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The microalga *Dunaliella* and its applications: a review

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ABSTRACT

Green microalgae in the genus *Dunaliella* have become increasingly important in biotechnology and industry. The high adaptability of *Dunaliella* to high salinity, as well as its fast growth and production of several metabolites have triggered interest. The attention of industry relates to its ability to synthesize several high-value compounds, such as β-carotene, lipids, glycerol, vitamins, and proteins. In addition, due to its tolerance to high salinity, contamination is reduced, and it can grow in open systems. *Dunaliella salina* can accumulate up to 25% dry weight in lipids and is the most efficient natural source of β-carotene. This review highlights the general characteristics of the genus, associated with its history, morphology, reproduction, occurrence, and taxonomy. The metabolic pathways for carotenoid and lipid synthesis are described. Relevant information on the most common strains is provided as well as the most widely used growth systems and conditions, and the expression systems under development. Applications of *Dunaliella* in several areas of the industry are also highlighted. Thus, this review can serve as a basis for future work and for the development of environmentally friendly, simple, and highly cost-effective production methods.

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Introduction

Algae have shown great potential in achieving sustainable and efficient solutions. In addition to their importance in ecosystems, involved in the main biogeochemical cycles (Du, Wang, You, & Zhao, 2013), they have also been widely used in industry. Specifically, microalgae are recognized as potential resources for applications in the food industry, for pharmaceutical compounds, as environmental solutions and for the production of specific compounds, among others. These organisms have many advantages, such as low production costs, high photosynthetic efficiency, high resistance, and simple genetics (Chisti, 2007; Koh & Ghazoul, 2008; León-Bañares, González-Ballester, Galván, & Fernández, 2004). The great diversity of substances, such as pigments, lipids or proteins, and mechanisms that are present in microalgae result partly from their wide diversity of origins, with multiple metabolic and physiological strategies (Iglesias et al., 2019). Thus, microalgal biotechnology has developed quickly in the last three decades (Spolaore, Joannis-Cassan, Duran, & Isambert, 2006).

Our aim here is to report on the genus *Dunaliella*, a well-known microalga used in many industrial applications. We describe the general characteristics of *Dunaliella* are described and also address its ecology, occurrence, metabolism, scientific relevance, most used species and strains, growth conditions and growing systems, genetic manipulation and specific biotechnological applications. The number of publications associated with the term [*Dunaliella*] was analysed in the PubMed database (National Center for Biotechnology Information, US National Library of Medicine), from the year 2000 to 2019. According to Fig 1, in recent years, scientific interest and consequently the number of publications related to this genus have been increasing.

General description

Dunaliella cells were identified for the first time by Michel F. Dunal (Dunal, 1838) in salt pans in the south of France and described as reddish unicellular algae. In 1905, Emanoil C. Teodoresco proposed the genus (Teodoresco, 1905). A distinguishing feature of these microalgae is their high resistance to various environmental factors, and there is therefore a high diversity of strains available for the industry. The study of particular strains has increased our understanding of the physiological adaptation mechanisms to high salinity, low pH and a wide range of temperatures (Oren, 2014). Many *Dunaliella* species have been isolated from environments with high salinity and a wide range of concentrations of other chemical

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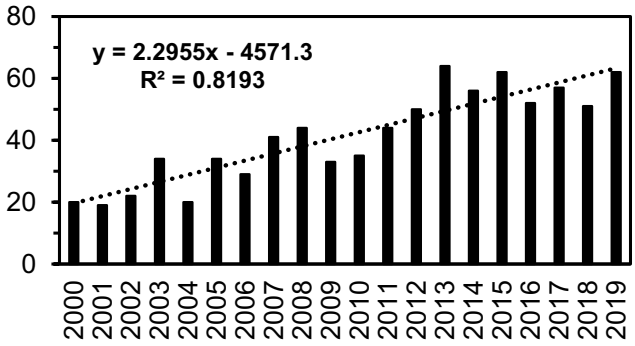

Figure 1. Number of publications related to the term [Dunaliella] between the years 2000–2019, in PubMed database (NCBI), and respective linear regression.

components (Yao et al., 2016). *Dunaliella* species have already been grown in salinities from 0.5% to 35%, so they are widely used in the industry, grown in extreme environments (Hadi, Shariati, & Afsharzadeh, 2008). *Dunaliella*'s adaptation mechanisms to different salt concentrations have been shown to be essentially based on the ability to alter the intracellular concentration of glycerol, a well-known compatible solute (Ghoshal, Mach, Agarwal, Goyal, & Goyal, 2002). On the other hand, data also indicate that this genus has an exceptional ability to remove sodium ions, in high salinity environments, through a redox sodium pump (Katz & Pick, 2001).

Dunaliella species have also been shown to be resistant to high concentrations of other compounds, such as glycerol, and to high light intensities, e.g., 2000 µmol photons m^{-2} s^{-1}. *Dunaliella acidophila* can grow in extremely acidic environments (pH 0–1) and *Dunaliella antarctica* resists temperatures below zero (Hosseini Tafreshi & Shariati, 2009; Pick, 1999), demonstrating its great adaptation capacity.

This genus occurs in extreme environments around the world (Oren, 2009). It has been isolated from cobwebs in the Atacama Desert, which can be used as a model for studying the evolution of aquatic organisms to land organisms (Cereceda, Larrain, Osses, Farías, & Egaña, 2008), in saline soils in the Great Salt Plains, Oklahoma (Buchheim, Kirkwood, Buchheim, Verghese, & Henley, 2010) and in three Antarctic lakes (Wright & Burton, 1981). *D. salina*, *D. viridis* and *D. parva* are found in the Great Salt Lake, USA (Oren, 2014). *D. salina* is also found in Lake Tyrrell in Australia (Oren, 2014), in lakes in Iran (Hadi, Shariati, & Afsharzadeh, 2008), and in halite crystals in Death Valley, USA, approximately 10–34 ky and 100 ky old (Schubert, Timofeeff, Lowenstein, & Polle, 2010).

Morphologically, *Dunaliella* cells are biflagellate, can be ovoid, spherical, pyriform, fusiform, or ellipsoidal, and can vary between 5 to 25 µm in length and 3 to 13 µm in width (Hosseini Tafreshi & Shariati, 2009). These cells do not have a rigid cell wall, being delimited by a thin and elastic membrane that allows more effective morphological adaptation, according to the osmotic pressure variations of the external environment (Ben-Amotz & Avron, 1990; Ben-Amotz, 1993). The cells contain a single, central, cup-shaped chloroplast with a central pyranoid surrounded by starch grains (Borowitzka & Siva, 2007).

Dunaliella cells multiply by longitudinal division, and sexual reproduction by isogamy can occur, where two cells merge, forming a zygote (Oren, 2005). It has also been shown that these cells can lead to the formation of palmelloid or aplanospore cells, if the environmental conditions inhibit growth (Borowitzka & Siva, 2007; Polle, Tran, & Ben-Amotz, 2009).

Knowing that *Dunaliella* species are mostly photoautotrophic, the presence of a wide variety of pigments in the cells is expected. In addition to chlorophyll *a* and *b*, several carotenoids such as α and β-carotene, neoxanthin, lutein, violaxanthin and zeaxanthin are found (Ben-Amotz & Avron, 1989*a*).

Regarding the taxonomy, the genus *Dunaliella* has been quite controversial (Polle, Tran, & Ben-Amotz, 2009). *Dunaliella* species belong to the phylum Chlorophyta, class Chlorophyceae, order Chlamydomonadales and family Dunaliellaceae. In the last few years, reassessments of the genus have been carried out, with 29 species being listed, of which 17 are halophiles: *D. parva*, *D. salina, D. pseudosalina, D. ruineniana, D. gracilis, D. bioculata, D. carpatica, D granulata, D. baasbeckingii, D. minuta, D. media, D. minutissima, D. terricola, D. viridis, D. asymmetrica, D. peircei* and *D. turcomanica*. The most researched species are *D. salina, D. tertiolecta, D. primolecta, D. viridis, D. bioculata, D. acidophila, D. parva* and *D. media* (Borowitzka & Siva, 2007). *Dunaliella bardawil* is considered to be a subspecies of *Dunaliella salina* (Gonzalez, Coleman, Gomez, & Montoya, 2001).

