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Review article

A new insight into cell walls of Chlorophyta

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ABSTRACT

Green microalgae, and Chlorophyta in particular, are considered as a renewable source of valuable chemicals for biofuel, nutraceutical or pharmaceutical industries, among others. Microalgae store most of their valuable components inside the cell behind a thick and resistant cell wall. Energy- or solvent-consuming steps are therefore needed to overcome this physical barrier and efficiently extract the desired compounds. Moreover, in a biorefinery context, the wall itself represents a valuable co-product. Although accurate knowledge of microalgal cell walls would be useful for product and process development and overall cost reduction, walls are still poorly understood. High discrepancies in structure and/or composition are regularly described within families, genera or species due to phenotypic plasticity, life cycle stage or erroneous taxonomical classification. Massive progress has been made in this latter research area, which gives, when compiled with the studies focusing on cell walls, a better understanding of micro-algal cell walls. This review focuses on the detailed cell wall structure and composition of Chlorophyta with a particular focus on, but not limited to, industrial micro-algae.

1. Introduction

Algae and especially green microalgae are considered an important source for sustainable production of high-value added chemicals and numerous other marketable products. Green microalgae accumulate carotenoids, vitamins or unsaturated fatty acids in appreciable amounts [1–3]. Particularly, the microalgae *Botryococcus, Chlorella, Scenedesmus, Chlamydomonas, Haematococcus, Dunaliella,* and, to a lesser extent *Tetraselmis* are currently subjects of industrial product development (Table 1) as well as intense research to improve productivity of their target molecules.

Except for rare cases, microalgae store their valuable compounds inside the cell often protected by a thick cell wall [13]. A wall disruption step is therefore needed to enhance extraction yields [14–16]. The main factors determining the suitability of a disruption process are the ability to overcome the cell wall barrier, to save the products of interest, the scalability from laboratory to industrial scale and the operating costs [17]. Moreover, unadjusted process parameters deeply affect the process assessments and thus the overall cost [17–19]. Over the last decades, due to environmental concerns, multifarious innovative processes have been developed in plant and algal biorefinery attempting to minimize energy consumption and pollutant usage [20–22].

Among these, enzyme-assisted aqueous extraction (EAE) presents several major advantages such as low energy requirements, mild and pollutant free operating conditions although high cost [20,23]. Its disruption efficiency and cost are dependent on the qualitative and quantitative accuracy of the enzymatic activities chosen to loosen the microalgal cell wall toughness and preserve the targeted molecules [20,23]. Therefore, a precise knowledge of the cell walls is unavoidable for cost reduction and high effect of the enzymatic processes [20]. Cell walls themselves are a source of components, such as polysaccharides, that, as by-products in a biorefinery context, are valuable for paper, food, fiber industries or biofuel production [24,25].

Although insufficient *per se*, taxonomy is a strong tool to aptly hypothesize the wall composition of algae and their related species to reduce the costs and time of process development by avoiding some time consuming biochemical analyses [26]. In addition, as reviewed by Champenois et al., accurate taxonomic classification is of great importance for product development [27]. Indeed, the access of microalgae in the EU food market depends on their Registration in the Novel Food Catalogue [27]. Hitherto very few green algae are registered and *e.g.,* among the three *Chlorellae* registered, one species, namely *C. luteoviridis,* has been since transferred out of the genus *Chlorella* while another one, *C. pyrenoidosa*, taxonomically no longer exists, its formerly assigned strains being transferred to different species, different genera or even within different classes (*e.g., Scenedesmus vacuolatus, Graesiella emersonii, Auxenochlorella protothecoides, etc.*) which, in all cases, are not registered in the Novel Food Catalogue [28].

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Table 1
Industrial interests of the Chlorophytes microalgae.

Microalgae	Industrial interests	Industry	References
Botryococcus	Hydrocarbons	Energy	[4]
Chlorella	Biomass, Lipids, Pigments	Food Industry, Energy	[5]
Scenedesmus	Biomass, Lipids, Pigments	Pharmaceuticals, Food, Cosmetics	[5]
Chlamydomonas	Cellular factory	Biotechnology, Pharmaceuticals	[6,7]
Haematococcus	Pigments, Astaxanthin	Pharmaceuticals, Food, Feed Industries	[8,9]
Dunaliella	Pigments, β-Carotene	Food, Feed Industries	[5]
Tetraselmis	Biomass, Vitamin E, EPA	Feed Industry	[10–12]

However, Chlorophyte wall composition, which is markedly different from land plants [29,30] is not well understood. Dramatic variability in cell wall composition and structure have been reported within a genus, a species and even within a strain or depending of life stage of the cell [31–33]. The very recent emergence of molecular phylogeny has brought deep revisions particularly in the organization of Chlorophyta [34]. Consequently, most of the work done on the wall composition and structure of Chlorophytes, which has been made in the past half-century, has been made under now revised genera or species names and thus is confusing. An updated review is therefore needed. Although the phylogeny will surely continue to evolve, the taxonomical revisions already allow a better comprehension of the cell walls of Chlorophyta.

