid-induced neurotoxicity. Cell Signal 17(10): 1338-1349. https://doi.org/10.1021/np500275x

26. Alfonso A, Vieytes MR, Ofuji K, Satake M, Nicolau KC, et al. 2005. Azaspiracid-1 in female NMRI mice. Toxicon 56(2): 109-116. https://doi.org/10.1016/j.toxicon.2012.04.351

25. Satake M, Ofuji K, Naoki H, James KJ, Furey A. 1998. Azaspiracid, a new marine toxin having unique spiro ring assemblies, isolated from new irish mussels, Mytilus edulis. J Am Chem Soc 120: 9967-9968. https://doi.org/10.1021/ja981413z

24. Furey A, O’Doherty S, O’Callaghan K, Lehane M, James KJ. 2010. Cell volume decrease as a link between Azaspiracid-induced cytotoxicity and c-Jun-N-Terminal kinase activation in cultured neurons. Cell Death Dis 1(2): 219-224. https://doi.org/10.1038/cddis.2010.28

23. Jauffrais T, Marcaillou C, Herrenknecht C, Truquet P, Séchet V, et al. 2003. Bioactive macrolides from the marine dinoflagellate Amphidinium carterae. Curr Med Chem Anti-cancer Agents 3(3): 449-470. https://doi.org/10.2174/156801103775805248

22. Cao Z, LePage KT, Frederick MO, Nicolau KC, Murray TF. 2010. Effects of azaspiracids 2 and 3 on intracellular cAMP, [Ca++] and pH. Chem Res Toxicol 17(10): 1338-1349. https://doi.org/10.1021/tx3014862

21. Alfonso A, Yafou A, Louza MC, de la Rosa LA, Leira F, et al. 2002. Azaspiracid-1, a potent, nonapoptotic new phycotoxin with several cell targets. Cell Signal 14(8): 703-716. https://doi.org/10.1016/s0898-6568(02)00015-3

20. Alfonso A, Yafou A, Vieytes MR, Ofuji K, Satake M, et al. 2004. Azaspiracid-4 inhibits Ca++ entry by stored operated channels in human T lymphocytes. Biochem Pharmacol 69(11): 1627-1636. https://doi.org/10.1016/j.bcp.2005.03.022

19. Song H, Li J, Lu CL, Kang L, Xie L, et al. 2011. Tetrodotoxin alleviates acute renal injury in rats. Toxicon 57(8): 1239-1246. https://doi.org/10.1016/j.toxicon.2016.06.010

18. Djamgoz MBA, Coombes RC, Schwab A. 2014. Ion transport and cancer: from initiation to metastasis. Philos Trans R Soc Lond B Biol Sci 369(1638): 20130092. https://doi.org/10.1098/rstb.2013.0092

17. Hagen NA, Laponte B, Ong-Lam M, Dubuc B, Walde D, et al. 2011. A multicentre open-label safety and efficacy study of tetradotoxin for cancer pain. Curr Oncol 18(3): 109-116. https://doi.org/10.3747/ co.v18i3.732

16. Taylor FJR. 1987. Dinoflagellates: an introduction in the biology of Dinoflagellates. Taylor FJR (ed) Blackwell Scientific Publications: Oxford,UK, pp 1-13.

15. Marasovic I, Pucher-Petkovic T, Petrova-Karadjova V. 1990. Karenia: the biology and health considerations. J Phycology 26(3): 247-251. https://doi.org/10.1111/j.1529-8817.1990.8817225.x

14. Chang Y, Li J, Liang J, Gu J, Sun L, et al. 2017. Antifungal and antibacterial activities of Amphidium carterae. J Nat Prod 80(6): 1524-1527. https://doi.org/10.1002/np.20075x

13. Litaker RW, Vandersea MW, Kibler SR, Madden VJ, Noga EJ, et al. 2002. Life cycle of the heterotrophic dinoflagellate Pfiesteria piscicida (Dinophyceae). J Phycol 38(3): 442-463. https://doi.org/10.1046/j.1529-8817.2002.01242.x