According to the NCBI database, the genomes of five strains of this genus have already been sequenced: *D. salina* CCAP 19/18 (GenBank: GCA_002284615.1), in 2017, *Dunaliella* sp. M2 (GenBank: GCA_004335885.1), *Dunaliella* sp. RO (GenBank: GCA_004335775.1), *Dunaliella* sp. WIN1 (GenBank: GCA_004335645.1) and *Dunaliella* sp. YS1 (GenBank: GCA_004335685.1), in 2019.

Industrially relevant metabolites

Dunaliella cells synthesize a wide range of products, among which carotenoids and lipids are the most

relevant. Some processes related to these components and their metabolism will be described.

Carotenoids

Some species, such as *D. salina* and *D. bardawil*, have the ability to accumulate high amounts of carotenoids (Ye, Jiang, & Wu, 2008). The most common type of carotenoid in *Dunaliella* cells is β-carotene, but α-carotene, lutein, zeaxanthin, cryptoxanthin and neoxanthin can also be found (Ben-Amotz, Katz, & Avron, 1982). Under specific growth conditions, some strains have the capacity to accumulate more than 15% of β-carotene by dry weight (Hosseini Tafreshi & Shariati, 2009). The high concentrations of β-carotene give the cells a red colouration (Oren, 2005).

Specifically, β-carotene has been a widely used component in the industry; thus, several strategies have been developed to maximize its production. Diverse *Dunaliella* strains have been used for the production of β-carotene in several countries, such as Australia, the USA and China (Borowitzka, 1999). *Dunaliella salina* is considered to be the most efficient natural source to produce β-carotene (Lamers, Janssen, De Vos, Bino, & Wijffels, 2008). For the induction of β-carotene accumulation, *Dunaliella* cells should be exposed to high light intensities and growth conditions that usually reflect low growth rates, such as extreme temperatures, high salinities, and limited nitrogen (Lamers, Janssen, De Vos, Bino, & Wijffels, 2008). β-carotene is a component of photosynthesis and is accumulated in lipid globules in the inter-thylakoid spaces of the chloroplasts in *Dunaliella*, under stress conditions (Hadi, Shariati, & Afsharzadeh, 2008).

The functions of carotenoids have been studied in the previous work. Knowing their conjugated polyene structure, these components play a very important role in the photosynthetic reaction, in terms of energy and oxygen transport (Siefermann-Harms, Joyard, & Douce, 1978), also preventing chlorophyll photo-damage (Ben-Amotz & Shaish, 1992). On the other hand, carotenoids can also suppress singlet oxygen and free radicals (Ye, Jiang, & Wu, 2008). More than 700 types of carotenoids have been described in natural resources (Feltl, Pacakova, Stulik, & Volka, 2006). Most of the carotenoids are C40 isoprenoids, that is, composed of eight isoprene units (Naik, Chanemougasoundharam, Khurana, & Kalloo, 2003). Specifically, β-carotene can be found in several structures since the configuration of each double bond can occur naturally as trans or cis. The most common isomers are all-trans, 9-cis, 13-cis and 15-cis (Patrick, 2000). *Dunaliella* cellular β-carotene is composed of a mixture of all-trans (42%), 9-cis (41%), 15-cis (10%) and other isomers (6%) (Borowitzka & Borowitzka, 1989).

Most of the β-carotene industrial production (approximately 85%) is still carried out by chemical synthesis (Ye, Jiang, & Wu, 2008). However, synthetic β-carotene is essentially made up of all-trans isomers, whereas in *Dunaliella* cells, high amounts of 9-cis isomers can be found, which have a much more relevant antioxidant effect (Shaish et al., 2006).

Regarding carotenoid metabolism, studies indicate that it is strongly related to the synthesis of chlorophyll (Fu et al., 2013; Lamers et al., 2010). The synthesis of each of these components can be competitive or cooperative, knowing that these processes are controlled by related metabolic pathways (Song et al., 2018).

The first step in the metabolic pathway for carotenoid synthesis is the synthesis of GGPP (geranylgeranyl pyrophosphate), by the catalyst enzyme GGPS (geranylgeranyl pyrophosphate synthase), from the addition of three IPP molecules (isopentenyl pyrophosphate) to a DMAPP molecule (Dimethylallyl pyrophosphate), via sequential reactions (Hirschberg, 2001). GGPP is the precursor to carotenoids (Xu et al., 2022).

For GGPP synthesis to be possible, IPP molecules have to be produced. Studies indicate the existence of two relevant metabolic pathways for the synthesis of IPP in plants and algae: the mevalonate (MVA) pathway and the non-mevalonate pathway (1-deoxy-D-xylulose 5-phosphate (DXP) pathway or 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway) (Lange, Rujan, Martin, & Croteau, 2000). The mevalonate pathway, described in 1950, starts with acetyl-CoA (Bloch, 1992), with the participating enzymes located in the cytosolic compartment. The vast majority of cytoplasmic IPP is synthesized by this pathway (Newman & Chappell, 1999). On the other hand, the non-mevalonate pathway, described in 1980, is responsible for the synthesis of most of the IPP present in plastids. It starts with the conjugation of D-glyceraldehyde 3-phosphate (GA3P) and pyruvate via intermediates. The pathway follows this order: 1-deoxy-D-xylulose 5-phosphate (DXP), 2-C-methyl-D-erythritol 4-phosphate (MEP), 4-diphosphocytidyl−2-C-methyl-D-erythritol (DPME), 4-diphosphocytidyl−2-C-methyl-D-erythritol 2-phosphate, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (MEcDP) and 4-hydroxy −2-methylbut−2-enyl pyrophosphate (HMBPP) (Seemann, Tse Sum Bui, Wolff, Miginiac-Maslow, & Rohmer, 2006). Finally, IPP, DMAPP and, consequently, GGPP are generated. This pathway has been described in bacteria (Rohmer, Knani, Simonin, Sutter, & Sahm, 1993), algae (Schwender, Seemann, Lichtenthaler, & Rohmer, 1996) and plants (Lichtenthaler, Schwender, & Müller, 1998). Genes, enzymes, and other components participate in the non-mevalonate pathway (Xu et al., 2022).

From GGPP, a pathway follows where several carotenoids are synthesized. The synthesis of carotenoids from GGPP is quite common in several organisms. Initially, two GGPP molecules are conjugated to obtain the first colourless carotenoid, phytoene, from the enzyme phytoene synthase (PSY). This enzyme is indicated as an essential element in the synthesis of carotenoids and regulation of carbon flow in this process (Shewmaker, Sheehy, Daley, Colburn, & Ke, 1999). Studies indicate that isomerization reactions occur in this step (Ben-Amotz, Lers, & Avron, 1988). As soon as phytoene is formed, denaturation reactions occur, obtaining, in this order: phytofluene, ζ-carotene, neurosporene and lycopene. From the formation of lycopene, two pathways for the formation of cyclic or acyclic carotenoids can be followed (Schmidt-Dannert, 2000). β-carotene is a cyclic carotenoid, so the most prevalent pathway in *Dunaliella* cells is the one that promotes the formation of cyclic carotenoids. In the cyclization process, cyclohexene rings are added to one or both ends of the lycopene molecule, from the enzyme lycopene cyclase (LYC), and the formation of δ-, α-, γ- and β-carotene takes place. Two types of the LYC enzyme have already been described, the lycopene β-cyclase (LYC-B) and lycopene ε-cyclase (LYC-E). LYC-B adds a β ring at one or two ends of the lycopene to form γ-carotene (monocyclic) or β-carotene (dicyclic) molecules. LYC-E only has the ability to add an ε ring to one end of the lycopene generating the δ-carotene (monocyclic) (Cunningham et al., 1996). For the production of α-carotene, an ε ring (by LYC-E) at one end and a β ring (by LYC-B) at the other must be added to the lycopene. It is possible to conclude that *Dunaliella* cells have both cyclases, as these cells contain α-carotene and β-carotene. From these two pathways, other components can be generated, such as α-cryptoxanthin, zeinoxanthin and lutein from α-carotene, and β-cryptoxanthin, zeaxanthin, antheraxanthin, violaxanthin and neoxanthin from β-carotene according to Fig 2. The metabolic processes previously mentioned were described in detail by Ye, Jiang, & Wu (2008).

