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Sterols Biosynthesis in Algae

Mariane Bittencourt Fagundes and Roger Wagner

Abstract

Sterols are secondary metabolites, they are considered bioactive, due to their recognized activity as antioxidants, anticarcinogenic, cardiovascular protectors, and antiviral capacity. These triterpenoids can be found in a wide range of concentrations in different algae strains, being the variations related to external factors. In the world, there are millions of algae, some strains have the ability to produce high-value phytosterols, like stigmasterol, and sitosterol, however, others could lead to cholesterol production. For this reason, understand the principal factors involved in sterols biosynthesis, allows us to appoint the algae strain for industrial application and escalating these specific compounds production. Some algae are capable to produce sterols from mevalonic acid pathway, other strains present the methylerythritol 4-phosphate (MEP), or 1-deoxy-D-xylulose-5-phosphate (DOXP) as the main pathway, each one is responsible for the production of plans of intermediary compounds. In this sense, this chapter summarizes current knowledge of the biosynthetic pathways responsible for different sterols formation, as well as, describe main sterols that could be isolated from algae metabolism.

Keywords: macroalgae, microalgae, cyanobacteria, phytosterols

1. Introduction

Marine biota has a diversified metabolism, possessing worldwide most complex and unexplored organisms, and maybe the richest source of important compounds, bioactive molecules, that could lead in benefits for distinct areas in human life [1]. In this way, exploring these microorganisms in the context of their biochemistry is an important step, not only for drug discovery, or nutraceuticals, but also to understand their evolution. This affirmative comes from a question never totally elucidated about the molecular origin, and its association with algae sterol metabolism, named as “sterolomic”. This approach could present important information’s about the cell membrane, without them does not exist cellular protection and organization [2].

Cellular membrane composition is major composed by phospholipids, and between sterols cholesterol, in terms of animal cell organization, however, plants possess phytosterols replacing cholesterol, and the most interesting information in the microalgae metabolic system is associated with the capability of some strains producing both classes of sterols. In this chapter, we are going to synthesize aspects about algae principal sterols metabolic pathways, and the ways that they can be manipulated to produce specific compounds.

2. Algae metabolism: sterols discovery

The literature brings information's about diverse algae sterolomic profile, so in this chapter let us begin with the most curious and strong algae, considered the earliest life forms in the world, the prokaryotes microalgae (cyanobacteria). These strains are also known as blue-green algae, they are widely distributed in the world, due to their robustness. Cyanobacteria are considered by biologists a variation from bacteria and eucaryotic strains, which could lead in a production of sterols related with vegetal, and also animal kingdom [3].

Cyanobacteria for this reason, can occur in marine environments with a huge salt variation, in cold waters as Antarctic system, and hot waters, could also proliferate in desert sand and rocks, providing a major response from their metabolic systems modifications according to the natural evolution. These cyanobacteria can produce different metabolites according to the habitat that they are living, for this reason, merging the information's we can understand that they can present many metabolic pathways leading to different end-sterols products. Their resistance comes from their plasmatic membrane associated mostly with structures named hopanoids, that are very similar to sterols, and are responsible for the flexibility of cyanobacteria cellular membrane [4].

The major discussion on the literature is the unknown ability of these organisms producing sterols. Many years ago, some researches described the possibility to exist only hopanoids in their structure, in fact, with the advance in tandem mass spectrometry, nuclear magnetic resonance analysis associated with new extraction techniques it was discovered the presence of sterols in their membrane. Thus, metabolism involved in sterols biosynthesis by cyanobacteria are not totally elucidated.

In the history context, the first works showing sterols production in cyanobacteria were in a filamentous cyanobacteria named as *Phormidium luridum* in 1968 [5], in this study it was isolated unsaturated sterols, like as 24 ethyl sterols, following this research's other studies investigated a way to produce this metabolite in large scale, considering the fact that this cyanobacterium has resistance in front of other microorganisms, inferring a remarkable capability for industrial application.

In the ninety's the researchers Sallal, Nimer, and Radwan [6] studied other cyanobacteria strains, and verified that after dark incubation, sterols concentration increased. In in agreement to this study, Fagundes et al. [7] showed higher concentrations of sterols (β -sitosterol, stigmasterol, and cholesterol), for *Phormidium autumnale* cultured in heterotrophic system, being the inoculum without the presence of these compounds. In general, cyanobacteria are manly photosynthetic, but some strains can growth in heterotrophic conditions, in this context, it can be concluded that more studies on this particular area are necessary for further acquire more comprehension for biotechnological application.