12. Lovko VJ, Vogelbein WK, Shields JD, Haas LW. 2003. A new larval fish bioassay for the pathogenicity of Pfiesteria spp (Dinophyceae). J Phycol 39(3): 600-609. https://doi.org/10.1046/j.1529-8817.2003.02106.x

11. Moeller PDR, Morton SL, Mitchell BA, Siverten SK, Fairey ER, et al. 2001. Current progress in isolation and characterization of toxins isolated from Pfiesteria piscicida. Environ Health Prospect (Suppl 5): 739-743. https://doi.org/10.1289/ehp.01109.5739

10. Taylor FJR. 1987. Dinoflagellates: an introduction in the biology of Dinoflagellates. Taylor FJR (ed) Blackwell Scientific Publications: Oxford,UK, pp 1-13.

9. Mimouni U, Ullmann L, Pasquet V, Mathieu M, Picot L, et al. 2012. The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. Curr Pharm Biotechnol 13(15): 2733-2750. https://doi.org/10.2174/138920112804724828

8. Alfonso A, O’Doherty S, O’Callaghan K, Lehane M, James KJ. 2010. Azaspiracid poisoning (AZP) toxins in shellfish: toxicological and health considerations. Toxicology 267(1-2): 1-17. https://doi.org/10.1016/j.toxicon.2008.03.010

7. Kobayashi J, Shimbo K, Kubota T, Tsuda M. 2003. Bioactive macrolides from marine dinoflagellates. J Nat Prod 66(10): 1313-1319. https://doi.org/10.1021/np0300490

6. Hagen NA, Laponte B, Ong-Lam M, Dubuc B, Walde D, et al. 2011. A multicentre open-label safety and efficacy study of tetradotoxin for cancer pain. Curr Oncol 18(3): 109-116. https://doi.org/10.3747/ co.v18i3.732

5. Djamgoz MBA, Coombes RC, Schwab A. 2014. Ion transport and cancer: from initiation to metastasis. Philos Trans R Soc Lond B Biol Sci 369(1638): 20130092. https://doi.org/10.1098/rstb.2013.0092

4. Lattes K, Venegas P, Lagos N, Lagos, Pedraza L, et al. 2009. Local infiltration of Gonyautoxin is safe and effective in treatment of chronic tension-type headache. Neurol Res 31(3): 228-233. https://doi.org/10.1179/174313209X380829

3. Djamgoz MBA, Coombes RC, Schwab A. 2014. Ion transport and cancer: from initiation to metastasis. Philos Trans R Soc Lond B Biol Sci 369(1638): 20130092. https://doi.org/10.1098/rstb.2013.0092

2. Taylor FJR. 1987. Dinoflagellates: an introduction in the biology of Dinoflagellates. Taylor FJR (ed) Blackwell Scientific Publications: Oxford,UK, pp 1-13.

1. Alfonso A, Yafou A, Louza MC, de la Rosa LA, Leira F, et al. 2002. Azaspiracid-1, a potent, nonapoptotic new phycotoxin with several cell targets. Cell Signal 14(8): 703-716. https://doi.org/10.1016/s0898-6568(02)00015-3
competitive ELISA to detect brevetoxins from *Karenia brevis* (Formely *Gymnodinium breve*) in seawater shellfish and mammalian body fluid. *Ecorv Health Perspect* 110(2): 179-189. https://doi.org/10.1097/ehp.0211079

49. Magana HA, Contreras C, Villareal TA. 2003. A historical assessment of *Kareina brevis* in the westn Gulf of Mexico. *Harmful Algae* 2(3):163-171. https://doi.org/10.1016/S1568-9883(03)00026-X

50. Porter H, Di, J, Beet A, Kirkpatrick B, Reich A, et al. 2014. The human health effects of Florida Red Tide (FRT) blooms: an expanded analysis. *Ecorver Intet 68*: 144-153. https://doi.org/10.1016/j.econv.2014.03.016