Lipids

In addition to carotenoids, *Dunaliella* is a source of lipids, reaching 25% in dry weight (Liu et al., 2013). In general, the production of these compounds is also associated with non-favourable growth conditions, such as temperature, light intensities (Gim et al., 2014) salinities (Takagi, Karseno, & Yoshida, 2006), or with the presence of specific compounds in the medium such as sodium tungstate (Benhima et al., 2018)

Lipids are essential components in cell structure and storage, and the variation of the lipid content in microalgae is usually due to a response to changes in the medium composition. Fatty acids (FA) allow the separation of the cellular content from the extracellular medium (Matsumoto, Shioya, & Nagashima, 1984). Sterols are among the compounds of greatest interest, usually used to determine the nutritional value of microalgal food products, used as biomarkers of the presence of organic matter in sediments (Volkman et al., 1998), and as indicators of cell proliferation, modulating the activity of membrane-bound enzymes (Francavilla, Trotta, & Luque, 2010).

Large amounts of fatty acids are found in *D. salina*, with about 50% of these being mono- or polyunsaturated (Cakmak, Kaya, & Asan-Ozusaglam, 2014), principally palmitic (C16: 0), alpha linolenic (C18: 3), and oleic acid (C18: 1). Alpha-linolenic acid is one of the best-known ω−3 fatty acids, associated with benefits in brain development, and in the prevention of cardiovascular diseases (Cakmak, Kaya, & Asan-Ozusaglam, 2014).

Some studies indicate that in eukaryotic algae, the synthesis of fatty acids is simpler than in plants (Goncalves, Wilkie, Kirst, & Rathinasabapathi, 2016). Three carbon pathways have been identified for biosynthesis in chloroplasts: 1- De novo synthesis directly from CO_2 assimilation by the Calvin cycle, the formation of acetyl-CoA by pyruvate dehydrogenase and its assimilation via fatty acid synthesis cycle (Fig 3) (Coleman & Lee, 2004; Ohlrogge & Browse, 1995); 2- fatty acid acyl transfer from pre-formed polar lipids, particularly from chloroplast galactoglycerolipids (Lippold et al., 2012); 3- Synthesis from the degradation of pre-formed starch (Fig 3) (Pick & Avidan, 2017). Pick and Avidan (2017) suggest that, in *Dunaliella*, about two-thirds of the triacylglycerol come from the third route, one-third from the first, and some residual amounts come from the second route.

Other products

This genus is also used as a glycerol source. *Dunaliella*, in hypersaline conditions, produces glycerol that helps to maintain the integrity of the membrane and proteins, with some strains accumulating more than 50% dry weight (Ben-Amotz & Avron, 1990), and to maintain osmotic balance. These organisms have the ability to secrete glycerol into the medium that functions as a carbon dioxide scavenger (Chow et al., 2013). The mechanisms of glycerol production can vary depending on the

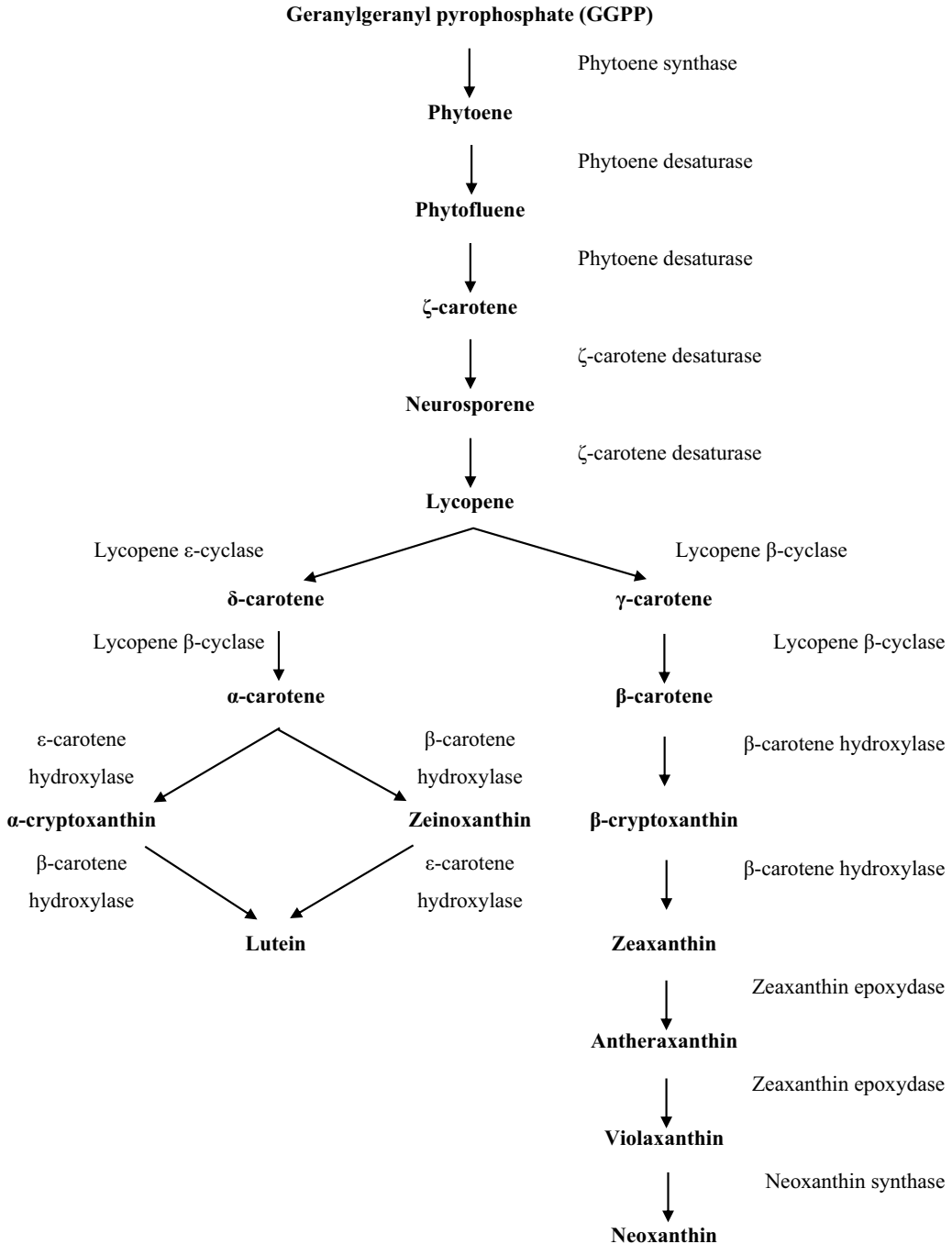

Figure 2. Metabolic pathway of carotenoid production in Dunaliella (Hirschberg et al., 1997; Hirschberg, 2001; Sandmann, 2001; Ye, Jiang, & Wu, 2008).

growth conditions, with higher production rates when cells are exposed to limited light (Ng, Low, Chow, & Lee, 2014) and high salinities. *D. salina* produces higher glycerol levels than other *Dunaliella* species (Hadi, Shariati, & Afsharzadeh, 2008).