Eukaryotic microalgae are reported in the literature as the most prominent strains for sterols production, and they are important to make feasible membrane cell permeability, and maintain structural protection [8, 9]. In this sense, the study of sterols biosynthesis started in eukaryotic cells, standing out in numerous hypothesis, and one of them is related to life adaptation on earth, showing that these molecules were produced in this cell as a protective response to reactive species of oxygen [10]. The first study in eukaryotic microalgae was in 1960 with *Scenedesmus*, showing as the major compound chondrillasterol [11], years later the same researchers Iwata and Sakurai [12] reported ergosterol as the most abundant sterol for *Chlorella*. In terms of macroalgae, the (brown) species *Ulva lactuca*, and *Cytoceira adriatica* from Adriatic Sea, were analyzed by the authors Kapetanovic et al. [13], showing that these species were the main sterols cholesterol and fucosterol for both algae.

In summary algae strain choice directly reflects in their potential for commercial application, for this reason, the knowledge of algal productivity, and the biotechnological treatment applied for each alga is important. So, understand the metabolic pathways for the full comprehension of sterols, and their intermediary metabolites formed provides important information for future culture modifications enhancing specific compounds [14]. For this, depending on the triterpenoid produced they can be applied for medical proposes, which is a great alternative since in the last decade we have the challenge for the isolation of new compounds, in front of many problems associated nowadays with diseases' outbreaks. Algae possess a diverse metabolic system; their sterol composition is interesting due to the fact that they show in their composition unconventional structural variations [15]. The main structure consist of a tetracyclic, with a fused-ring skeleton, with the presence of a hydroxyl group at the carbon 3 (head group- 3β), and biochemical modifications at the carbon C24 (in sterol side chain), besides modifications found in the tetracyclic nuclei, and also their side chain with different alkylation's patterns [15].

Nowadays, there are studies focusing on unconventional sterols bioactivity like the sterols isolated from *Isochrysis galbana*, being cholest-5-24-1,3-(acetyloxy)- 3β -ol, ergost-5-en-3- β -ol, and 24-oxocholesterol acetate. Other study identified unconventional sterols in *Sargassum fusiforme*: saringosterol, 24-hydroperoxy-24-vinyl-cholesterol, 29-hydroperoxy-stigmasta-5,24 (28)-dien- 3β -ol, 24-methylene-cholesterol, 24-keto-cholesterol, and 5α , 8α -epidioxyergosta-6,22-dien- 3β -ol all associated with anti-atherosclerotic function [16].

Industrial initiative for algae biomass application started in 20 centuries with the investment in many programs for algae research. The principal countries producing algae biomass and their products are shown in the **Figure 1**. Their major focus are on biofuels, or commercializing the biomass powder, and in terms of fine-chemicals the market is based on pigments, being only two sterols commercially produced from algae, fucosterol and desmosterol [17]. With this in mind, is important highlight that sterols are important bioactive metabolites that are normally isolated from non-renewable source, comprehend the metabolic sterols pathways and the ways to modify their production, presenting algae as a new source of sterols to the world, could lead to a sustainable sterols production.