51. Shimoda T, Krazanowski JI, Nelson R, Martin DF, Polson J, et al. 1988. *In vitro* red tide effects on human bronchial smooth muscle. *J Allergy Clin Immunol* 91(6):1187-1191. https://doi.org/10.1016/0091-6749(88)90889-5

52. Sas KM, BaatzJE. 2010. Brevetoxin-2 induces an inflammatory response in an alveolar macrophage cell line. *Int J Hyg Environ Health* 213: 352-358. https://doi.org/10.1016/j.ijheh.2010.06.007

53. Backer LC, Fleming LE, Rowan A, Cheng YS, Benson JM, et al. 2003. Recreational exposure to aerosolized brevetoxins during Florida red tide events. *Harmful Algae* 2(1): 19-28. https://doi.org/10.1016/S1568-9883(03)00005-2

54. Backer LC, Kirkpatrick B, Fleming LE, Cheng YS, Pierce R, et al. 2005. Occupational exposure to aerosolized brevetoxins in an animal model of asthma. *Am J Respir Crit Care Med* 171(1): 26-34. https://doi.org/10.1164/rccm.200406-735OC

55. Woodcock AH. 1948. Note on concerning human respiratory irritation associated with high concentrations of plankton and mass mortality of marine organisms. *Scorn Found J Marine Res* 7: 56-62.

56. Pierce RH, Henry MS, Blum PC, Lyons J, Cheng YS, et al. 2003. Brevetoxin concentrations in marine aerosol: human exposure levels during a *Karenia brevis* harmful algal bloom. *Ball Environ Contam* 70(1): 161-165. https://doi.org/10.1007/s00128-002-0170-y

57. Abraham WM, Bourdelais AJ, Sabater JR, Ahmed A, Lee TA, et al. 2005. Airway responses to aerosolized brevetoxins in an animal model of asthma. *Am J Respir Crit Care Med* 171(1): 26-34. https://doi.org/10.1164/rccm.200406-735OC

58. Asai S, Krazanowski JI, Anderson WH, Martin DF, Polson JB, et al. 2002. Effects of the red tide toxin, *Phylocodinis brevis* on canine tracheal smooth muscle: a possible new asthma-triggering mechanism. *J Allergy Clin Immunol* 69(5): 418-428. https://doi.org/10.1016/S0091-6749(82)90116-6

59. Hofmann E, Wrench PM, Sharples FP,希尔ier RG, Wlte W, et al. 1967. 19-28. https://doi.org/10.1016/S1568-9883(03)00005-2

60. Ishikawa C, Jomori T, Tanaka J, Senba M, Mori N. 2016. Peridinin, *Cryptocodinium cohnii* and *Heterocapsa circularisquama*. *Aquat Toxicol* 201: 119-128. https://doi.org/10.1016/j.aquatox.2018.06.004

61. Mendes A, Reis A, Vasconcelos R, Guerra P, Lopes Da Silva T. 2009. *Gymnodinium colhu* with emphasis on DHA production: a review. *J Appl Phycol* 21: 199-214. https://doi.org/10.1007/s10811-008-9351-3

62. Lauriezo P. 2010. Marine polyaccharides in pharmaceutical applications: an overview. *Mar Drugs* 8(9): 2435-2465. https://doi.org/10.3390/md8092435

63. Gardeva E, Toshkova R, Yossifova L, Minkova K, Gigaova L. 2012. Cytotoxic and apoptogenic potential of red microalgal polyaccharides. *Biotechnol Biof D$p 26(4): 3167-3172. https://doi.org/10.5504/biotechbio.2012.0035

64. de Jesus Raposo MF, de Morais RM, de Morais AM. 2013. Health applications of bioactive compounds from marine microalgae. *Life Sci* 93(15): 479-486. https://doi.org/10.1016/j.lfs.2013.08.002

65. Gauthier L, Tison-Rosbery J, Morin S, Mazzella N. 2019. Metabolome response to anthropogenic contamination on microalgae: a review. *Metabolomics* 15(1): 8. https://doi.org/10.1007/s11306-019-1626-9