Dunaliella is also used for the production of proteins, which can represent between 50% and 80% of dry biomass (Becker, 2007; Sui, Muys, Vermeir, D'Adamo, & Vlaeminck, 2019). *D. acidophila* grows at very low pH and has been used for the production of the enzyme H^+-ATPase, which is one of the main proteins in its membrane (Matalin et al., 2021; Sekler & Pick, 1993). Some strains of *D. salina* have large amounts of adenosylcobalamin and are identified as a possible source of vitamin B12, accumulating about

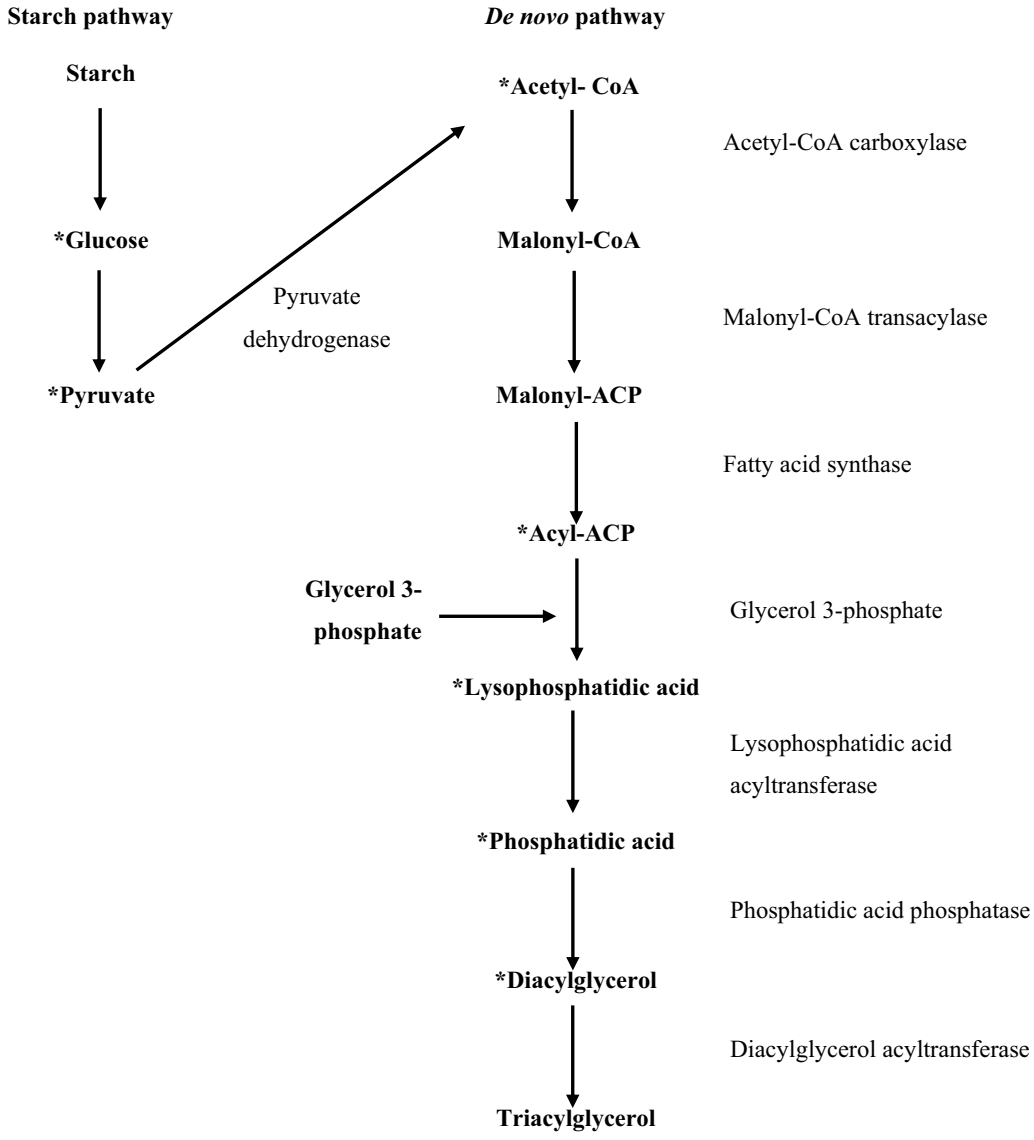

Figure 3. The starch and De novo pathway of Triacylglycerol synthesis in green algae. (*the metabolic pathways were simplified to a better understanding) (Coleman & Lee, 2004; Lippold et al., 2012; Ohlrogge & Browse, 1995; Pick & Avidan, 2017).

50% of dry weight (Kumudha & Sarada, 2016). *D. tertiolecta* is used to synthesize vitamin E, xanthophyll and violaxanthin that have antiproliferative activities (Pasquet et al., 2011).

Under particular growth conditions, some species of *Dunaliella* have antimicrobial activity against several microorganisms (Kocberber Kilic, Erdem, & Donmez, 2018). Chang et al. (1993) demonstrated that the crude extract of *D. primolecta* shows antimicrobial activity against *Staphylococcus aureus, Enterobacter aerogenes, Bacillus subtilis* and *Bacillus cereus*. The production of these compounds occurred under osmotic stress (Kocberber Kilic, Erdem, & Donmez, 2018). Lutein and ferulic acid were the main compounds responsible for the antimicrobial response (Chang et al., 1993).

Commonly used species and strains in the industry

Several species of *Dunaliella* have been studied and widely used in recent years. *D. salina* is one of the most used microalgae in the industry. Its most significant feature is its ability to accumulate large amounts of β-carotene and lipids (Yilancioglu et al., 2014). In addition, aliphatic and isoprenoid hydrocarbons, sterols, phospholipids and glycolipids have been identified in *D. salina*. *D. salina* has been the target of several studies as a physiological model and for growth optimization (Besson & Guiraud, 2013; Béchet et al., 2018; Khadim et al., 2018). Thus, numerous strains of this species have been developed and registered, partly due to their halotolerance, that prevents the growth of most competitors

Table 1. Strains used in identified studies, of different species of the genus *Dunaliella*. (Strain availability: "CCAP" - Culture Collection of Algae and Protozoa; "DF" - the Marine Biological Association; "SAG" - SAG Culture Collection; "LB" - UTEX Culture Collection; "RCC" - Roscoff Culture Collection; "CCMP"- NCMA Bigelow Laboratory for Ocean Sciences; "ATCC"- American Type Culture Collection).

	Species	Strain	Reference
1	*D. salina*	CCAP 19/18	(Park, Lee, & Jin, 2013)
2		CCAP 19/26	(Besson, Formosa-Dague, & Guiraud, 2019)
3		DF40	(Monte et al., 2020)
4		SAG 184.80	(Sui, Muys, Vermeir, D'Adamo, & Vlaeminck, 2019; Vanitha, Narayan, Murthy, & Ravishankar, 2007)
5		LB 1644	(Atasever-Arslan, Yilancioglu, Bekaroglu, Taskin, & Cetiner, 2015)
6		RCC3579	(Iglesias et al., 2019)
7		V–101	(Kumudha & Sarada, 2016)
8		MS002	(EL Arroussi et al., 2018)
9		(Lake Tuz, Turkey; Yucatan, Mexico)	(Cakmak, Kaya, & Asan-Ozusaglam, 2014; García, Freile-Pelegrín, & Robledo, 2007)
10	*D. tertiolecta*	LB 999	(Lin & Lee, 2017; Lin, Shen, & Lee, 2018; Ng, Low, Chow, & Lee, 2014)
11		CCMP1320	(Thakkar, Mitra, & Wei, 2016)
12		ATCC 30,929	(Takagi, Karseno, & Yoshida, 2006)
13	*D. bardawil*	LB 2538	(Park, Lee, & Jin, 2013)
14		(Sambar Lake, India)	(Vanitha, Narayan, Murthy, & Ravishankar, 2007)
15	*D. acidophila*	SAG 19.85	(Sekler & Pick, 1993)
16		RT22	(Puente-Sánchez, Olsson, & Aguilera, 2016)
17	*D. maritima*	CCAP 19/1	(Khramov, Matalin, Karpichev, Balnokin, & Popova, 2019)
18	*D. viridis*	(Yucatan, Mexico)	(García, Freile-Pelegrín, & Robledo, 2007)
19	*Dunaliella sp.*	RCC5	(Iglesias et al., 2019)

or predators (Ben-Amotz, Shaish, & Avron, 1991), as indicated in Table 1.