The goal of the present work is therefore to clarify the data by considering the new taxonomical revisions while reviewing the cell walls of microalgae. As a comprehensive review on cell walls of the Chlorophyta is a gigantic undertaking, this work focuses mainly on industrially valuable Chlorophytes, particularly because the amount and quality of data is satisfactory and, as described above, because those algae are the subjects of product development that could involve a disruption process. This review thus focuses on the green microalgae of industrial interest and spread across the classes of Chlorophyta including, but not limited to, *Botryococcus, Chlorella, Scenedesmus, Chlamydomonas, Haematococcus, Dunaliella* and *Tetraselmis.*

2. Taxonomy of Chlorophyta

Chlorophyta, comprising the majority of the green algae, belong together with the Streptophyta to the Viridiplantae. The phylum accommodates a wide variety of algae from the smallest known eukaryote (*i.e., Ostreococcus*) to symbiotic algae inclusive of giant unicells with multiple nuclei, single cells and multicellular forms [35,36]. The monophyly of Chlorophyta is widely accepted while its subdivision is however still under debate [34]. Chlorophytes were subdivided in four distinct lineages, Chlorophyceae, Trebouxiophyceae, Ulvophyceae and Prasinophyceae [37,38]. Recent works demonstrated that this classification was not fitting its evolutionary history [34]. The previous works were based on morphological and ultrastructural criteria. The advent of molecular phylogeny, based on ribosomal RNA, actin, chloroplast and mitochondrial genes, brought new elements of analysis [34]. Indeed, species complexes, namely morphospecies including different biological species, have been revealed [34] as well as some cryptic species [39–42]. In addition, emergence of polyphasic approaches, which is a combination of morphological, ultrastructural observations under different conditions and different life stages with molecular and multi-gene comparisons, give a better comprehension of the phylogeny of green algae [34,43,44]. The recent taxonomic investigations led therefore to the evolution of the division model within Chlorophyta [36]. Most revisions occurred at species and genera

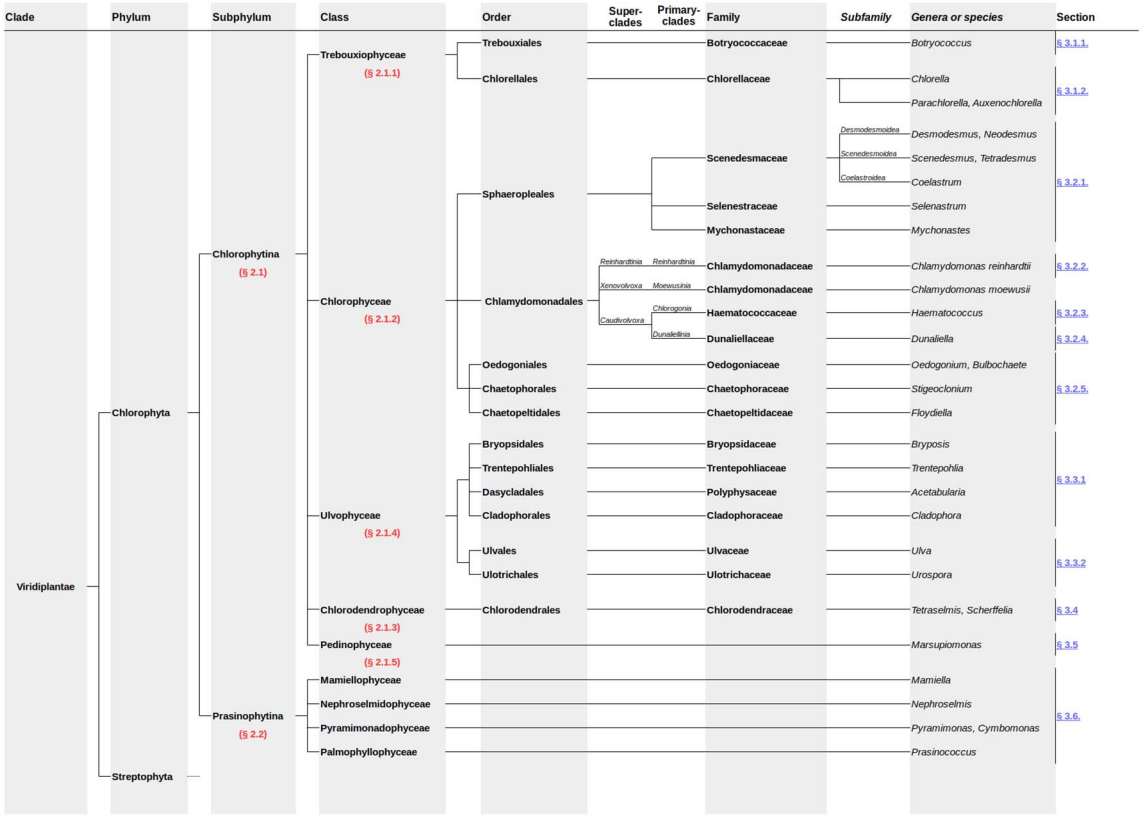

Fig. 1. Overview of the main lineages of Chlorophytes (modified from [43,53]). Number in brackets refer to the sections of the review dealing with the phylogeny of the subphylum or class; Underlined numbers refer to the sections dealing with the cell walls.