Available online at www.sciencedirect.com

ScienceDirect

European Journal of Protistology 75 (2020) 125720

European Journal of PROTISTOLOGY

www.elsevier.com/locate/ejop

Bioactive molecules from protists: Perspectives in biotechnology

Adriana Vallesi[a,*], Sandra Pucciarelli[a,*], Federico Buonanno[b], Angelo Fontana[c], Marco Mangiagalli[d]

[a] *School of Biosciences and Veterinary Medicine, Università degli Studi di Camerino, Camerino (MC), Italy*
[b] *Laboratory of Protistology and Biology Education, Department of E.C.H.T. Università degli Studi di Macerata, Macerata, Italy*
[c] *Bio-Organic Chemistry Unit, CNR-Institute of Biomolecular Chemistry, Pozzuoli, Napoli, Italy*
[d] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy*

Received 23 February 2020; received in revised form 11 May 2020; accepted 15 May 2020
Available online 4 June 2020

Abstract

For hundreds of years, mankind has benefited from the natural metabolic processes of microorganisms to obtain basic products such as fermented foods and alcoholic beverages. More recently, microorganisms have been exploited for the production of antibiotics, vitamins and enzymes to be used in medicine and chemical industries. Additionally, several modern drugs, including those for cancer therapy, are natural products or their derivatives. Protists are a still underexplored source of natural products potentially of interest for biotechnological and biomedical applications. This paper focuses on some examples of bioactive molecules from protists and associated bacteria and their possible use in biotechnology.

Keywords: Bioactive molecules from protists; Natural products; Cold-adapted enzymes; Ice binding proteins; Climacostol; Secondary metabolites

Introduction

Protists are fundamental components of microbial communities in every aquatic and terrestrial habitat worldwide, from the ocean depths to the highest mountain peaks. Inhabiting all environments, including extreme ones characterized by low or high temperatures (Oliverio et al. 2018; Valbonesi and Luporini 1993), high salinity (Harding et al. 2017), and low or high pH values (Ong'ondo et al. 2013), protists have evolved an amazing biodiversity and unique mechanisms of adaptation which involve synthesis of specific molecules required to thrive in these environments. In addition, protists use chemical signals to face predators and capture prey (Buonanno et al. 2013), communicate within the same species or among different species (Frenkel et al. 2014; Ianora et al. 2011; Luporini et al. 2016a; Luporini et al. 2016b), and mediate interactions between symbiotic organisms (Amin et al. 2012; Pohnert et al. 2007; Roy et al. 2013). This large array of products offers a new and unexplored source of bioactive compounds with potential biotechnological applications in industrial sectors related to food, cosmetics and human health (Assunção et al. 2017; Peyrat et al. 2019; Singh et al. 2017; Sun et al. 2015).

The demand for bioactive molecules with desirable properties is constantly growing, and the development of modern technological methods for molecular purification and the increasing availability of genome data provide new approaches for the discovery of natural products and the study

*Corresponding authors.
E-mail addresses: adriana.vallesi@unicam.it (A. Vallesi), sandra.pucciarelli@unicam.it (S. Pucciarelli).

https://doi.org/10.1016/j.ejop.2020.125720
0932-4739/

of their applications. The main classes of bioactive molecules are primary metabolites (*e.g.* amino acids, enzymes, lipids, vitamins *etc.*) essential for the growth of microorganisms (Singh et al. 2017; Sun et al. 2015), and secondary metabolites, which are small organic compounds produced during the stationary phase of growth, with antimicrobial and cytotoxic activities (Demain 1999).

This review reports recent advancements in this specific field of protistological research discussed at the symposium "Bioactive molecules from protists: perspectives in biotechnology" during the VIII European Congress of Protistology – ISOP joint meeting (Rome, 2019). Natural products isolated from diatoms, dinoflagellates and ciliates have shown great potential in various fields. The cold-adapted enzymes from Antarctic ciliates and the ice binding proteins synthesized by their associated bacteria display peculiar physical-chemical features that make them exploitable for industrial purposes. In diatoms, specific classes of lipids act as signals of cell death and growth termination; manipulation of lipid biosynthesis can thus be used to control diatom growth for biomass production, making these microalgae a promising font of biofuels, food raw materials and other value-added products. Secondary metabolites synthesized by numerous aquatic ciliates for chemical defense and/or offence in predator–prey interactions are now emerging as potential chemotherapeutic agents with immune-modulatory and anti-cancer activities.

Enzymes and ice-binding proteins from protists and their associated bacteria

Enzymes play a key role in biotechnological and industrial applications. In some cases, industrial processes present harsh conditions (*e.g.* low or high temperatures, acidic or basic pHs, the presence of organic solvents) or require high or low substrate specificity (Sarmiento et al. 2015). In this context, enzymes from extremophilic organisms are particularly attractive; for instance, enzymes active at low temperatures find application in detergency, food industry and molecular biology (Collins and Margesin 2019; Mangiagalli et al. 2020; Sarmiento et al. 2015), while thermophilic enzymes find application in food and in pulp and paper industries, as well as in molecular biology (Sarmiento et al. 2015). The enzymes obtained from protists and characterized to date, while few in number, indicate the great potential of these microorganisms as powerful source for new biomolecules.

The characterization of enzymes from "extreme" protists is interesting not only for industrial applications, but also for the study of the mechanisms of molecular evolution and adaptation to the environment. For instance, studies carried out on cold-active α-amylase and superoxide dismutase from the psychrophilic Antarctic ciliate *Euplotes focardii* provided insights into the relationship between activity at low temperature and stability to heat (Pischedda et al. 2018; Yang et al. 2013). In particular, *E. focardii* superoxide dismutases are active at 4 °C and retain residual activity even after incubation at 50 °C (Fig. 1A) (Pischedda et al. 2018).

Ice binding proteins (IBPs) represent another class of molecules with application in food industries and in cryopreservation of biological materials (Kim et al. 2017; Mangiagalli et al. 2020). These proteins prevent cell freezing by depressing the freezing point of water below the melting point and inhibit ice recrystallization by binding to the surface of ice crystals (Fig. 1) (Bar Dolev et al. 2016; Davies 2014; Vance et al. 2019; Voets 2017). Different IBPs have been identified from psychrophilic protists including Antarctic diatoms (Bayer-Giraldi et al. 2010; Xiao et al. 2014), unicellular algae (Raymond 2014; Raymond and Morgan-Kiss 2013; Raymond and Remias 2019) and unicellular fungi (Arai et al. 2019; Cheng et al. 2016; Kondo et al. 2012). A new IBP (*Efc*IBP) has been identified and extensively characterized from the metagenomic analysis of the bacterial consortium associated to the Antarctic ciliate *E. focardii* (Mangiagalli et al. 2017; Mangiagalli et al. 2018; Pucciarelli et al. 2015). Biochemical characterization of recombinant *Efc*IBP showed that this protein is one of the best-performing IBPs described to date in inhibiting the growth of large harmful ice crystals (Mangiagalli et al. 2017). This finding indicates that even protist-associated bacteria may prove valuable in the search for new molecules to be used in industrial applications.