(A) *bardawil* (considered by some authors as a subspecies of *D. salina*; Gonzalez, Coleman, Gomez, & Montoya, 2001) has been grown mainly for the production of fatty acids.
(B) *tertiolecta* has also shown great biotechnological potential. This species has been the subject of many studies due to its exceptional ability to accumulate lipids, glycerol and starch (Slocombe et al., 2015). It has many advantages, such as high growth rates, efficient use of carbon dioxide and high halotolerance, among others (Chow et al., 2013; Katz, Paz, & Pick, 2019).
(C) *acidophila* is one of the most representative extreme acidophilic organisms (Aguilera & Amils, 2005). It can grow at a pH below 1.0, maintaining its intracellular pH at 7.0 (Pick, 1999).
(D) *maritima* shows high production of ATPases (Khramov, Matalin, Karpichev, Balnokin, & Popova, 2019).
(E) *viridis* has applications in the aquaculture feed industry (García, Freile-Pelegrín, & Robledo, 2007). Table 1 lists some strains that have been used commercially.

Production methods

The growing biotechnological applications of microalgae have increased interest in the research and development of growth systems and processes associated with industrial production, including a need to make microalgal biorefineries profitable (Monte et al., 2020). In the production of *Dunaliella*, systems and processes that promote the extraction of the largest number of components in the most efficient way possible must be developed (Chew et al., 2017). After laboratory evaluations define the potential of a given species, the design of the process, the scale up of the indicated photobioreactors and the development of techniques for the extraction and harvesting of the target components are carried out. This scale-up step is vital for the feasibility of future applications. The main parameters that influence microalgal productivity are mixing rate, culture depth, inoculum volume and the growth conditions (Kroumov et al., 2016).

Growth systems

Numerous microalgal growth systems have been developed, such as large open ponds, circular ponds, raceway ponds, cascade ponds, large bags, tanks, heterotrophic fermenters, and several kinds of closed photobioreactors (Borowitzka, 1999; Pulz, 2001). Within these, two main types can be distinguished: open-grown systems and closed-growth systems. The main distinction between these is the control of the conditions to which the cultures are exposed. In closed systems, the conditions are controlled more efficiently. Open growth systems have been the most widely used in recent years (Vigani et al., 2015). Open ponds represent the most economical and conventional system for microalgae growth (Ben-Amotz & Avron, 1989*a*). There are two main types of

open growth systems, very large ponds (extensive mode) of up to 250 ha (Borowitzka & Borowitzka, 1990), and smaller paddle wheel stirred raceway ponds (Ben-Amotz, 1995). For the growth of *Dunaliella* in open systems, it is favourable to establish structures in locations with a warmer and drier climate, preferably close to locations with a high concentration of NaCl. It is also important that there are no sources of contamination nearby. These systems are advantageous as they are considerably cheaper and the media can be quite selective, given the resistance to high salinity of *Dunaliella* cells (Borowitzka & Borowitzka, 1988). However, there are some disadvantages associated with this type of systems, such as the difficulty of controlling the temperature of the medium and the quality of the light to which the cells are exposed, so the productivity tends to be lower (Ben-Amotz & Avron, 1989*a*).

Photobioreactors are very advantageous systems for controlling culture parameters and are used for the growth of microalgae, cyanobacteria, and plants. There are three basic designs in the development of closed-growth systems: flat plate bioreactors, tubular photobioreactors and ultrathin immobilized configurations (Borowitzka, 1999; Pulz, 2001; Tredici & Zitelli, 1997). Tubular photobioreactors have been shown to have higher biomass productivities than flat plate bioreactors, but the latter have distinct advantages due to low oxygen accumulation, greater surface area in relation to volume, the possibility of applying turbulent flow, lower cost, easy maintenance, and bio-encrustation control (Bergmann, Ripplinger, Beyer, & Trösch, 2013). In general, closed systems allow precise control of growth conditions, enhancing the ability to precisely control light quality and the photoperiod (Lamers, Janssen, De Vos, Bino, & Wijffels, 2008). The main disadvantage of these systems is the high cost, in relation to open systems.

Growth conditions

Species of the genus *Dunaliella* have great resilience to external factors. Studies indicate that some species can survive at temperatures from 0°C to 45°C, with optimum growth occurring between 25°C and 35°C (Ben-Amotz, 1995; Jin & Polle, 2009). Borowitzka & Borowitzka (1987) demonstrated that very low nighttime temperatures (such as in Hutt Lagoon, Australia) can slowdown growth, thus decreasing the process yield. On the other hand, temperatures close to 40°C promote carotenoid synthesis, but also decrease the growth rate (Borowitzka & Borowitzka, 1989). Temperatures above 40°C can lead to the release of glycerol into the environment, which may be a carbon source for microbial contaminants (Ben-Amotz, 1995). The conditions used for the laboratory growth of some species, in some studies, are described in Table 2. The average temperature used was 24.7°C, and the most used temperature was 25°C.

Dunaliella species can resist pH values between 1, in the case of *D. acidophila*, up to 11, but for optimal growth, they require a pH close to 9 (Hosseini Tafreshi & Shariati, 2009). In intensive production systems, the pH is kept close to 7.5 as it tends to increase with the CO_2fixation process (Ben-Amotz, 1995). Considering Table 2, the average pH used was 7.6 and the most used pH value was 7.5.

In the studies indicated in Table 2, the most used medium was Modified Johnson's medium (Johnson, Johnson, MacElroy, Speer, & Bruff, 1968), followed by F/2 medium (Guillard, 1975) and Walne's medium (Walne, 1970). Carbon dioxide and bicarbonate are used as carbon sources for the growth of *Dunaliella* (usually photoautotrophic). Ben-Amotz & Avron (1989*a*) indicated the possibility of using sodium bicarbonate as a carbon source. As a source of nitrogen, nitrate is considered the most efficient. Other sources of nitrogen, such as ammonia salts or urea, are not appropriate and can lead to cell death (Borowitzka, 1990). To increase biomass and protein synthesis, a favourable availability of nitrogen is necessary (Uriarte, Farías, Hawkins, & Bayne, 1993). The source of phosphorus that leads to better results is monopotassium phosphate or monosodium phosphate. For optimal growth, *Dunaliella* cells also need sulphate and several ions, such as $K^+, Ca^{2+}, Mg^{2+}, Cl^-, Na^+$, chelated iron and trace elements (Hosseini Tafreshi & Shariati, 2009). For culture conservation, cultures are usually kept on agar media plates or liquid media, subculturing every 1–2 months. Studies also indicate the possibility of preservation at very low temperatures for up to 12 months (Taylor & Fletcher, 1998). Colusse, Mendes, Duarte, Carvalho, & Noseda (2020) directly compare the effect on growth, biochemistry, and costs of the most used media in *D. salina* cultivation. el Agawany, Kaamoush, El-Zeiny, & Ahmed (2021) demonstrated the effects of heavy metals on *D. tertiolecta* production.

Dunaliella cells usually grow under autotrophic conditions and light is their most common source of energy. Usually, the cell growth increases with photoperiod (Sui, Muys, Vermeir, D'Adamo, & Vlaeminck, 2019). Cell growth and carotenoid synthesis clearly depend on the quantity and intensity of the light. Ben-Amotz & Avron (1989*b*) demonstrated that the synthesis of carotenoids does not depend on the wavelength of the absorbed radiation but depends fundamentally on its irradiance.

Table 2. Growth conditions applied to different species of the genus Dunaliella, in the analysed studies.