66. Anderson DM, Glibert PM, Burkholler JM. 2002. Harmful algal blooms and eutrophication: nutrient sources, consequences, and consequences. *Estuaries* 25: 704-726. https://doi.org/10.1007/10.1016/j.estuar.2009.11.005

67. Ferreiro SF, Carrera C, Vilarrino N, Louzao MC, Santamaria G, et al. Acute cardio toxicity evaluation of the marine biotoxins OA, DTX-1 and YTX. *Toxins (Basel)* 7(4): 1030-1047. https://doi.org/10.3390/toxins7041030

68. Davidson K, Baker C, Higgins C, Higman W, Swan S, et al. 2015. Potential threats posed by new or emerging marine biotoxins in UK waters and examination of detection methodologies used for their control: Cicilii imines. *Mar Drugs* 13(12): 7087-7112. https://doi.org/10.3390/md13127057

69. Otero P, Alfonso A, Alfonco C, Arozio R, Molgos J, et al. 2011. First direct fluorescence polarization assay for the detection and quantification of spiruloids in mussel samples. *Anal Chim Acta* 701(2): 200-208. https://doi.org/10.1016/j.aca.2011.05.034

70. Fux E, McMillan B, Bire A, Hess P. 2007. Development of an ultra-performance liquid chromatography-mass spectrometry method for the detection of lipophilic marine toxins. *J Chromatogr A* 1169(1-2): 273-280. https://doi.org/10.1016/j.chroma.2007.05.016

71. Adarme-Vega TC, Lim DKY, Timmins M, Vernen F, Li Y, et al. 2012. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. *Microb Cell Fact* 11: 96. https://doi.org/10.1186/1475-2859-11-96

72. Gong Y, Wan X, Jiang M, Hu C, Hu H, et al. 2014. Metabolic engineering of microorganisms to produce omega-3 very long-chain polyunsaturated fatty acids. *Prog Lipid Res* 56: 19-35. https://doi.org/10.1016/j.plipres.2014.07.001

73. Nauroth JM, Liu YC, Blum PC, Ferris EB, et al. 2010. Docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA-n-6) algal oils reduce inflammatory mediators in human peripheral mononuclear cells in vitro and paw edema in vivo. *Lipids* 45(5): 375-384. https://doi.org/10.1016/j.lipids.2009.11.005

74. Sprague M, Bientonz MR, Thomas TR. 2017. Microbial and genetically engineered oils as replacements for fish oil in aquaculture feeds. *Biotechnol Lett* 39(11): 1599-1609. https://doi.org/10.1007/s10529-017-2402-6

75. Ganuza E, Beinetez-Tsanta T, Aroliak E, Vega-Orellana L, Ganga R, et al. 2008. *Cryptodinium colhu* and *Schizochytrium sp*. as potential substitutes to fisheries-derived oils from sea bream (*Sparus aurata*) microdiets. *Aquaculture* 277(1-2): 109-116. https://doi.org/10.1016/j. aquaculture.2008.02.005

76. Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussungul JH,
et al. 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenerg Res 1: 20–43. https://doi.org/10.1007/s12155-008-9008-8

82. Kamat PK, Rai S, Nath C. 2013. Okadaic acid induced neurotoxicity: an emerging tool to study Alzheimer’s disease pathology. Neurotoxicology 37: 163–172. https://doi.org/10.1016/j.neuro.2013.05.002

83. Pieters R, Hunger SP, Boos J, Rizzari C, Silverman L, et al. 2011. L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. Cancer 117(2): 238–249. https://doi.org/10.1002/cncr.25489

84. Xu F, Oruna-Concha MJ, Elmore JS. 2016. The use of asparaginase to reduce acrylamide levels in cooked food. Food Chem 210: 163–171. https://doi.org/10.1016/j.foodchem.2016.04.105

85. Benson J, Hahn F, March T, McDonald J, Sopori M, et al. 2004. Inhalation toxicity of brevetoxin 3 in rats exposed for 5 days. J Toxicol Environ Health A 67(18): 1443–1456. https://doi.org/10.1080/15287390490483809