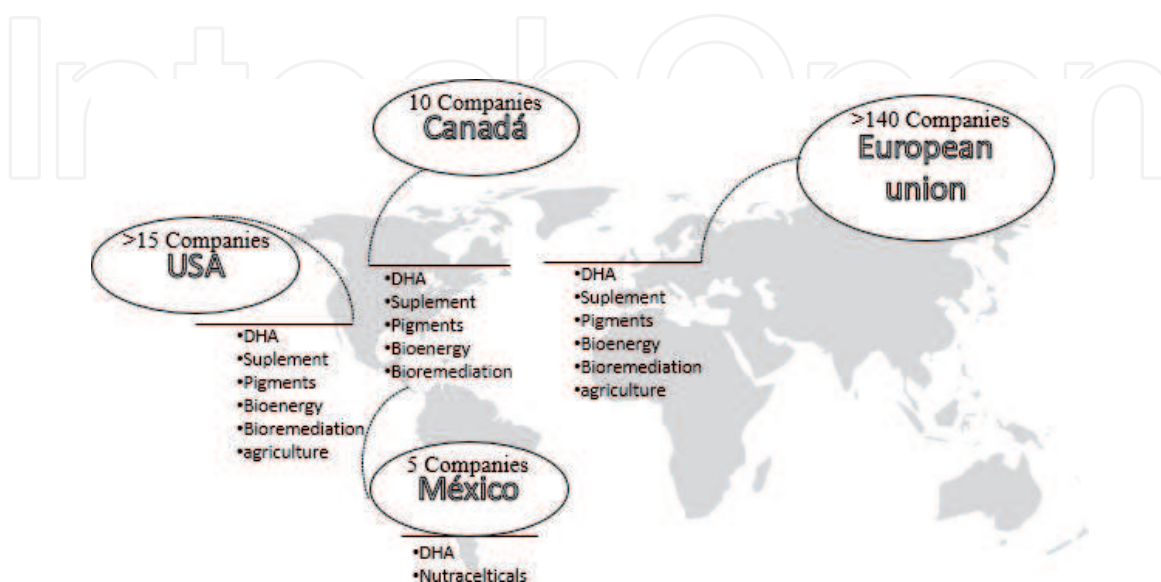


Figure 1.
Principal countries with important algae biotechnology companies' and their products. DHA - docosahexaenoic acid.

3. Algae sterols metabolic pathways

Sterols biosynthesis started by two main pathways the mevalonic acid (MVA), and by the 1-deoxy-D-xylulose-5-phosphate/2-C-methyl-D-erythritol-4-phosphate (MEP), recently discovered [18], also known as non-mevalonate pathway. The objective of these two pathways is produce an isoprenoid structure, a molecule of 5 carbons isopentenyl diphosphate (IPP), and dimethylallyl pyrophosphate (DMAPP), that are considered the sterol building block. MVA pathway occurs in cytosol, when MEP in the plastids, however the pathways activation are different according to the algae classification, being that some algae with the presence of both pathways' biochemical machinery MEP and MVA and others with only one of them active [19].

Understand the pathways involved for sterols production in algae is difficult, due to a huge phylogenetic heterogeneity found in strains. Since today still have research's showing for the first time the active pathway in some algae, like the observed by Scodelaro Bilbao et al. [20] studying *Haematococcus pluvialis*. A deeper discussion about numerous algae and the two possible active pathways can be found at the review from the authors Lohr et al. [21].

The prokaryotic cell, are known for possess MEP as the active isoprenoid producer, and for the ancestor reason, probably they were responsible for introducing this metabolism in eukaryotic strains. The MEP pathway is described as the major used for sterols production in algae, being green algae (*Chlorophyta*), with only MEP active for sterol production due to the loss of MVA pathway in the algae cellular evolution [21], as in many algae system both pathways occur, for this reason the pathways are depicted in the **Figure 2**.

The pathways are divided in two segments, the first one can be observed at the **Figure 2A**, which represents the transformation of DMAPP and IPP to squalene, this step consists in the MVA, and MEP. MVA pathway occurs in the cell cytosol until a condensation of two molecules of acetyl-CoA with the catalysis of acetoacetyl-CoA thiolase, after occurs other condensation forming 3-(*s*)-hydroxy-3-methylglutaril-CoA (HMG-CoA) by the action of 3-(*s*)-hydroxy-3-methylglutaril coenzyme A synthase. After that, the conversion to 3-(*R*)-mevalonate trough a reduction occurred by a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reductive diacylation by the enzyme HMG-CoA reductase [22]. The following steps consist in the conversion of MVA to mevalonate-5-diphosphate, catalyzed by mevalonate kinase (MK), and mevalonate-5-diphosphate kinase (MVADP), with the insertion of two ATP molecules, being the last step the conversion by isopentenyl diphosphate isomerase to the formation of DMAPP.