Nº	Species	T (ºC)	pH	Medium	Light		NaCl (g l^{-1})	Reference
					Period (h)	Irradiance (µmol photons m^{-2} s^{-1})		
1	***D. salina***	20	7.5	Modified Johnson's medium	12; 24	55	116.90	(Sui, Muys, Vermeir, D'Adamo, & Vlaeminck, 2019)
2		25	7.0	Modified Johnson's medium	16	90–120	9.90–233.80	(Hadi, Shariati, & Afsharzadeh, 2008)
3		28	7.5	Modified Johnson's medium	16	80	29.25	(Khadim et al., 2018)
4		29	7.5	Modified Johnson's medium	12	80	100.00–350.00	(García, Freile-Pelegrín, & Robledo, 2007)
5		20	8.0	F/2 medium	16	-	35.00	(Chae, Kim, & An, 2019)
6		20	8.0	F/2 medium	12	50	35.00	(Gao et al., 2014)
7		24	7.8	F/2 medium		(Natural light)	35.00	(Song et al., 2018)
8		20	8.5	Walne's medium	12	150	35.10–122.70	(Francavilla, Trotta, & Luque, 2010)
9		25	8.5	Walne's medium	24	150	233.80	(EL Arroussi et al., 2018)
10		30	8.5	AS−100 medium		(Natural light)	87.70	(Vanitha, Narayan, Murthy, & Ravishankar, 2007)
11		25	7.5	Modified AS−100 medium	16	45	-	(Kumudha & Sarada, 2016)
12		20	8.2	Roscoff seawater	14	150	33.00	(Iglesias et al., 2019)
13		20–21	7.4	Complete Nutritive Conway	12; 16	40	100.00	(Besson, Formosa-Dague, & Guiraud, 2019)
14		25	7.5	Artificial seawater	24	40–400	87.70	(Park, Lee, & Jin, 2013)
15		26	7.5	(Custom)	-	-	87.70	(Monte et al., 2018)
16		25–35	7.0–8.0	-	-	-	90.00–120.00	(Monte et al., 2020)
17	***D. tertiolecta***	25	7.5	ATCC−1174 medium	14	50	29.20	(Lin, Shen, & Lee, 2018)
18		25	7.5	ATCC−1174 medium	14	50	29.20	(Lin & Lee, 2017)
19		25	7.5	ATCC−1174 medium	24	100	29.20–116.90	(Ng, Low, Chow, & Lee, 2014)
20		24	8.0	F/2 medium	24	120–150	35.00	(Benhima et al., 2018)
21		20	8.5	Walne's medium	12	150	35.10–122.70	(Francavilla, Trotta, & Luque, 2010)
22		19	8.1	Artificial seawater (SOW)	12	120	35.00	(Thakkar, Mitra, & Wei, 2016)
23		24	7.5	BG−11(−N) Medium	24	120–150	29.25	(Pick & Avidan, 2017)
24		28–30	8.0	Modified NORO medium	24	65–150	29.20–58.40	(Takagi, Karseno, & Yoshida, 2006)
25	***D. bardawil***	25	7.5	Artificial seawater	24	40–400	87.70	(Park, Lee, & Jin, 2013)
26		30	8.5	AS−100 medium		(Natural light)	87.70	(Vanitha, Narayan, Murthy, & Ravishankar, 2007)
27	***D. viridis***	25	7.0	Modified Johnson's medium	16	90–120	9.90–233.80	(Hadi, Shariati, & Afsharzadeh, 2008)
28		29	7.5	Modified Johnson's medium	12	80	10.00–35.00	(García, Freile-Pelegrín, & Robledo, 2007)
29	***D. acidophila***	25	0.5	(Custom)	-	-	26.90	(Sekler & Pick, 1993)
30	***D. parva***	25	7.5	Modified Johnson's medium	16	50	87.70	(Ismaiel, El-Ayouty, Said, & Fathey, 2018)
31	***Dunaliella sp.***	20	7.5	Modified Johnson's medium	24	-	25.00	(Çelekli & Dönmez, 2006)
32		30	7.5	Modified Johnson's medium	24	154	10.00–25.00	(Kocberber Kilic, Erdem, & Donmez, 2018)
33		24	8.5	Walne's medium	12	30	29.20–292.21	(Jayappriyan, Rajkumar, & Rengasamy, 2011)
34		29	7.8	F/2 medium	24	80	40.00–80.00	(Dahmen-Ben Moussa et al., 2018)

In the growth tests referenced in Table 2, the average of the photoperiod is 18 h, and the most common photoperiod was 24 h. The average photon irradiance is 103 µmol photons m^{-2} s^{-1} and the most used irradiance was 80 µmol photons m^{-2} s^{-1}. Recent studies indicate the possibility of growing *D. salina* under heterotrophic and mixotrophic conditions for the production of lipids. In this case, the most used carbon sources are glucose, acetate, and glycerol (Capa-Robles, García-Mendoza, & Paniagua-Michel, 2021; Chavoshi & Shariati, 2019; Gonabadi, Samadlouie, & Shafafi Zenoozian, 2022).

Dunaliella cells grow in environments with high concentrations of NaCl. This characteristic is extremely advantageous since most of the contaminating organisms, harmful for *Dunaliella*, do not resist high salinities (Ben-Amotz & Avron, 1989*a*; Butinar, Sonjak, Zalar, Plemenitaš, & Gunde-Cimerman, 2005). The optimal growth of *Dunaliella* is obtained between concentrations of 58.5 g l^{-1} (1 M) and 116.9 g l^{-1} (2 M) of NaCl (Hadi, Shariati, & Afsharzadeh, 2008). In the studies indicated in Table 2, the average NaCl concentration was 74.6 g l^{-1}, and the most used concentration was 87.7 g l^{-1} (1.5 M). *D. salina* increases its synthesis of carotenoids and glycerol, when grown in high salinities (Hadi, Shariati, & Afsharzadeh, 2008). These cells respond to the osmotic stress by regulating the flow of carbon between the synthesis of starch in chloroplasts and the production of glycerol in the cytoplasm (Bental, Pick, Avron, & Degani, 1990). Cycil et al. (2021) also demonstrated that *D. salina* grows exposed to low atmospheric pressures.

Harvesting and component extraction

The development of a sustainable and economically viable extraction and harvesting process for microalgal components is a major challenge. The cost represents between 20% and 40% of the total production cost (Mata, Martins, & Caetano, 2010). As such, a universal technique cannot be defined; each process depends on the species used, its characteristics and the component to be extracted (Uduman, Qi, Danquah, Forde, & Hoadley, 2010). In the case of *Dunaliella* cells, there are three main characteristics that influence the harvesting and extraction process: the absence of a rigid cell wall, the high salinity of the medium and the low cell densities (Monte et al., 2020).

Harvesting of microalgae can occur by centrifugation, filtration, flocculation, and flotation (Garg, Li, Wang, & Schenk, 2012). Component extraction is usually done via flocculation by pH modulation (Besson & Guiraud, 2013; Vandamme, Foubert, & Muylaert, 2013), electrolysis (Wan et al., 2015), metallic cations (Pirwitz, Rihko-Struckmann, & Sundmacher, 2015), or cationic polymers like chitosan or modified starch (Vandamme, Foubert, Fraeye, Meesschaert, & Muylaert, 2012). Carotenoids can be extracted from algal biomass or dry powder, using these methods: extraction in conventional organic solvents (Ruane, 1974), extraction of the carotene from the algae in an edible oil (Nonomura, 1987), separation of β-carotene isomers via CO_2 supercritical fluid extraction (Gamlieli-Bonshtein, Korin, & Cohen, 2002), or selective extraction by biocompatible organic solvents in two-phase bioreactors (Hejazi et al., 2002). These conventional processes are not very efficient, with high costs and energy consumption. In the case of cell harvesting methods, cells are often damaged, as is the case with centrifugation and filtration (Pragya, Pandey, & Sahoo, 2013). Monte et al. (2018) described a membrane-harvesting process for *D. salina* that allows cell pre-concentration, significantly increasing the efficiency of cell harvesting compared to other harvesting processes. Besson, Formosa-Dague, & Guiraud (2019) also described a process of cell harvesting of *D. salina* by flocculation/flotation that can be applied on an industrial scale. This method allows flocculation without the need for chemical flocculants, thus reducing cell damage and contamination.