Biomass, carotenoids and fatty acids from microalgae

Microalgae are widespread photosynthetic microorganisms inhabiting marine and freshwater environments, and are of great interest in biotechnology as a source of biomass and bioactive molecules (*e.g.* carotenoids, fatty acids and antioxidants) used as additives in food, cosmetic and animal feed industries (Assunção et al. 2017; Borowitzka 2013; Gangl et al. 2015).

Diatoms have pivotal roles in biological and geochemical marine cycles (Armbrust 2009; Benoiston et al. 2017). Because these protists gain their energy from photosynthesis and use dissolved CO_2 as a building block (Reinfelder et al. 2004), they have developed very efficient mechanisms for maintaining high metabolic plasticity that allows them to balance growth, production of functional compounds and accumulation of storage products (Adelfi et al. 2019; Cutignano et al. 2006; D'Ippolito et al. 2004; Gerecht et al. 2011; Lamari et al. 2013). Diatom blooms are at the very basis of the marine food web and they are primary producers of essential molecules such as eicosapentaenoic acid (EPA) that are transferred though the web to higher organisms, including human beings (Brett et al. 2009; Larson et al. 2013). The natural ability of diatoms to produce large biomass has also prompted interest in the commercial exploitation of these products (Katiyar et al. 2017; Lebeau and Robert 2003).

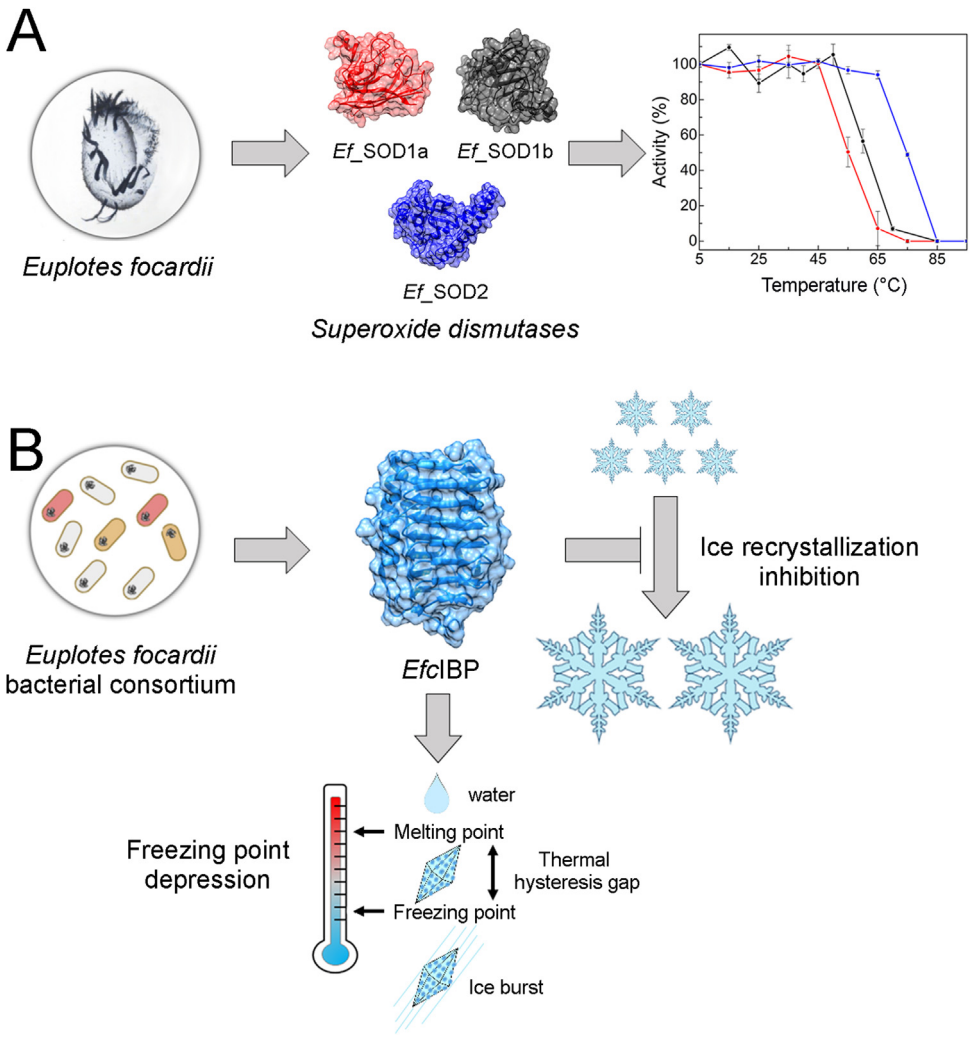

Fig. 1. Proteins from *E. focardii* and its bacterial consortium. (A) Superoxide dismutases (SODs) from *E. focardii*. The three different SODs identified in the Antarctic ciliate *E. focardii* show remarkable activity at 4 °C, coupled with unusual heat stability. Figure adapted from Pischedda et al. (2018). (B) Binding of *Efc*IBP to ice crystals induces the freezing point depression and stabilized the small ice crystals inhibiting the growth of the large ones.

In general, large-scale cultures of microalgae started more than 50 years ago in open ponds. These cultivations do not compete in land and space with terrestrial crops, and could contribute to mitigate the negative effects of carbon dioxide and other pollutants on the environment. The main studies in this field have traditionally focused on engineering solutions to reduce capital and operating expenditures, as well as costs of downstream processes such as harvesting and dewatering (Assunção et al. 2017; Ugwu et al. 2008). However, recent researches have been directed to both the selection of resilient species and the development of biological methods to improve production yields (Klok et al. 2014; Rodolfi et al. 2009; Vaezi 2015). From this point of view, the biodiversity offered by the estimated 100,000 diatom species living in the Earth's waters can be a wealth of new strains and source of different target products (D'Ippolito et al. 2015). Lately, studies on the cosmopolitan diatom species *Thalassiosira weissflodgi* have demonstrated the feasibility of year-round outdoor cultivation, with no need to control ambient temperature and light irradiance (Vella et al. 2019). Biomass (4 g/m^2 d) and oil (0.5 g/m^2 d) productivity of this cultivation was lower than that of indoor reference cultures (12 g/m^2 d and 4 g/m^2 d, respectively), but significant improvements can be achieved by adjustments to culture conditions such as nutrient quality and availability, salinity, pH, and dissolved oxygen and carbon dioxide.