In terms of MEP pathway, the first step is a thiamin diphosphate-dependent condensation between D-glyceraldehyde 3-phosphate and pyruvate forming 1-Deoxy-D-xylulose-5-phosphate by the enzyme 1-deoxy-d-xylulose-5-phosphate synthase (DXS), following an isomerization to 2-C-methyl-o-erythritol-4-phosphate (MEP) by the enzyme 1-Deoxy-D-xylulose-5-phosphate (DXR) reducto-isomerase [18]. After, MEP and cytidine 5'-triphosphate are coupled, being catalyzed by 4-diphosphocytidyl-2-C-methylerythritol (MCT) synthetase, forming methylerythritol cytidyl diphosphate. The other enzymes involved in MEP pathway are in the sequence: 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase (CMK) for 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol 2-phosphate formation. After, 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (MDS) which forms the 2-C-methyl-D-erythritol-2,4-cyclic diphosphate, 4-hydroxy-3-methylbut-2-enyl diphosphate synthase (HMBPP- synthase), leading to (2E)-4-hydroxy-3-methylbut-2-enyl diphosphate, and 4-hydroxy-3-methylbut-2-enyl diphosphate

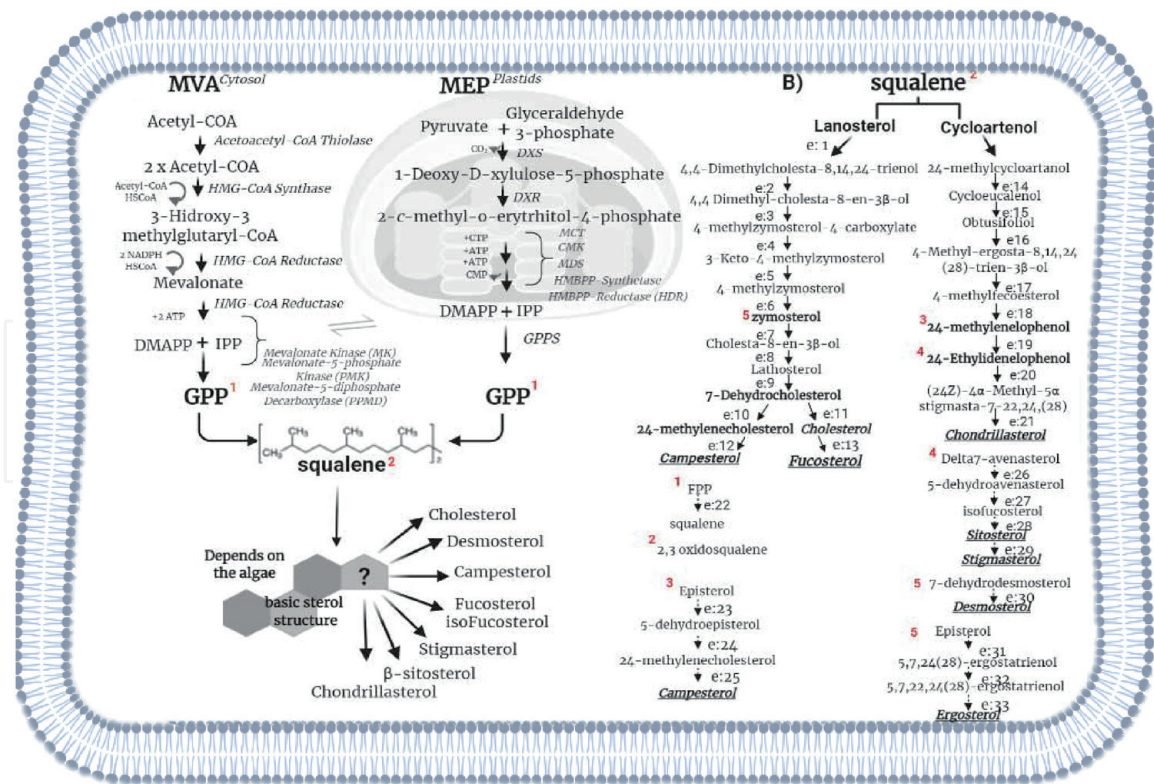


Figure 2.