Multi-product biorefineries

The concept of "Blue Biotechnology" has recently gained relevance, and consequently, the development of biorefineries that allow the extraction of several products in a single industrial production has also attracted interest (Nishshanka, Anthonio, Nimarshana, Ariyadasa, & Chang, 2022). Marine microalgae have been widely included in these processes and methods for multi-product extraction from *Dunaliella* species have already been described. Francavilla, Kamaterou, Intini, Monteleone, & Zabaniotou (2015) verified the production of a total lipid fraction rich in β-carotene, phytosterols and fatty acids in *D. tertiolecta*, and evaluated the possibility of extracting, from the residues of the first process, 45% of bio-oil and 29% biochar by pyrolysis. Harvey & Ben-Amotz (2020) demonstrated the production of 9-cis β-carotene in *D. salina*, and verified that, after the clean extraction of this compound, the residual biomass still included 50% of the lipids present in the cells. In addition, this residual biomass could also be used to produce animal feed. Monte et al. (2020) proposed a method for extracting several components of *D. salina*. A possible biorefinery has been developed that allows efficient fractional extraction of carotenoids, lipids, glycerol and proteins.

This sustainable design allowed the collection of 85% of carotenoids, 94% of polar lipids and 86% of glycerol.

Recombinant gene expression

Dunaliella has been shown to be an advantageous candidate for the synthesis of high-value products. The delay in the use of most eukaryotic algae in these systems is mainly due to the lack of knowledge of specific promoters for the efficient expression of the introduced genes. Until recently, there has not been much research on the genetic transformation and metabolism of *Dunaliella*. As a consequence of advances in genetic engineering and with the need for higher quality and yield of recombinant proteins, some species such as *D. salina, D. tertiolecta and D. parva* have aroused great interest (Feng, Li, Xu, & Qi, 2014; Ismaiel, El-Ayouty, Said, & Fathey, 2018; Lin, Shen, & Lee, 2018).

Thus, this genus has been the target of mutagenesis and genetic manipulation to increase the amounts of carotenoids and other high-value products. Recombination technologies allowed heterotrophy by autotrophic algae, thus allowing an increase in biomass per litre and the economic value of the algae (Jin & Melis, 2003). Jin & Polle (2009) created strains of *D. salina* with a mutation that allowed the manipulation of the content of carotenoids, especially zeaxanthin, producing 20 times more zeaxanthin than the wild type (Jin & Melis, 2003). Recently, a transgenic strain of *D. tertiolecta* was characterized, with an increase in medium chain length fatty acids (MCFAs) levels (Lin, Shen, & Lee, 2018). This compound is used in the production of insect pheromones (Tillman, Seybold, Jurenka, & Blomquist, 1999), detergents (Knaut & Richtler, 1985), antibiotics (Laakel et al., 1994) and surfactants (Ohlrogge, 1994). In *D. parva* transformation with an external PSY gene (phytoene synthase gene) resulted in carotenoids with greater antioxidant capacity (Ismaiel, El-Ayouty, Said, & Fathey, 2018).

The most used systems in recombination are *Escherichia coli*, yeast, mammalian cells, or transgenic animals. However, all have disadvantages that affect yield and production cost. Expression systems with *E. coli* and yeasts are low-cost, however, these have a reduced ability to make post-translational modifications (Gonçalves et al., 2013; Liu et al., 2013). Systems of transgenic animals and mammalian cells are complex and costly and show low yield (Aricescu & Owens, 2013; Guan et al., 2013). Thus, *Dunaliella* is a competitive alternative to produce recombinant compounds (Barzegari et al., 2010). As it is a microalga that does not have a rigid cell wall (Borowitzka & Siva, 2007), the transformation process is easier, requiring less time to scale-up production (Feng, Xue, Liu, & Lu, 2009). Also, it is possible to perform the harvest by removing the product of interest and taking advantage of residuals as secondary products (Hejazi, Holwerda, & Wijffels, 2004). As a haploid microorganism, the introduced genes are always expressed, whether dominant or recessive (Jones & Sparks, 2009). Although reproduction is mainly due to vegetative cell division, sexual reproduction can occur (Borowitzka & Siva, 2007). When sexual reproduction occurs between individuals with different inserted genes, the offspring can receive several genes (Primrose, 1991); however, it is not desirable in the production of multi-chain antibodies. Furthermore, post-translational processing occurs naturally in eukaryotic organisms, which is an advantage. Also, recombinant proteins can be easily removed with cell lysis procedures (Feng, Li, Xu, & Qi, 2014). The great advantage in comparison with other species is that there are no escape mechanisms for genes, making transgenic individuals easy to control and with a low probability of escape of transformed cells into the environment (Barzegari et al., 2010).

This genus has been the target of several transformation methods, including electroporation (Sun et al., 2005), particle bombardment (Tan, Qin, Zhang, Jiang, & Zhao, 2005), glass beads (Feng, Xue, Liu, & Lu, 2009), and lithium acetate/polyethylene glycol (PEG) mediated method (Chai, Chen, Xu, & Xu, 2013).

Many *Dunaliella* genes have been isolated, characterized, and transformed (He et al., 2020). Some of these genes express DNA photolyases with the function of repairing DNA damage caused by UV radiation (Yi et al., 2006), a nitrate transporter gene named DsNRT2Æ1 (He et al., 2004), plastid glycerol 3-phosphate dehydrogenases (He et al., 2007), x3 fatty acid desaturases (Lyukevich, Mouradyan, & Los, 2003), the 5-enolpyruvylshikimate–3-phosphate synthase (Yi et al., 2007), and the sodium-dependent phosphate transporter, DvSPT1 (Li et al., 2006). In *D. maritima*, the DmHA2 ATPase gene was isolated as responsible for the production of Na^+-ATPase (Khramov, Matalin, Karpichev, Balnokin, & Popova, 2019).

The genes responsible for carotenogenesis of a carotenogenic strain were identified, extracted, and used to genetically manipulate the species (Zhu, Jiang, & Chen, 2008). However, the genes involved in this pathway and its regulation are not fully understood (Jin & Polle, 2009).

Industrial applications

Due to its advantageous characteristics mentioned previously, *Dunaliella* is one of the most used species in the biotechnological industry, and its applications are observed in diverse sectors, such as carotenoid

production, food and pharmaceutical industry, bioactive compounds, bioremediation, bioindicators, biofuel and antifouling.

Carotenoids

Currently, carotenoids are the main product obtained from *Dunaliella* (Duan et al., 2023). In the industry, these components are applied as dyes, and with the growing demand for natural, healthy, and colourful products, carotenoids are highly valued as natural additives for food and cosmetics (Ye, Jiang, & Wu, 2008). These compounds are also applied in animal feeding to improve the colour manipulation of ornamental fish (Pulz & Gross, 2004) and eggs (Moulton & Burford, 1990). According to a Fortune Business Insights report the world market for carotenoids reached US $1.4 billion in 2019 and should reach US $1,8 billion by 2027.

β-carotene can be obtained naturally or synthetically, however, the natural form has greater antioxidant and anti-tumour capabilities (Hu, Lin, Lu, Chou, & Yang, 2008). Microalgae are one of the natural sources with the highest production, as well as some vegetables, fruits, and fungi (Dufossé et al., 2005). *Dunaliella* cells accumulate hundreds of times more β-carotene than a carrot cell (Klausner, 1986) in addition to being of higher quality.

The therapeutic effects of β-carotene are mainly due to its function as a protector of cells against harmful free radicals and to stimulate the immune system (Ben-Amotz, 1996). In addition, β-carotene is known to be oxidized by liver enzymes that produce vitamin A, improving vision and epithelial tissues (Hosseini Tafreshi & Shariati, 2009). This component is also effective in controlling cholesterol levels (Törnwall et al., 2004), in the prevention and treatment of diseases, such as cataracts (Gupta et al., 2003), cardiovascular problems (Shaish et al., 2006), and certain cancers (Mayne et al., 1994; Michaud et al., 2000; van Poppel, 1993). A recent study proved the effectiveness of using *Dunaliella* as a nutraceutical in rats with diet-induced obesity and hepatic lipid accumulation, without affecting food intake (Xu et al., 2022; Yamashita et al., 2020).