As in other microalgae, nutrient limitation triggers product changes in diatoms (*i.e.*, reduced levels of silicon increase oil accumulation) (Botte et al. 2018), but the response seems to vary from one species to another. Molecular and biochemical approaches are other possible tools for controlling product synthesis. In the last decade, studies have begun to outline the eco-physiological processes that regulate diatom growth, including a number of lipid-based mechanisms involved in both cell death programs and allelopathy (Bidle 2016; Fontana et al. 2007; Franklin et al. 2006). These mecha-

nisms can be employed to direct synthesis of specific products at high rates or to control biomass productivity (Bacellar Mendes and Vermelho 2013; Hamilton et al. 2014). For instance, sterol sulfates have been shown to be implied in physiological cell death in the marine diatom *Skeletonema costatum*. The biochemical inhibition of their synthesis has a beneficial effect on cells with a significant increase of the cell number and the amount of produced biomass in comparison to control cultures under the same conditions (Gallo et al. 2017, 2018, 2020).

Among products extracted from microalgae, β-carotene is synthesized in large amounts from the halophilic alga *Dunaliella salina* (Borowitzka 2013), and astaxanthin, one of the most powerful antioxidants, from the freshwater alga *Haematococcus pluvialis* (Ambati et al. 2014). Species such as *D. salina, T. weissflogii* and *Cyclotella cryptica* are also known for their ability to store triacyl-glycerides, which represent 60–80% of the total lipid content (D'Ippolito et al. 2015; Scott et al. 2010). This finding suggests that microalgae could be exploited for production of biodiesel, which is proving to be a viable alternative to fossil fuels.

Secondary metabolites and drug discovery from aquatic protists

Aquatic organisms live in a particularly competitive environment, and for this reason have evolved a great number of adaptive strategies, many of which are devoted to managing predator–prey interactions. While some organisms have specific offensive/defensive morphological structures, others synthesize toxic secondary metabolites which are typically used for chemical defense and/or offence. Secondary metabolites are small organic molecules whose molecular weight usually is less than 1000 Da; they perform different physiological or ecological functions in the organism that produces them, and show a considerable structural diversity and "privileged scaffolds", with molecular characteristics adapted to the specific interaction with cellular targets. The amazing chemical and structural diversity of these bioactive molecules is unequalled by any synthetic library, providing the basis for the design of a variety of new effective drugs (Carugo and Draetta 2019; Catalani et al. 2016).

The marine environment hosts a wide variety of organisms in terms of physiological characteristics and adaptability. Since the late sixties (Carroll et al. 2020), marine organisms have been the subject of several bioprospecting studies for the identification of new therapeutic agents (Newman and Cragg 2020). Currently, 13 approved drugs are based on marine natural products and more than 20 molecules are in the pharmaceutical pipeline (Altmann 2017; Carroll et al. 2020; Liang et al. 2019; Newman and Cragg 2016). The majority of these compounds are used as anticancer agents and are produced from or were originally identified in invertebrates (mostly sponges and molluscs), while protists are still largely unexplored, mainly because it is difficult to have sufficient material for bioassay-guided purification and structure elucidation. In effect, the process of drug discovery involves pharmacological tests with raw extracts followed by isolation and characterization of the components responsible for the activity. In the specific case of marine products, the presence of high salt concentrations in cell extracts requires appropriate sample preparation and fractionation procedures (Broach and Thorner 1996; Cobice et al. 2015; Cutignano et al. 2015; Hughes et al. 2011; Zhang et al. 2016), and large amounts of starting material are usually needed to purify the active molecules. Although it is difficult to obtain these amounts from protists, there are some exceptions.

Dinoflagellates and diatoms produce several different biotoxins that are traditionally grouped on the basis on their effects upon humans: paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrheic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP), azaspiracid poisoning (AZP), and ciguatera fish poisoning (CFP) (Carroll et al. 2020; Fu et al. 2017; Kitchen et al. 2018; Rasmussen et al. 2016; Sakai and Swanson 2014; Van Dolah 2000; Wang 2008). Many of these compounds are used as powerful tools for research in cell biology and have been suggested for several pharmaceutical applications related to blockage of ion channels and transmission of electrical impulses in neurons and muscle cells (Assunção et al. 2017; Gerwick and Moore 2012; Harvey 2008; Newman and Cragg 2004; Romano et al. 2017).

Gambierol, gymnodimine, yessotoxin and other biotoxins have immunotherapeutic activity and have been proposed as immunosuppressants in dysfunctional immune system diseases, including neurodegenerative diseases (Assunção et al. 2017). The mechanisms of action of these molecules are rather diverse and include reversible down-regulation of the T cell receptor complex in T lymphocytes by yessotoxin and okadaic acid (Martín-López et al. 2011), potassium channel-dependent inhibition of resting T lymphocytes (Rubiolo et al. 2015), or activation of proinflammatory pathways in human macrophages (Crinelli et al. 2012). In addition, the symbiotic dinoflagellates of the genus *Amphidinium* produce two large families of molecules known as amphidinols and amphidinolides that have antibacterial and antifungal properties (Kobayashi 2008; Kobayashi and Tsuda 2004).

Immunomodulatory activity is the main characteristic of Sulfavant A (Fig. 2A), a synthetic derivative of α-sulfoquinovosides of the diatom *T. weissflodgii* (Manzo et al. 2017). Alpha-sulfoquinovosides are major components of chloroplast membranes in this diatom and are common in many photosynthetic organisms. Sulfavant induces the maturation of dendritic cells with up-regulation of phenotypic markers in a dose-dependent manner and enhances cell surface expression of CD83, a critical marker for *in vivo* priming of naive T cells. Used *in vivo* in B16F10 mice, Sulfavant A increases antigen-specific immune protection and significantly delays the onset and development of melanoma tumor