Algae sterols different pathways, a: Mevalonic acid pathway, and B: Non-mevalonic acid pathway (methylerythritol 4-phosphate): HMG-CoA: Beta-Hydroxy-beta-methylglutaryl-coenzyme a, ATP: Adenosine triphosphate, GPP: Geranyl pyrophosphate, FPP: Farnesyl pyrophosphate, e:1: Lanosterol synthase, e:2: Sterol 14 α -demethylase, e:3: Methylsterol monooxygenase, e:4: Sterol-4 α -carboxylate 3-dehydrogenase, e:5: 3-keto steroid reductase, e:6: Methylsterol monooxygenase, e:7: Cholesterol Delta-isomerase, e:8: Cholesterol delta-isomerase, e:9: Delta7-sterol 5-desaturase, e:10: Delta7-sterol C5 desaturase, e:11: 7-dehydrocholesterol reductase, e:12: Delta24-sterol reductase, e:13: 24-sterol reductase, e:14: 3-beta-hydroxysteroid 3-dehydrogenase, e:15: Cycloeucaenol cycloisomerase, e:16: Sterol 14 α -demethylase, e:17: Delta14-sterol reductase, e:18: Cholesterol Delta-isomerase, e:19: 24-methylenesterol C-methyltransferase, e:20: 4- α -monomethylsterol monooxygenase, e:21: 7-dehydrocholesterol reductase, e:22: Farnesyl-diphosphate farnesyltransferase, e:23: Delta24(24(1))-sterol reductase, e:24: 7-dehydrocholesterol reductase, e:25: Delta24-sterol reductase, e:26: 3-beta-hydroxysteroid 3-dehydrogenase, e:27: 7-dehydrocholesterol reductase, e:28: Delta24-sterol reductase, e:29: Sterol 22-desaturase, e:30: 7-dehydrocholesterol reductase, e:31: Delta7-sterol 5-desaturase, e:32: Sterol 22-desaturase, e:33: Delta-24(24(1))-sterol reductase.

reductase (HMBPP-Reductase) being formed (2E)-4-hydroxy-3-methylbut-2-enyl diphosphate. The last step consists in the building blocks IPP and DMAPP and their coupling through isopentenyl-diphosphate isomerase [18, 23].

In the literature, there are numerous data, in which sometimes contrast about the biosynthesis of the isoprene units. MEP pathway was detected for the first time in bacteria, however further evidence has shown that in eukaryotes which performs photosynthesis found compounds from this metabolic pathway [24]. Normally a cyanobacteria which possess a metabolic system similar to bacteria produce phytosterols by MEP pathway, and also other authors describe that photosynthetic eukaryotic strain produce phytosterols only from MEP pathway [25]. On the other hand, MVA pathway normally is used for the production of cholesterol in animals, and also the green macroalgae sterols, in last case it occurs due to their metabolic similarity with higher plants, differently occurred with green microalgae from Chlorophyceae as described by Volkman [8, 9].

Geranyl pyrophosphate (GPP) is formed by the isoprenoids DMAPP and IPP, and through the diverse condensations leading to a presqualene compound, followed by the formation of squalene through farnesyl-diphosphate farnesyltransferase, and through squalene monooxygenase, or an alternative squalene epoxidase newly discovered [26]. These two pathways transform squalene into squalene

2,3-epoxide which is the lanosterol or cycloartenol intermediary, formed when squalene is oxidized by the enzyme squalene monooxygenase.

The following stages for different sterols isolated in algae are presented at the **Figure 2B**, being considered the anaerobic postsqualene pathway step. The biosynthesis occurs through cycloartenol pathway, however some strains produce cholesterol by lanosterol pathway. In the case of ergosterol the same pathway is activated for other microorganisms, but it is different for algae, starting their pathway by cycloartenol as observed in a study performed with *Chlamydomonas reinhardtii* [27]. Fucosterol is produced mainly by lanosterol pathway as observed by Gallo et al. [28] in diatoms, and sitosterol followed by a C22 desaturation leading to stigmaterol both produced until cycloartenol pathway, the same occurs with desmosterol and chondrillasterol. Cholesterol is represented in the pathway figure produced by lanosterol, however there is research proving that this compound production also occurs by cycloartenol-dependent pathway [29].

4. Ways to manipulate sterol biosynthesis

Algae sterols can be easily manipulated to enhance their concentration, however, only few studies show the culture manipulation for this objective. In the algae metabolism commonly, the major changes occur when algae are cultured by nutrient limitation/modification. Photosynthetic system modifications consists in changing light intensity, and carbon dioxide amount, in terms of heterotrophic culture the exogenous carbon source can be considered the most important influence in sterols biosynthesis activation, salinity can be other factor important to sterol enhancer in algae [14].