Food and feed industry

Due to the high production of protein, fatty acids, β-carotene, and the lack of cell walls, *Dunaliella* is an excellent feed for aquaculture and cattle, and a food source for humans (Hosseini Tafreshi & Shariati, 2009). *Dunaliella* products have been identified as important alternatives for ruminant and crustacean feed due to their high mineral and vitamin content, stimulating optimum growth (Madeira et al., 2017) and increasing the weight and survival capacity of shrimps (Supamattaya, Kiriratnikom, Boonyaratpalin, & Borowitzka, 2005). Furthermore, due to antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Candida albicans*, and *Aspergillus niger, Dunaliella* extracts can prevent contamination during the production of food products (Herrero, Jaime, Martín-Álvarez, Cifuentes, & Ibáñez, 2006).

There is also great potential for the use of components extracted from *Dunaliella* species in the development and production of food and feed supplements (Camacho, Macedo, & Malcata, 2019). Considering the β-carotene and xanthophylls extracted from these species, relevant antioxidant and anticancer capacities have already been demonstrated (Jayappriyan, Rajkumar, Venkatakrishnan, Nagaraj, & Rengasamy, 2013; Singh, Baranwal, & Reddy, 2016). The use of *Dunaliella* for protein production in the food industry is still not very common; however, the great quantity, quality, and nutritional value indicate it as a sustainable source of protein, with an amino acid content similar to soy. Currently, despite its great potential as a protein source for human consumption, it has mainly been used in feed supplements (e.g., feeding ornamental fish, shrimp and birds) (Sui & Vlaeminck, 2020). The direct use of *Dunaliella* biomass in food products is also potentially advantageous, having positive effects on the treatment of cardiovascular diseases and cancer, with anti-inflammatory properties (Silva et al., 2021).

It was also verified that the exopolysaccharides of *D. salina* are potential alternatives for the protection and improvement of agricultural crops, acting as attenuators of biotic and abiotic stress. Positive results have been obtained in tomato plants under osmotic stress (Arroussi et al., 2018).

Pharmaceutical industry and bioactive compounds

Dunaliella produces a large number of bioactive compounds, enzymes, and vitamins that are of high value in applications in the pharmaceutical industry. *D. salina* produces a unique enzyme, dihydroxy acetone reductase, which has already been commercialized (Ben-Amotz & Avron, 1990). The extract has antioxidant, antimicrobial, and antiaging activity (Cakmak, Kaya, & Asan-Ozusaglam, 2014; Havas et al., 2022). *D. tertiolecta* extracts demonstrated muscle relaxation, antiserotonin, antihypertensive, analgesic, bronchodilator, polysynaptic block, and antioedema activities (Borowitzka, 1995; Villar,

Laguna, Calleja, & Cadavid, 1992). Moreover, *D. primolecta* contains several substances with antibiotic activity (Chang et al., 1993). Pentapharm (Basel, Switzerland) developed a bioactive compound from *D. salina* that stimulates cell proliferation (Stolz & Obermayer, 2005).

In two species of *Dunaliella* (*D. tertiolecta and D. salina*) phytosterols (about 1% of dry weight) were identified and isolated (Francavilla, Trotta, & Luque, 2010). These compounds are of great value for the pharmaceutical industry as they are precursors of some bioactive molecules and act in the prevention of coronary heart disease through the reduction of cholesterol (Fernandes & Cabral, 2007). In addition, these compounds have anti-inflammatory activity and may have anti-cancer and anti-oxidative properties (Francavilla, Trotta, & Luque, 2010).

The protective effects of *D. salina* against fibrosarcoma cells are already known (Raja, Hemaiswarya, & Rengasamy, 2007); this species has selective cytotoxic potential in malignant neuroblastoma cells (Atasever-Arslan, Yilancioglu, Bekaroglu, Taskin, & Cetiner, 2015). However, *in vivo* studies are still needed to expand knowledge of the cytotoxic and anti-tumour mechanisms.

Bioremediation

The treatment of wastewater involves several steps, one of which may involve the use of microorganisms to metabolize organic matter. Microalgae can contribute to this process by removing inorganic nitrogen, phosphorus and CO_2, products generated by these microorganisms (Talbot & de la Noüe, 1993). Some species of *Dunaliella* can remove 98% of the metals in a closed system after one week of exposure (Dahmen-Ben Moussa et al., 2018). *D. tertiolecta* has heavy metal-binding peptides (phytochelatins) and can be used in bioremediation to remove heavy metals from the environment (Tsuji et al., 2002). Thus, it is a candidate for the third treatment phase of saline wastewater due to its capacity to accumulate NH_4^+ and PO_4^{3-} and heavy metals, such as copper and arsenic (Takimura, Fuse, Murakami, Kamimura, & Yamaoka, 1996; Yamaoka, Takimura, Fuse, & Murakami, 1999).

Bioindicators

Dunaliella tertiolecta is a good bioindicator for assessing the ecotoxicity of anthropogenic components in the environment. This species, as well as other algae, is highly sensitive to effluents from urban areas and industries (Lewis, Weber, & Stanley, 1998). Sacan & Balcioglu (2006) studied the effects and responses of seaweed when grown in a medium with aluminium and effluent from a pharmaceutical industrial production. *D. primolecta* was the most advantageous alga as a biomarker for four representative herbicides (Santín-Montanyá, Sandín-España, García Baudín, & Coll-Morales, 2007). *D. salina* has also been used as a model to evaluate the toxicity of a typical mutagenic phenol (Chen, Jiang, & Lin, 2007), and the effects of high concentrations of microplastics (Chae, Kim, & An, 2019).

Biofuel production

Microalgae are identified as potential sources of biomass for biofuel production due to their high lipid content and rapid growth (Chisti, 2007). However, these organisms are still not widely applied due to being economically nonviable at large scale (Benhima et al., 2018). However, the interest in applying microalgae in the production of biodiesel is increasing (Huntley & Redalje, 2007). Thus, some species of *Dunaliella*, such as *D. tertiolecta, D. primolecta and D. salina*, are strong candidates for these applications, as they can accumulate about 40% of their dry weight in the form of lipids and show high growth rates and high CO_2 absorption rates (Hosseini Tafreshi & Shariati, 2009; Sathya et al., 2023).

Antifouling

Biofouling is a worldwide phenomenon that causes enormous damage to submerged structures every year (Acevedo et al., 2013). To mitigate this, paints with toxic and biocidal components are commonly used (Rittschof, Lai, Kok, & Teo, 2003). However, these compounds have high toxicity for the surrounding environments and are very persistent in time (Thomas & Brooks, 2010). Thus, natural and environmentally friendly compounds with antifouling properties have been developed. In this context, *D. salina* emerges as a potential producer of these compounds (Gao et al., 2014). Gao et al. (2014) verified that the extract of *D. salina*, composed of unsaturated and saturated 16- and 18-carbon fatty acids, has antifouling properties against diatom species and barnacle larvae.

Conclusion

Dunaliella is a genus resistant to several extreme environments, highlighting hypersaline, low pH and low temperature conditions, often reflected in the production of compounds, such as carotenoids, lipids, and glycerol. In addition to these, *Dunaliella* species

synthesize compounds with antimicrobial and antiproliferative characteristics. Among the species of this genus, *Dunaliella salina* is the most studied and applied in the industry, followed by *D. tertiolecta*, *D. parva*, and *D. viridis*. These species are usually easy to grow, with open growth systems being the most used method in industrial production. When grown in photobioreactors, different conditions are used depending on the purpose of the production.

With the advent of genetic engineering, these species were considered as a biological platform to produce several recombinant products, presenting advantages when compared to other organisms, such as yeast or bacteria. When compared to cell lines of mammals and live animals, some *Dunaliella* species show rapid growth and higher yield with less risk of contamination. Genetic engineering has also allowed the identification and isolation of genes of interest, thus new strains have been developed with mutations in production pathways of carotenoids and other high-value products.

Dunaliella has several potential applications in the pharmaceutical and cosmetic industries, through bioactive compounds, and the food industry, as it is a rich source of protein and food supplements. These microalgae are also applied in antifouling paints, biofuel production, and bioremediation. Thus, it is expected that the high biotechnological potential associated with this genus, as reviewed here, will be reflected in a growing scientific and economic interest so that more ecological, efficient and innovative industrial solutions can be developed.

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No potential conflict of interest was reported by the authors.

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