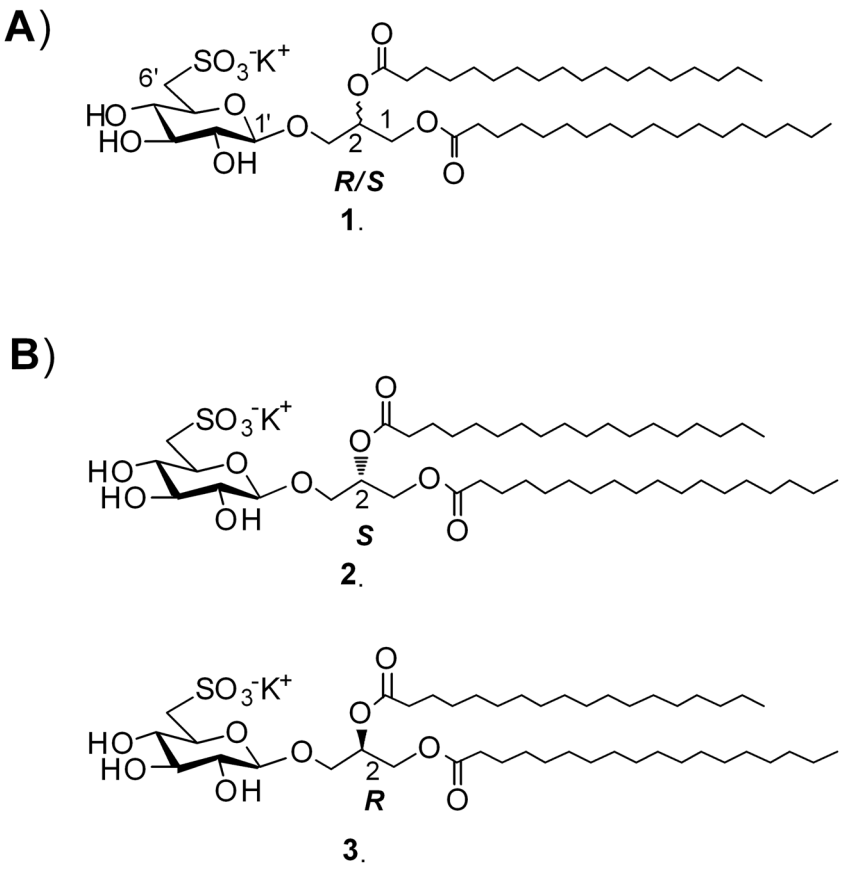

Fig. 2. Chemical structures of Sulfavants. (A) Sulfavant A (1); (B) Sulfavant S (2) and R (3).

when the molecule is associated with the human peptide gp10025-33. The boost of the immune response by Sulfavant has thus suggested its use as vaccine adjuvant; since Sulfavant has a mechanism of action independent from Toll-Like Receptors, it is potentially much less toxic than other adjuvants. Recently, other analogs with activity 1000 times more potent than Sulfavant A have been reported (Fig. 2B) (Manzo et al. 2019). In addition to antigen and adjuvant, vaccine formulation can include several additives such as stabilizers, preservatives, solubilizers and surfactants. The amphiphilic molecule of Sulfavant A and the attitude to form self-aggregation make this compound able to form stable suspensions without the addition of other ingredients (Manzo et al. 2019). These chemo-physical properties of Sulfavant A and analogs affect the immunological efficiency and can offer a technical advantage in the vaccine formulation.

The toxin climacostol and its derivates exemplify how the combination of chemistry and biology has allowed the production of new molecules of pharmacological interest. Climacostol (5-[(2*Z*)-non-2-en-1-yl] benzene-1,3-diol) is synthesized by the heterotrich freshwater ciliate *Climacostomum virens* for defense against unicellular or multicellular predators (Buonanno et al. 2013; Miyake et al. 2003). This molecule exhibits antimicrobial and cytotoxic activities against pathogenic bacteria, fungi and protozoa (Fig. 3A) (Buonanno and Ortenzi 2010; Buonanno and Ortenzi 2018; Petrelli et al. 2012). Moreover, it is able to inhibit the proliferation of human and rodent cell lines through the induction of DNA damage and apoptosis (Buonanno et al. 2008; Perrotta et al. 2016; Quassinti et al. 2013).

Climacostol has been available as a synthetic compound since 1999 (Masaki et al. 1999; Masaki et al. 2004), and more recently it has been chemically synthesized as a pure compound in the natural and most bioactive Z-configuration (Fiorini et al. 2010), allowing researchers to design and produce different synthetic analogs (Buonanno et al. 2019; Catalani et al. 2019). The addition of a methyl group to the aromatic ring of Climacostol enhances its antimicrobial and cytotoxicity activities, while the addition of a hydroxyl group in the same position induces apoptosis in protozoa (Fig. 3B) (Buonanno et al. 2019). On the other hand, the addition of a methoxymethyl ether protective group to the aromatic ring of Climacostol resulted in the synthesis of a prodrug (Fig. 3B) which can be activated by acidic conditions (pH < 7). This prodrug appears to be particularly useful for delivering the non-toxic protected Climacostol to acidic pathological multicellular or unicellular targets such as tumor cells and parasites, where it shifts to the active form. Tested *in vitro* in melanoma cells and *in vivo* in *Drosophila*