For this reason, algae culture nutrient changes for phytosterols production have been mostly reported as phosphorous and nitrogen concentration. In relation to nitrogen, Zhang, Sachs, & Marchetti [30] analyzed freshwater and marine algae and they showed a reduction of 20% in sterols production when observed a nitrogen limitation for *Eudorina unicocca* and *Volvox aureus*, the reduction was similarly was observed in *Botryococcus braunii* [31], and for *Schizochytrium* sp. [32]. On the other hand, phosphorous modifications in the culture lead to a different result, the authors Piepho et al. [33] studied concentrations of 50 mM as the highest phosphorous concentration, and 10 mM as the lowest phosphorous amount. However, the phosphorus concentration was different according to the strain, being the low phosphorous concentration 1 mM for *Scenedesmus*, 5 mM for *Cryptomonas* and *Chlamydomonas* and 10 mM P for *Cyclotella*, due to each specie requirements, being the major sterol concentration found in a high-phosphorous culture system [33].

In the same line, the authors Chen et al. [34], verify for the strains *Thalassiosira oceanica*, *Rhodomonas salina*, *Isochrysis galbana*, and *Acartia tonsa*, the effect of different iron concentration added to the culture system, in fact in this experiment it was observed that the highest levels of Fe were capable to increase the total sterols, with the exception of *Isochrysis galbana*.

The effect of salt stress showed that the concentration of total free sterols increased with higher levels of NaCl in *Nitzschia laevis* [35], being the same observed in *Dunaliella salina* [36, 37]. The same compartment was observed in *Pavlova lutheri*, the changes were not observed in their total sterol composition, but in the individual sterols concentration, the enhance of salt modify the algae membrane, avoiding an excessive flux of Na⁺ and Cl⁻ ions into cells by increasing the membrane rigidity, helping the microorganism increasing high salt concentrations [38]. The nutrient composition from the culture as already mention has a huge influence on sterols, in another study the authors Fagundes et al. [7, 39], showed

that *Phormidium autumnale* cultured with different carbon sources, glucose, sucrose, and different industrial wastes can accumulate more sterols, compared to the inoculum, and that each culture system shows a diverse composition.

Other factor of influence in sterols composition is the UV-C radiation doses, Ahmed and Schenk [40] proved that for *Pavlova lutheri* algae the sterols increase occurred by treating the algae with UV-C radiation, however the insertion of hydrogen peroxide does not show any effect. With regards to the photosynthetic system, there is few studies showing that after high light intensities the cell sterols content increase in three microalgae [33, 41].

The authors Pereira et al. [42], also showed that light intensities of 30, 60, 140, 230, and 490 mmol photons m⁻² s⁻¹ were tested for two Chlorophyceae *Scenedesmus quadricauda*, *Chlamydomonas globose*, Cryptophyceae *Cryptomonas ovata*, and the Mediophyceae (Bacillariophyta) *Cyclotella meneghiniana*, showing the best production in the highest sterol intensity. The authors explained this increase by some theories, being correlated with the algae species, as described in the biosynthesis topic some algae produce sterols from MVA pathway, and others from MEP, according to the study green algae that uses only MEP for sterols synthesis, being MEP linked to the chloroplast. For this reason, hypothetically related to the photosynthesis, being the explanation for the higher intensities of sterols found in *S. quadricauda* and the diatom *C. meneghiniana*, for this more studies needs to be performed with different strains to understand sterols metabolism.

Genetically modify strains to produce sterols are gaining attention, but also is a new strategy to turn these metabolic rich systems a source of sterols. According to D'Adamo et al. [43], they introduced in *Phaeodactylum tricornutum* three enzymes from a plant *Lotus japonicus*, the modifications were responsible for mRNA expression levels, increasing the expression of the native mevalonate and, consequently sterol biosynthesis pathway was stimulated, being responsible for the expression of important triterpenoids.

5. Final considerations

Algae sterols are a new segment for being studied, they are different according to the strain, and their environment, due to the fact that external factors affect the cellular membrane, as so, the sterol concentration. In this chapter, the most important sterols end-pathway products described are: Fucosterol, β -sitosterol, stigmasterol, ergosterol, cholesterol, chondrillasterol, and desmosterol. Still today there are research's discovering pathways for algae, due to the fact that algae are spread through the world, and can be isolated in simple access places or complex ones, being responsible for the metabolic variations. The studies involving algae sterols are ascending for industrial application, so, understand their origin is an important factor for future prospective.

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