

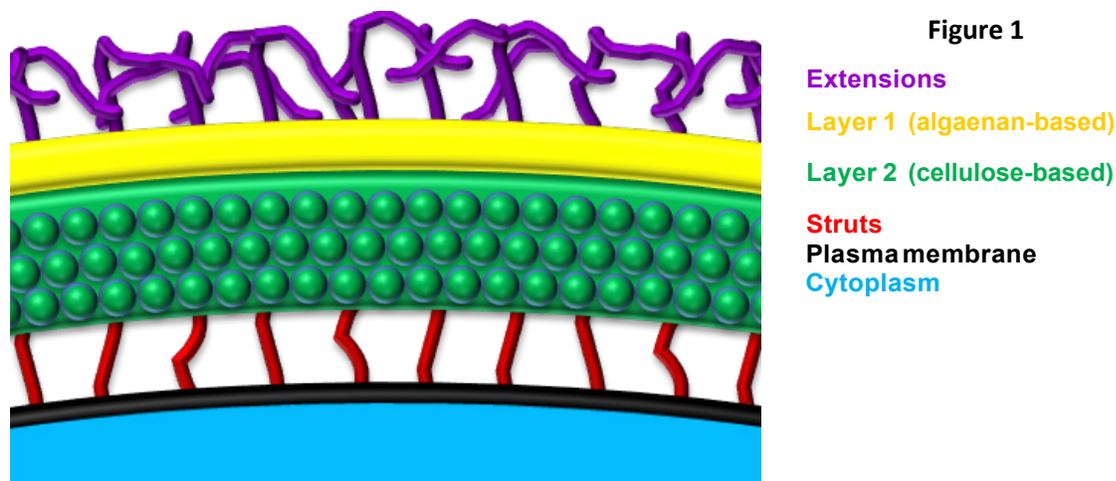
150. Ultrastructure and Composition of the *Nannochloropsis gaditana* Cell Wall

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Project Goals: Some of the oleaginous eukaryotic microalgae that best thrive in open-pond growth facilities, like the chlorophyte *Scenedesmus* and the eustigmatophyte *Nannochloropsis*, are endowed with “recalcitrant” cell walls that presumably confer resistance to predation and desiccation during growth but are refractory to breakage and drying and hence to product extraction—notably the extraction of triacylglyceride (TAG). These walls have been shown to contain cross-linked long-chain hydrocarbons, generically called “algaenans,” that defy solubilization in the laboratory. We report a comprehensive analysis of *N. gaditana* wall ultrastructure and composition, documenting that its two major components are algaenan and cellulose, and present the first spectral profiles of non-denatured algaenan using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. We also identify and characterize *N. gaditana* genes encoding proteins that may participate in the biosynthesis and secretion of these wall components— genes that might be engineered such that expression conferring robustness during growth is switched off at time of harvest.

Quick-freeze deep-etch electron micrographs of native walls or shed mother walls of *N. gaditana* document a two-layered structure (Fig. 1). When walls are instead prepared from linear-phase cells subjected to multiple passes through a French Press, layer 2 is converted from a dense to a loose configuration. Whereas the dense configuration is refractory to cellulase digestion, the loose configuration is completely sensitive, generating pure preparations of layer 1 that have not been subjected to any denaturants. Biochemical and spectral analyses of non-digested and digested wall preparations document that layer 1 is dominated by algaenan, forming a seal around the cell, and that layer 2 is dominated by cellulose. We hypothesize that the native dense layer-2 configuration represents a form of crystalline cellulose that is resistant to most cellulases and provides additional hardness to the organism.



Spectra of non-denatured layer 1 are consistent with previous analyses of denatured material (1, 2) which concluded that *Nannochloropsis* algaenan consists of straight-chain, aliphatic hydrocarbons that are cross-linked via ether bridging and contain few, if any, branched methyl groups. This is consistent with algaenan synthesis proceeding by the crosslinking of fatty alcohols/aldehydes/acids synthesized by either fatty acid synthases (FASs) or polyketide synthases (PKSs), rather than via terpenoid biosynthetic pathways.

Inspection of the *N. gaditana* genome identified 6 putative polyketide synthase (PKS) genes, and domains encoding components of a type II FAS complex. Five of these PKS genes are conserved in the *N. oceanica* genome. The *PKS1* gene contains a thioesterase module, indicating free fatty acids as the final product, whereas the other 5 genes do not encode this module. The *PKS4-6* genes encode a fatty acyl-reductase (FAR) domain instead of a thioesterase domain, indicating that fatty aldehydes—or potentially fatty alcohols if a four-electron reduction is catalyzed—are released from the acyl-ACP (acyl-carrier protein) rather than fatty acids. The *PKS2/3* genes contain terminal non-ribosomal peptide synthetase (NRPS) domains; these participate in a myriad of reactions wherein a diversity of nucleophiles, including amino acids, can attack the thioester linkage on ACP to establish a new covalent linkage with the acyl group formerly bound to the PKS ACP. The lack of dehydratase (DH) and enoyl reductase (ER) domains in *PKS2/3* suggests that internal alcohols are retained in the products of these enzymes after keto reductase (KR) reduction, at the β -position of the C_2 extension(s), to the fatty acids that are loaded onto these PKSs by fatty acyl-AMP ligase (FAAL).

Intriguingly, the NRPS modules in *PKS2/3* contain predicted membrane-spanning domains that could allow a growing algaenan chain to exit on the extracellular side of the plasma membrane as fatty acyl groups are polymerized, which could explain how intracellular metabolic precursors are polymerized into longer-chain algaenan precursors that are deposited on the cell exterior. Additionally, *PKS1*, 3, and 4 are adjacent to genes encoding ABC transporters (pathway genes are frequently clustered in the *Nannochloropsis* genome), which may also play a role in the export of some lipophilic algaenan precursors.

In sum, 6 PKSs have been identified that are promising candidates for roles in algaenan synthesis, and putative mechanisms for the secretion of algaenan precursors have been identified. Future experiments will explore the effects of knocking out or knocking down one or more of these genes. If the mutants display compromised wall structure, then we will attempt to construct strains wherein the gene(s) are expressed during growth but blocked near the time of harvest.

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2. Gelin, F., J.K. Volkman, C. Largeau, S. Derenne, J.S.S. Damste, and J.W. De Leeuw (1999). Distribution of aliphatic, nonhydrolyzable biopolymers in marine microalgae. *Organic Geochemistry*, **30**, 147-159.

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