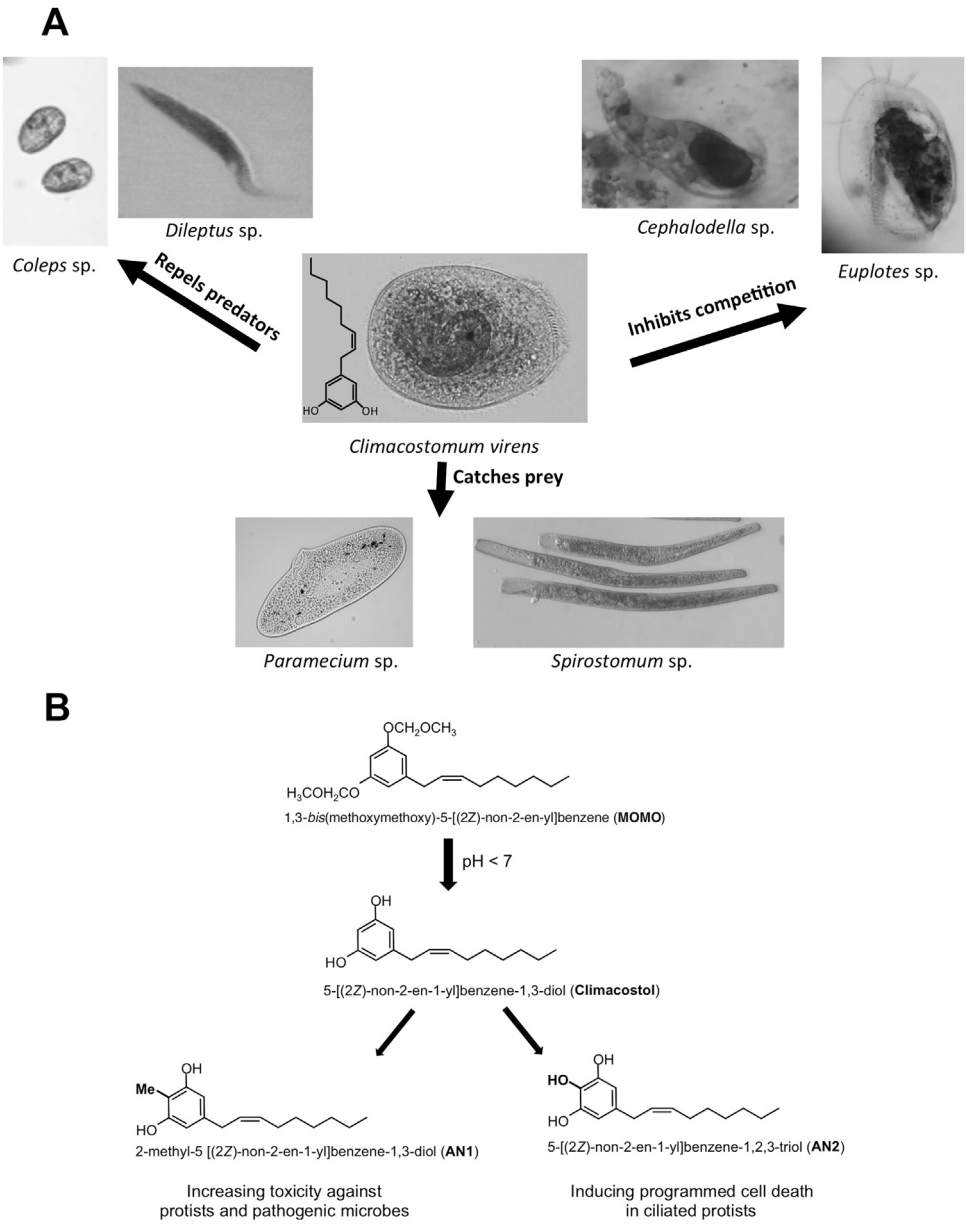

Fig. 3. Structure and activity of Climacostol, the toxic secondary metabolite produced by *Climacostomum virens*. (A) Natural functions of Climacostol. (B) The MOM-protected Climacostol (MOMO) shifts in active Climacostol if exposed to pH < 7, and the two analogs of Climacostol and their functions.

melanogaster fruit flies, this modified molecule is able to reduce viability by induction of apoptosis (Catalani et al. 2019).

Conclusion and perspective

Due to their biodiversity and ubiquity, protists are a gold mine for the discovery of new bioactive molecules which are still almost unknown. In many cases, the paucity of information about natural products and secondary metabolites from protists depends mainly on the lack of adequate techniques for obtaining the required biomass for molecular isolation and identification. Along with technological improvements, genetic and biochemical manipulations seem to be the next steps to develop the biotechnological use of natural products. In addition, the growing number of protist genomes and DNA sequences deposited in public databases will pave the way for finding new molecules by a genome mining approach.

We do not know to what extent molecules from protists will be used in biotechnological applications as biologically active (pharmaceutical) and functional (nutraceutical and cosmetic) compounds. In any case, the discovery of new molecules may contribute to better understanding of the mechanisms of adaptation to different environments, including extreme ones.

Author contributions

All authors contributed equally to this work.

Acknowledgments

AF thanks the colleagues from the Bioorganic Chemistry Unit, namely Carmela Gallo, Emiliano Manzo, Giuliana d'Ippolito, Genoveffa Nuzzo and Raffaele De Palma.

FB thanks: Elisabetta Catalani, Davide Cervia, Francesca Proietti Serafini, Simona Picchietti, Anna Maria Fausto of the Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Università degli Studi della Tuscia, Viterbo, Italy; Simone Giorgi, Gabriele Lupidi, Federico Vittorio Rossi, Enrico Marcantoni of the School of Sciences and Technologies, Section of Chemistry, Università di Camerino; Claudio Ortenzi of the Laboratory of Protistology and Biology Education, Department of E.C.H.T. Università di Macerata, Macerata, Italy.

SP and MM thank the H2020-MSCA-RISE program and the Progetto Nazionale di Ricerche in Antartide (PEA), AV the support of the University of Camerino (Fondo di Ateneo per la Ricerca 2018). The authors would also like to thank Sheila Beatty for editing the English usage in the manuscript.

References

Adelfi, M.G., Vitale, R.M., d'Ippolito, G., Nuzzo, G., Gallo, C., Amodeo, P., Manzo, E., Pagano, D., Landi, S., Picariello, G., et al., 2019. Patatin-like lipolytic acyl hydrolases and galactolipid metabolism in marine diatoms of the genus *Pseudo-nitzschia*. BBA – Mol. Cell Biol. Lipids 1864, 181–190.

Altmann, K.H., 2017. Drugs from the oceans: marine natural products as leads for drug discovery. Chimia (Aarau) 71, 646–651.

Ambati, R., Phang, S.M., Ravi, S., Aswathanarayana, R., 2014. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications – a review. Mar. Drugs 12, 128–152.

Amin, S.A., Parker, M.S., Armbrust, E.V., 2012. Interactions between diatoms and bacteria. Microbiol. Mol. Biol. Rev. 76, 667–684.

Arai, T., Fukami, D., Hoshino, T., Kondo, H., Tsuda, S., 2019. Ice-binding proteins from the fungus *Antarctomyces psychrotrophicus* possibly originate from two different bacteria through horizontal gene transfer. FEBS J. 286, 946–962.

Armbrust, E.V., 2009. The life of diatoms in the world's oceans. Nature 459, 185–192.

Assunção, J., Guedes, A.C., Malcata, F.X., 2017. Biotechnological and pharmacological applications of biotoxins and other bioactive molecules from dinoflagellates. Mar. Drugs 15, 393.

Bacellar Mendes, L.B., Vermelho, A.B., 2013. Allelopathy as a potential strategy to improve microalgae cultivation. Biotechnol. Biofuels 6, 152.

Bar Dolev, M., Braslavsky, I., Davies, P.L., 2016. Ice-binding proteins and their function. Ann. Rev. Biochem. 85, 515–542.

Bayer-Giraldi, M., Uhlig, C., John, U., Mock, T., Valentin, K., 2010. Antifreeze proteins in polar sea ice diatoms: diversity and gene expression in the genus *Fragilariopsis*. Environ. Microbiol. 12, 1041–1052.

Benoiston, A.S., Ibarbalz, F.M., Bittner, L., Guidi, L., Jahn, O., Dutkiewicz, S., Bowler, C., 2017. The evolution of diatoms and their biogeochemical functions. Philos. Trans. R. Soc. B: Biol. Sci. 372, 20160397.

Bidle, K.D., 2016. Programmed cell death in unicellular phytoplankton. Curr. Biol. 26, R594–R607.

Borowitzka, M.A., 2013. High-value products from microalgae—their development and commercialization. J. Appl. Phycol. 25, 743–756.

Botte, P., D'Ippolito, G., Gallo, C., Sardo, A., Fontana, A., 2018. Combined exploitation of CO_2 and nutrient replenishment for increasing biomass and lipid productivity of the marine diatoms *Thalassiosira weissflogii* and *Cyclotella cryptica*. J. Appl. Phycol. 30, 243–251.

Brett, M.T., Kainz, M.J., Taipale, S.J., Seshan, H., 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proc. Natl. Acad. Sci. U. S. A. 106, 21197–21201.

Broach, J.R., Thorner, J., 1996. High-throughput screening for drug discovery. Nature 384, 14–16.

Buonanno, F., Catalani, E., Cervia, D., Proietti Serafini, F., et al., 2019. Bioactivity and structural properties of novel synthetic analogues of the protozoan toxin Climacostol. Toxins 11, 42.

Buonanno, F., Harumoto, T., Ortenzi, C., 2013. The defensive function of trichocysts in *Paramecium tetraurelia* against metazoan predators compared with the chemical defense of two species of toxin-containing ciliates. Zool. Sci. 30, 255–261.

Buonanno, F., Ortenzi, C., 2010. The protozoan toxin climacostol and its derivatives: cytotoxicity studies on 10 species of free-living ciliates. Biologia 65, 675–680.

Buonanno, F., Ortenzi, C., 2018. Predator–prey interactions in ciliated protists. In: Sebata, A. (Ed.), Extremophilic Microbes and Metabolites – Diversity, Bioprespecting and Biotechnological Applications. InTech Open Editions, London, UK.

Buonanno, F., Quassinti, L., Bramucci, M., Amantini, C., et al., 2008. The protozoan toxin climacostol inhibits growth and induces apoptosis of human tumor cell lines. Chem.-Biol. Interact. 176, 151–164.

Carroll, A.R., Copp, B.R., Davis, R.A., Keyzers, R.A., Prinsep, M.R., 2020. Marine natural products. Nat. Prod. Rep. 37, 175–223.

Carugo, A., Draetta, G.F., 2019. Academic discovery of anticancer drugs: historic and future perspectives. Annu. Rev. Cancer Biol. 3, 385–408.

Catalani, E., Buonanno, F., Lupidi, G., Bongiorni, S., et al., 2019. The natural compound climacostol as a prodrug strategy based on pH activation for efficient delivery of cytotoxic small agents. Front. Chem. 7, 463.

Catalani, E., Serafini, F.P., Zecchini, S., Picchietti, S., et al., 2016. Natural products from aquatic eukaryotic microorganisms for cancer therapy: perspectives on anti-tumour properties of ciliate bioactive molecules. Pharmacol. Res. 113, 409–420.

Cheng, J., Hanada, Y., Miura, A., Tsuda, S., Kondo, H., 2016. Hydrophobic ice-binding sites confer hyperactivity of an antifreeze protein from a snow mold fungus. Biochem. J. 473, 4011–4026.

Cobice, D.F., Goodwin, R.J.A., Andren, P.E., Nilsson, A., Mackay, C.L., Andrew, R., 2015. Future technology insight: mass spec-

trometry imaging as a tool in drug research and development. Br. J. Pharmacol. 172, 3266–3283.
Collins, T., Margesin, R., 2019. Psychrophilic lifestyles: mechanisms of adaptation and biotechnological tools. Appl. Microbiol. Biotechnol. 103, 2857–2871.
Crinelli, R., Carloni, E., Giacomini, E., et al., 2012. Palytoxin and an ostreopsis toxin extract increase the levels of mRNAs encoding inflammation-related proteins in human macrophages via p38 MAPK and NF-κB. PLoS One 7, e38139.
Cutignano, A., D'Ippolito, G., Romano, G., Lamari, N., et al., 2006. Chloroplastic glycolipids fuel aldehyde biosynthesis in the marine diatom *Thalassiosira rotula*. Chembiochem 7, 450–456.
Cutignano, A., Nuzzo, G., Ianora, A., Luongo, E., et al., 2015. Development and application of a novel SPE-method for bioassay-guided fractionation of marine extracts. Mar. Drugs 13, 5736–5749.
Davies, P.L., 2014. Ice-binding proteins: a remarkable diversity of structures for stopping and starting ice growth. Trends Biochem. Sci. 39, 548–555.
Demain, A.L., 1999. Pharmaceutically active secondary metabolites of microorganisms. Appl. Microbiol. Biotechnol. 52, 455–463.
D'Ippolito, G., Sardo, A., Paris, D., Vella, F.M., et al., 2015. Potential of lipid metabolism in marine diatoms for biofuel production. Biotech. Biofuels 8, 28.
D'Ippolito, G., Tucci, S., Cutignano, A., Romano, G., Cimino, G., Miralto, A., Fontana, A., 2004. The role of complex lipids in the synthesis of bioactive aldehydes of the marine diatom *Skeletonema costatum*. Biochim. Biophys. Acta – Mol. Cell Biol. Lipids 1686, 100–107.
Fiorini, D., Giuli, S., Marcantoni, E., Quassinti, L., et al., 2010. A straightforward diastereoselective synthesis and evaluation of climacostol, a natural product with anticancer activities. Synthesis 9, 1550–1556.
Fontana, A., D'Ippolito, G., Cutignano, A., Romano, G., et al., 2007. LOX-induced lipid peroxidation mechanism responsible for the detrimental effect of marine diatoms on zooplankton grazers. Chembiochem 8, 1810–1818.
Franklin, D.J., Brussaard, C.P.D., Berges, J.A., 2006. What is the role and nature of programmed cell death in phytoplankton ecology? Eur. J. Phycol. 41, 1–14.
Frenkel, J., Vyverman, W., Pohnert, G., 2014. Pheromone signaling during sexual reproduction in algae. Plant J. 79, 632–644.
Fu, W., Nelson, D.R., Yi, Z., Xu, M., et al., 2017. Bioactive compounds from microalgae: current development and prospects. In: Atta-ur, R. (Ed.), Studies in Natural Products Chemistry, vol. 54. Elsevier, Amsterdam, pp. 199–225.
Gallo, C., d'Ippolito, G., Nuzzo, G., Sardo, A., Fontana, A., 2017. Autoinhibitory sterol sulfates mediate programmed cell death in a bloom-forming marine diatom. Nat. Commun. 8, 1–11.
Gallo, C., Landi, S., d'Ippolito, G., Nuzzo, G., Manzo, E., Sardo, A., Fontana, A., 2020. Diatoms synthesize sterols by inclusion of animal and fungal genes in the plant pathway. Sci. Rep. 10, 4204.
Gallo, C., Nuzzo, G., d'Ippolito, G., Manzo, E., Sardo, A., Fontana, A., 2018. Sterol sulfates and sulfotransferases in marine diatoms. Methods in Enzymology, vol. 605. Elsevier Inc., pp. 101–138.
Gangl, D., Zedler, J.A.Z., Rajakumar, P.D., Martinez, E.M.R., et al., 2015. Biotechnological exploitation of microalgae. J. Exp. Bot. 66, 6975–6990.
Gerecht, A., Romano, G., Ianora, A., D'Ippolito, G., Cutignano, A., Fontana, A., 2011. Plasticity of oxylipin metabolism among clones of the marine diatom *Skeletonema marinoi* (Bacillariophyceae). J. Phycol. 47, 1050–1056.
Gerwick, W.H., Moore, B.S., 2012. Lessons from the past and charting the future of marine natural products drug discovery and chemical biology. Chem. Biol. 19, 85–98.
Hamilton, M.L., Haslam, R.P., Napier, J.A., Sayanova, O., 2014. Metabolic engineering of *Phaeodactylum tricornutum* for the enhanced accumulation of omega-3 long chain polyunsaturated fatty acids. Metab. Eng. 22, 3–9.
Harding, T., Roger, A.J., Simpson, A.G.B., 2017. Adaptations to high salt in a halophilic protist: differential expression and gene acquisitions through duplications and gene transfers. Front. Microbiol. 8, 944.
Harvey, A.L., 2008. Natural products in drug discovery. Drug Discov. Today 13, 894–901.
Hughes, J.P., Rees, S.S., Kalindjian, S.B., Philpott, K.L., 2011. Principles of early drug discovery. Br. J. Pharmacol. 162, 1239–1249.
Ianora, A., Bentley, M.G., Caldwell, G.S., et al., 2011. The relevance of marine chemical ecology to plankton and ecosystem function: an emerging field. Mar. Drugs 9, 1625–1648.
Katiyar, R., Gurjar, B.R., Biswas, S., Pruthi, V., Kumar, N., Kumar, P., 2017. Microalgae: an emerging source of energy based bio-products and a solution for environmental issues. Renew. Sustain. Energy Rev. 72, 1083–1093.
Kim, H.J., Lee, J.H., Hur, Y.B., Lee, C.W., et al., 2017. Marine antifreeze proteins: structure, function, and application to cryopreservation as a potential cryoprotectant. Mar. Drugs 15, 27.
Kitchen, S.A., Bourdelais, A.J., Taylor, A.R., 2018. Interaction of a dinoflagellate neurotoxin with voltage-activated ion channels in a marine diatom. PeerJ 6, e4533.
Klok, A.J., Lamers, P.P., Martens, D.E., Draaisma, R.B., Wijffels, R.H., 2014. Edible oils from microalgae: insights in TAG accumulation. Trends Biotechnol. 32, 521–528.
Kobayashi, J., 2008. Amphidinolides and its related macrolides from marine dinoflagellates. J. Antibiot. (Tokyo) 61, 271–284.
Kobayashi, J., Tsuda, M., 2004. Amphidinolides, bioactive macrolides from symbiotic marine dinoflagellates. Nat. Prod. Rep. 21, 77–93.
Kondo, H., Hanada, Y., Sugimoto, H., Hoshino, T., Garnham, C.P., Davies, P.L., Tsuda, S., 2012. Ice-binding site of snow mold fungus antifreeze protein deviates from structural regularity and high conservation. Proc. Natl. Acad. Sci. U. S. A. 109, 9360–9365.
Lamari, N., Ruggiero, M.V., D'Ippolito, G., Kooistra, W.H.C.F., Fontana, A., Montresor, M., 2013. Specificity of lipoxygenase pathways supports species delineation in the marine diatom genus *Pseudo-nitzschia*. PLOS ONE 8, e73281.
Larson, J.H., Richardson, W.B., Knights, B.C., Bartsch, L.A., et al., 2013. Fatty acid composition at the base of aquatic food webs is influenced by habitat type and watershed land and use. PLOS ONE 8, e70666.
Lebeau, T., Robert, J.M., 2003. Diatom cultivation and biotechnologically relevant products. Part II: current and putative products. Appl. Microbiol. Biotechnol. 60, 624–632.
Liang, X., Luo, D., Luesch, H., 2019. Advances in exploring the therapeutic potential of marine natural products. Pharmacol. Res. 147, 104373.
Luporini, P., Alimenti, C., Pedrini, B., Vallesi, A., 2016a. Ciliate communication via water-borne pheromones. In: Witzany, G., Nowacki, M. (Eds.), Biocommunication of Ciliates. Springer, Berlin, pp. 159–174.

Luporini, P., Pedrini, B., Alimenti, C., Vallesi, A., 2016b. Revisiting fifty years of research on pheromone signaling in ciliates. Eur. J. Protistol. 55, 26–38.

Mangiagalli, M., Bar-Dolev, M., Tedesco, P., Natalello, A., et al., 2017. Cryo-protective effect of an ice-binding protein derived from Antarctic bacteria. FEBS J. 284, 163–177.

Mangiagalli, M., Sarusi, G., Kaleda, A., Bar Dolev, M., et al., 2018. Structure of a bacterial ice binding protein with two faces of interaction with ice. FEBS J. 285, 1653–1666.

Mangiagalli, M., Brocca, S., Orlando, M., Lotti, M., 2020. The "cold revolution". Present and future applications of cold-active enzymes and ice-binding proteins. New Biotechnol. 55, 5–11.

Manzo, E., Cutignano, A., Pagano, D., Gallo, C., et al., 2017. A new marine-derived sulfoglycolipid triggers dendritic cell activation and immune adjuvant response. Sci. Rep. 7, 6286.

Manzo, E., Gallo, C., Fioretto, L., Nuzzo, G., et al., 2019. Diasteroselective colloidal self-assembly affects the immunological response of the molecular adjuvant sulfavant. ACS Omega 4, 7807–7814.

Martín-López, A., Gallardo-Rodríguez, J., Sánchez-Mirón, A., García-Camacho, F., Molina-Grima, E., 2011. Immunoregulatory potential of marine algal toxins yessotoxin and okadaic acid in mouse T lymphocyte cell line EL-4. Toxicol. Lett. 207, 167–172.

Masaki, M.E., Harumoto, T., Terazima, M.N., Miyake, A., Usuki, Y., Iio, H., 1999. Climacostol, a defense toxin of the heterotrich ciliate *Climacostomum virens* against predators. Tetrahedron Lett. 40, 8227–8229.

Masaki, M.E., Hiro, S., Usuki, Y., Harumoto, T., et al., 2004. Climacostol, a defense toxin of *Climacostomum virens* (protozoa, ciliata), and its congeners. Tetrahedron 60, 7041–7048.

Miyake, A., Buonanno, F., Saltalamacchia, P., Masaki, M.E., Iio, H., 2003. Chemical defense by means of extrusive cortical granules in the heterotrich ciliate *Climacostomum virens*. Eur. J. Protistol. 39, 25–36.

Newman, D.J., Cragg, G.M., 2004. Marine natural products and related compounds in clinical and advanced preclinical trials. J. Nat. Prod. 67, 1216–1238.

Newman, D.J., Cragg, G.M., 2016. Drugs and drug candidates from marine sources: an assessment of the current "state of play". Planta Med. 82, 775–789.

Newman, D.J., Cragg, G.M., 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J. Nat. Prod. 83, 770–803.

Oliverio, A.M., Power, J.F., Washburne, A., Cary, S.C., et al., 2018. The ecology and diversity of microbial eukaryotes in geothermal springs. ISME J. 12, 1918.

Ong'ondo, G.O., Yasindi, A.W., Oduor, S.O., Jost, S., et al., 2013. Ecology and community structure of ciliated protists in two alkaline-saline Rift Valley lakes in Kenya with special emphasis on *Frontonia*. J. Plankton Res. 35, 759–771.

Perrotta, C., Buonanno, F., Zecchini, S., Giavazzi, A., et al., 2016. Climacostol reduces tumour progression in a mouse model of melanoma via the p53-dependent intrinsic apoptotic programme. Sci. Rep. 6, 27281.

Petrelli, D., Buonanno, F., Vitali, L., Ortenzi, C., 2012. Antimicrobial activity of the protozoan toxin climacostol and its derivatives. Biologia 67, 525–529.

Peyrat, L.A., Tsafantakis, N., Georgousaki, K., Ouazzani, J., Genilloud, O., Trougakos, I.P., Fokialakis, N., 2019. Terrestrial microorganisms: cell factories of bioactive molecules with skin protecting applications. Molecules 24, 1836.

Pischedda, A., Ramasamy, K.P., Mangiagalli, M., Chiappori, F., et al., 2018. Antarctic marine ciliates under stress: superoxide dismutases from the psychrophilic *Euplotes focardii* are cold-active yet heat tolerant enzymes. Sci. Rep. 8, 14721.

Pohnert, G., Steinke, M., Tollrian, R., 2007. Chemical cues, defence metabolites and the shaping of pelagic interspecific interactions. Trends Ecol. Evol. 22, 198–204.

Pucciarelli, S., Devaraj, R.R., Mancini, A., Ballarini, P., et al., 2015. Microbial consortium associated with the antarctic marine ciliate *Euplotes focardii*: an investigation from genomic sequences. Microb. Ecol. 70, 484–497.

Quassinti, L., Ortenzi, F., Marcantoni, E., Ricciutelli, M., et al., 2013. DNA binding and oxidative DNA damage induced by climacostol–copper (II) complexes: implications for anticancer properties. Chem.-Biol. Interact. 206, 109–116.

Rasmussen, S.A., Christian Andersen, A.J., Andersen, N.G., Nielsen, K.F., Hansen, P.J., Larsen, T.O., 2016. Chemical diversity, origin, and analysis of phycotoxins. J. Nat. Prod. 79, 662–673.

Raymond, J.A., 2014. The ice-binding proteins of a snow alga, *Chloromonas brevispina*: probable acquisition by horizontal gene transfer. Extremophiles 18, 987–994.

Raymond, J.A., Morgan-Kiss, R., 2013. Separate origins of ice-binding proteins in Antarctic *Chlamydomonas* species. PLOS ONE 8, e59186.

Raymond, J.A., Remias, D., 2019. Ice-binding proteins in a Chrysophycean snow alga: acquisition of an essential gene by horizontal gene transfer. Front. Microbiol. 10, 2697.

Reinfelder, J.R., Milligan, A.J., Morel, F.M.M., 2004. The role of the C4 pathway in carbon accumulation and fixation in a marine diatom. Plant Physiol. 135, 2106–2111.

Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., Tredici, M.R., 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol. Bioeng. 102, 100–112.

Romano, G., Costantini, M., Sansone, C., Lauritano, C., Ruocco, N., Ianora, A., 2017. Marine microorganisms as a promising and sustainable source of bioactive molecules. Mar. Environ. Res. 128, 58–69.

Roy, J.S., Poulson-Ellestad, K.L., Sieg, R.D., Poulin, R.X., Kubanek, J., 2013. Chemical ecology of the marine plankton. Nat. Prod. Rep. 30, 1364–1379.

Rubiolo, J.A., Vale, C., Martín, V., et al., 2015. Potassium currents inhibition by gambierol analogs prevents human T lymphocyte activation. Arch. Toxicol. 89, 1119–1134.

Sakai, R., Swanson, G.T., 2014. Recent progress in neuroactive marine natural products. Nat. Prod. Rep. 31, 273–309.

Sarmiento, F., Peralta, R., Blamey, J.M., 2015. Cold and hot extremozymes: industrial relevance and current trends. Front. Bioeng. Biotechnol. 3, 148.

Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., et al., 2010. Biodiesel from algae: challenges and prospects. Curr. Opin. Biotechnol. 21, 277–286.

Singh, R., Kumar, M., Mittal, A., Mehta, P.K., 2017. Microbial metabolites in nutrition, healthcare and agriculture. Biotech 7, 15.

Sun, X., Shen, X., Jain, R., Lin, Y., et al., 2015. Synthesis of chemicals by metabolic engineering of microbes. Chem. Soc. Rev. 44, 3760–3785.

Ugwu, C.U., Aoyagi, H., Uchiyama, H., 2008. Photobioreactors for mass cultivation of algae. Bioresour. Technol. 99, 4021–4028.

Vaezi, R., 2015. Enhancing the Production of Omega-3 Polyunsaturated Fatty Acids in Marine Diatoms. University of East Anglia.

Valbonesi, A., Luporini, P., 1993. Biology of *Euplotes focardii*, an Antarctic ciliate. Polar Biol. 13, 489–493.

Van Dolah, F.M., 2000. Marine algal toxins: origins, health effects, and their increased occurrence. Environ. Health Perspect. 108, 133–141.

Vance, T.D.R., Bayer-Giraldi, M., Davies, P.L., Mangiagalli, M., 2019. Ice-binding proteins and the 'domain of unknown function' 3494 family. FEBS J. 286, 855–873.

Vella, F.M., Sardo, A., Gallo, C., Landi, S., Fontana, A., D'Ippolito, G., 2019. Annual outdoor cultivation of the diatom *Thalassiosira weissflogii*: productivity, limits and perspectives. Algal Res. 42, 101553.

Voets, I.K., 2017. From ice-binding proteins to bio-inspired antifreeze materials. Soft Matter 13, 4808.

Xiao, N., Hanada, Y., Seki, H., Kondo, H., et al., 2014. Annealing condition influences thermal hysteresis of fungal type ice-binding proteins. Cryobiology 68, 159–161.

Yang, G., Aprile, L., Turturo, V., Pucciarelli, S., et al., 2013. Characterization and comparative analysis of psychrophilic and mesophilic alpha-amylases from *Euplotes* species: a contribution to the understanding of enzyme thermal adaptation. Biochem. Biophys. Res. Commun. 438, 715–720.

Zhang, G., Li, J., Zhu, T., Gu, Q., Li, D., 2016. Advanced tools in marine natural drug discovery. Curr. Opin. Biotechnol. 42, 13–23.

Wang, D.Z., 2008. Neurotoxins from marine dinoflagellates: a brief review. Mar. Drugs 6, 349–371.